EFFECTS OF MATERNAL DIETS WITH NEGATIVE DCAD PREPARTUM ON CALF GROWTH, HEALTH AND METABOLISM

By

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To my mother

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LIST OF ABBREVIATIONS

BHBA	Beta-hydroxybutyric acid
BW	Body weight
CO_2	Carbon dioxide
d	Day
DCAD	Dietary cation-anion difference
HCO_3^-	Bicarbonate
iCa	Ionized calcium
IGF	Insulin-like growth factor
IgG	Immunoglobulin G
K	Potassium
L	Long duration of feeding a diet with negative DCAD
LSM	Least squares means
Mg	Magnesium
Na	Sodium
NEFA	Non-esterified fatty acids
pCO_2	Partial pressure of carbon dioxide
pO_2	Partial pressure of oxygen
S	Short duration of feeding a diet with negative DCAD
SD	Standard deviation
SEM	Standard error of the mean
tCa	Total calcium

Abstract of Thesis Presented to the Graduate School
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The objective of Chapter 1 is to review research that has linked maternal nutrition to fetal programming in various species. Human epidemiological studies have provided extensive scientific information that allowed researchers to correlate adult diseases, such as diabetes and obesity, with fetal growth. Furthermore, malnutrition, either under or over nutrition during early, mid or late gestation can "program" the developing fetus. Studies in beef cattle and sheep have shown that nutrient deficiency and nutrient supplementations during late gestation can impact the offspring's reproduction, growth and performance postnatally. Despite the progress in fetal programming within livestock species, the limited available research in dairy cattle will be discussed in this chapter. The dairy industry utilizes several management and nutritional strategies to alleviate the metabolic stress associated with the onset of lactation in the transition period as well as to mitigate disorders such as hypocalcemia. This literature review summarizes the various strategies that are currently used to mitigate hypocalcemia, more specifically, it focuses on the use of acidogenic salts prepartum and the potential effects these diets may have on the calf's growth, metabolism and immunity.

Chapter 2 describes and discuss the findings of my experiment designed to evaluate the effects of maternal supplementation of acidogenic salts in growth, immunity, hematology parameters, energy and mineral metabolism of calves. The experimental design was a randomized block design with a 2x2 factorial arrangement of two negative DCAD levels, -70 or -180 mEq/kg, and two feeding durations, the last 21 days (d; short) or 42 d (long) prepartum. After birth, all calves were transported to the University of Florida Calf Unit and managed according to the University of Florida standard operating procedures. At birth and weaning, calves born to dams fed diets with negative DCAD for 42 d prepartum weighed less compared with calves born to dams fed the same diets with negative DCAD for 21 d (40.0 *vs*. 42.8 kg ± 0.8 and 76.7 *vs*. 81.5 ± 1.8 kg, respectively). However, calf body weight at 3 or 6 months of age did not differ with treatments. Calves born to dams fed -180 mEq/kg DCAD had greater ionized calcium (iCa) concentrations from birth to 3 d of age than calves born to dams fed -70 mEq/kg DCAD. At birth, calves born to -180 DCAD dams experienced a more defined metabolic acidosis compared with calves born to -70 DCAD dams, and by 3 d of age all calves there were no differences in the measures of acid-base balance. Calves born to -180 DCAD dams had smaller concentration of BHBA compared with calves born to -70 DCAD dams. Plasma immunoglobulin G (IgG) concentrations and apparent efficiency of absorption of IgG did not differ with maternal dietary treatments, thus, passive immune transfer was not impacted. Our data show that, in spite of slight alterations in calf growth, acid-base balance, mineral and energy metabolism during the neonatal period, feeding -70 and -180 mEq/kg DCAD diets during late gestation to dairy cows did not greatly impact the overall health and performance of the offspring.

CHAPTER 1
LITERATURE REVIEW

Maternal Effects on the Offspring

Development of the conceptus involves crucial periods of rapid cell division that occurs at different times of gestation and in various parts of the body which are essential for growth and maturation of tissues and organs (Barker, 1993). During these crucial developmental periods, maternal nutrition and stressors may result in epigenetic changes possibly leading to lifelong consequences on the offspring's metabolism, physiology and anatomy (Godfrey and Barker, 2000). Dr. Barker proposed the fetal programming theory during his pivotal study on fetal and infant growth in the Hertfordshire cohort study, which demonstrated that diseases and metabolic disorders in their children's adult life could have fetal origins. Human epidemiological studies stem from Barker's study supporting the concept of fetal programming. For example, the study done on the 1944 Dutch Famine of World War II demonstrated that malnutrition during periods of gestation can have consequences on fetal development, leading to coronary disease, insulin resistance and glucose intolerance (Roseboom et al., 2006). Additionally, this study reported that famine during the last trimester of gestation lead to a greater risk of infant mortality compared with those exposed during early and mid-gestation, indicating that malnutrition has different effects depending on the period of gestation (Roseboom et al., 2006). Researchers reported that rats born to dams fed a nutrient restricted diet led to alterations in the postnatal metabolism resulting in high systolic blood pressure (Langley-Evans et al., 1996), hyperinsulinism, hyperleptinemia and hyperphagia (Vickers et al., 2000), all of which may play key roles in hypertension and obesity.

Similar to humans and rodents, the number of research studies in livestock species exploring the concept of fetal programming and the effects it may have on the offspring's future health and productivity are increasing. Nutrients available for the offspring, *in utero,* is largely

determined by the way the cow partitions nutrients to support embryonic, placental, and fetal development in concert with her growth and performance, which can be determined by her production level and energy status (Banos et al., 2007). The placenta plays a key role in regulating fetal growth, thus, manipulation of maternal nutrition is an important factor that may influence the development and function of the fetal organ systems and metabolism. The transport of nutrients from the maternal surface of the placenta to the fetal surface of the placenta is a complex process, which controls the transport of nutrients with varies mechanisms such as a bidirectional or unidirectional simple diffusion or highly regulated active transport system. Developmental physiological events occur systematically during gestation and, therefore, effects of nutrition during different stages of gestation on the offspring vary greatly.

Maternal Nutrients During Gestation that Impact the Offspring

In cows, the first half of gestation is focused mainly on placental vascularization, placentome formation and fetal organogenesis processes that are essential for normal conceptus development (Funston et al., 2010). Maternal nutrition may affect the embryo even during early to mid-gestation regardless of the reduced embryo size during that period. Barker and colleagues found that maternal undernutrition in humans during the first half of gestation, followed by adequate nutrition from mid-gestation to term, resulted in infants of normal birth body weight to be proportionally longer and thinner than normal (Godfrey and Barker, 2000). In addition, the infant's disproportionate body size observed at birth was associated with increased risk of obesity, diabetes and coronary heart disease in the infant's adult life (Godfrey and Barker, 2000). Correspondingly, researchers have shown that when ewes are undernourished during the first 100 days of gestation, the fetus' cardiovascular system is altered which led to postnatal hypertension in lambs (Hawkins et al., 2000). During mid-gestation, 28 to 72 days of gestation, ewes subjected to a nutrient restriction diet of 50% of NRC requirements (NRC, 1985) had lambs with increased intramuscular

triglyceride content and a decreased lean-to-fat ratio compared with ewes that were not restricted (Zhu et al., 2006). In beef cows, nutrient restriction, exclusively, during 30 to 125 days of gestation, affected placental angiogenesis, possibly disturbing the amount of nutrients transporting through the maternal-fetal placenta (Vonnahme et al., 2007). One of the earliest events during embryonic and placental development is establishment of fetal and uteroplacental circulation (Patten, 1964). Factors affecting uteroplacental blood flow will impact the placental efficiency and, consequently, fetal growth (Funston et al., 2010). Additionally, beef cows given a nutrient restricted diet of 70% of NRC requirements (NRC, 2000) from early to mid-gestation, from 60 to 180 days of gestation, produced calves with reduced skeletal muscle fibers, impacting long-term growth, performance, and marbling quality (Du et al., 2010). Relative to the brain and heart, the skeletal muscles have a lower priority in nutrient partitioning (Bauman et al., 1982; Close and Pettigrew, 1989). Additionally, the fetal period is essential for skeletal muscle development because there is no net increase in the number of muscle fibers postnatally (Glore et al., 1982; Greenwood el at., 2000). In ruminant species, maternal nutrient restriction during early and mid-gestation can lead to long-term effects on the offspring's growth and performance.

During the second half of gestation, in the cow, the growth of the placenta slows while its function and metabolism increases dramatically with a substantial increase in transplacental exchange of nutrients which is necessary for the exponential growth of the fetus and fetal organ maturation. It has been noted that nearly 75% of the fetus growth occurs during that last 2 months of gestation (Robinson et al., 1977). Energy and protein requirements increase significantly during the last 4 to 8 weeks of gestation to support the increased growth rate of fetal tissues (Prior et al., 1979). In a human study, pregnant women exposed to the Dutch famine during mid or late gestation had infants with reduced birth BW and short body length; these characteristics were associated with increased risk of obesity later in adult life (Roseboom et al., 2006). During late

gestation, prenatal exposure to famine is associated with glucose intolerance in adults (Ravelli et al., 1998). A study in ewes demonstrated that undernutrition during the second half of gestation (90 to 142 d of gestation) resulted in decreased glucose and amino acid concentrations in the fetus, in addition to irreversible fetal growth retardation, which is linked with increased morbidity and mortality (Mellor and Murray, 1982). Ewes given restricted diets during late gestation (110 to 147 d of gestation), parallel with the rapid period of fetal growth, resulted in reduced birth BW and had the greatest impact on the weight at birth compared with early and mid-gestation (Gardner et al., 2007). Corah et al. (1975) demonstrated that beef cows consuming a diet with only 65% of calories recommended by the NRC (NRC, 1970), during the last 100 days of gestation, resulted in lighter calves and lower neonatal survival. Furthermore, the cows consuming a low-energy diet had calves with a higher incidence of being treated for scours and had decreased weaning weights compared with calves born to cows consuming a diet with 100% of NRC recommendations (Corah et al., 1975). Theoretically, similar effects of fetal programming would be expected to occur when dairy cows are nutrient restricted, however data in support of such occurrence is limited. It is worth mentioning that dairy cows are not as prone to suffer nutrient deficiency because of the different nutritional management strategies between the beef and dairy industries.

The late gestation period is critical for livestock because they need to prepare to transition into lactation, while simultaneously providing the highest nutrient demand to their growing fetus. It is common for dairy cows to undergo a period of negative nutrient balance in which the cow is expending more calories than she is consuming. Therefore, during late gestation, nutritional manipulations and management strategies, such as manipulations of the diet composition, are implemented to alleviate the increased nutritional needs and enhance the cow's performance. This may inadvertently impact the fetus and lead to short-term, long-term or even permanent effects that could improve or worsen their performance and health postnatally. In Gao et al. (2012), dairy cows

consuming a diet considered to contain inadequate caloric content, 1.26 Mcal of net energy of lactation (NE_L) per kg of dry matter, during the last 21 days prepartum had calves with compromised immunity compared with calves born to cows fed diets with either moderate (NE_L = 1.41 Mcal/kg dry matter) or high (NE_L = 1.55 Mcal/kg of dry matter) caloric content. A study that investigated protein supplementation in beef cows during the last trimester did not find differences between birth BW in calves born to dams given a protein supplementation and calves born to dams given a placebo supplementation (Martin et al., 2007). However, heifers born to dams given the protein supplementation had increased pregnancy rates compared with calves born to dams not supplemented with protein (Martin et al., 2007). The possible mechanism that altered conception in those beef calves is unknown, however, it has been shown in rats that a low protein diets results in persistent maternal hyperglycemia and contribute to changes in endocrine signaling leading to long-term changes in the offspring's reproductive system (Fernandez-Twinn et al., 2003). Beef steer progeny from protein supplemented dams were heavier at weaning, had heavier carcass weight and had increased intramuscular fat, resulting in greater meat quality compared with steer progeny not given protein supplements (Stalker et al., 2007; Larson et al., 2009). Holstein cows given selenium supplementation the last 60 days prepartum, had increased selenium concentrations in their calves' whole blood, plasma and liver at birth and 42 days of age compared with calves born the Holstein dams not supplemented with selenium (Abdelrahman and Kincaid, 1995). This is important because selenium deficiency can lead to myocardial degeneration and neonatal mortality (Cawley et al., 1978). Furthermore, selenium supplementation in dairy cows can reduce retained placenta (Trinder et al., 1973). Despite the different mechanisms controlling the transport of nutrients from the mother to the fetus, high concentrations of minerals may significantly influence the offspring even after the neonatal period. Moreover, hypercalcemic ewes, induced with calcium infusions, had lambs with significantly increased calcium concentrations compared with lambs

born to ewes not given the same calcium infusions (Abbas et al, 1987). This demonstrates that there is a carry-over effect from that dams to their labs.

The combination of fetal programming within different species has led to a better comprehension of maternal nutritional influences on the developing fetus, *in utero* and postnatally. However, cow-based experimental studies exploring the effects fetal programming have on production and performance of their calves are limited. Nutrient deficiency often occurs in beef cattle because forage-based diet availability varies because of seasonality of production, forage quality and mismanagement. On the other hand, dairy cattle are predisposed to nutritional manipulations, such as feeding acidogenic diets or other mineral supplementations which are used with the intention of enhancing performance and health but may potentially "program" the growing fetus. Investigating the effects of maternal nutritional supplementations during late gestation on performance of the offspring will strengthen recommendations for improvement in management strategies, growth efficiency and health in the dairy herd.

The Neonatal Period

The neonatal period is considered to be the first 28 days of life. The neonatal period is a dynamic state for the newborn because of the intrinsic adjustments to the extrauterine environment at the time of parturition. Once the fetus separates from the umbilicus during parturition, the neonate no longer has nutrient blood supply from the mother through the placenta and, therefore, causes a change from high pressure, low resistance to low pressure, high resistance in the neonatal respiratory and circulatory system. This causes asphyxia to rise, forcing the newborn calf to initiate respiration and increase oxygenated blood by lung inflation leading to decreased pulmonary vascular resistance (Detweiler and Riedesel, 1993; Kasari, 1994). Through these physiological changes, the neonate must be able to maintain adequate oxygen saturation, regulate acid-base balance, engage endogenous metabolic pathways for energy production and maintain body

temperature within physiological limits (Kasari, 1994). Furthermore, during late gestation the fetal calf utilizes glucose and lactate as energy sources (Comline and Silver, 1976). These sources may be used by the fetus as fuel for oxidation and source of carbon for net tissue accretion (Kasari, 1994).

Perinatal Mortality

If vital physiological functions are disturbed, this can lead to perinatal mortality. Perinatal mortality is defined as calves born alive, but die within 48 hours after birth. Perinatal mortality in Holstein calves presents a reoccurring concern in the United States that has led to a loss of more than $125 million per year (Berger and Meyer, 2004). Factors that can lead to increased perinatal mortality in calves include: low calf birth BW to cow BW ratio, birth weights greater than 42 kg, and gestation lengths that are shorter than 273.1 d (Johanson and Berger, 2003). In dairy cows it has been reported that heifers born to cows with a gestation length ranging from 270 to 282 days lived longer and had improved reproductive performance compared with heifers' of dams who had a shorter or longer gestation length compared with the average gestation length (Vieira-Neto et al., 2017). Normal gestation length is essential for the final stages of development in the calf, in addition, assist the calf in achieving a normal birth BW (40 ± 5.7 kg) which can prevent dystocia and mortality in both the calf and dam (Johanson and Berger, 2003). Birth BW is instrumental to determining short and long-term calf health, but it may not be the most efficient indicator of other health parameters such as immunity, acid-base status, and mineral and energy metabolism.

Neonates are born agammaglobulinemic and achieve passive immunity through ingestion of colostrum. The macronutrients in colostrum are then transported through the intestinal epithelium, which remains permeable to large molecules for approximately 24 hours after birth (Staley et al., 1985; Stott et al., 1979). Therefore, neonates rely on colostrum nutrients such as immunoglobulins, proteins, and growth factors, until the specific immune system of the neonate

matures. Blood immunoglobulin G (IgG) is an important indicator of passive immunity transfer in the neonate. For instance, a 24 hour old calf with blood IgG concentrations below 10 g/L (Quigley and Drewry, 1998) is highly susceptible to morbidity and mortality (Besser et al., 1994), and can affect long-term calf performance (Wittum et al., 1995). Failure of passive immunity can be caused by external factors including decreased colostral IgG concentration (Morin et al., 1997) and calf stress (Stott et al., 1979). Maternal related in utero factors may "program" the developing offspring and determine the calf's metabolism and health postnatally.

Acid-Base Balance and Buffer Systems

During the neonatal period, calves are predisposed to acid-base imbalances because of the rupture of the fetal membrane and the uterine contractions that occur during parturition, which alters respiratory components in the acid-base balance. Acid-base equilibrium is essential to maintain balance between chemical acids and bases, which are important for biological mechanisms, such as enzymatic activity. For example, ranges outside of normal can denature proteins and increase loss of function in enzymes. Hence, the acid-base balance is tightly regulated by buffer systems. A buffer system is a mixture of weakly dissociated acid and a salt of that acid designed to minimize changes in pH (Kasari, 1999). There are three basic mechanisms that are used to correct imbalances; the chemical buffering system, respiratory adjustment of blood carbon dioxide concentrations, and excretion of hydrogen ions (H^+) or bicarbonate ions (HCO_3^-) by the kidneys (Reece, 2009). The chemical buffering system includes the bicarbonate system, phosphate buffer system, and protein-peptide buffer system (Sherwood, 2012). The bicarbonate buffer system is extremely important in maintaining pH homeostasis in the blood because it is the first responder and primary buffer in the extracellular fluid for noncarbonic acids (Reece, 2009). Nearly 80% of carbon dioxide (CO_2) transport occurs in the form of HCO_3^- and is regulated following this equation ($CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$; Reece, 2009). The hydration reaction equation in

plasma favors the left side because it accounts for minimal transport of CO_2 and is favored within erythrocytes because of the presence of carbonic anhydrase, catalyzing the formation of H^+ and HCO_3^-. Therefore, venous blood has a lower pH than arterial blood (Reece, 2009). Erythrocytes play a key role in transporting CO_2 because it contains hemoglobin, one of the most plentiful proteins that are available for buffering H^+ during the hydration equation (Reece, 2009). Hemoglobin releases CO_2 intracellularly to attract H^+ ions and then diffuses CO_2 back into plasma as bicarbonate (Kasari, 1999). When erythrocytes are oxygenated then hemoglobin releases H^+ ions and combine with bicarbonate to form carbonic acid, thus, dissociating into CO_2 and H_2O.

Evaluating Acid-Base Status

Strategies to evaluate acid-base status include collecting venous or arterial blood and the parameters that are analyzed are pH, partial pressure of carbon dioxide (pCO_2), HCO_3^-, partial pressure of oxygen and base excess. Analyzing blood samples for the specific parameters such as pH, pCO_2, HCO_3^- and oxygen in the blood allows to evaluate the severity, classification and probable cause of the acidosis. However, it is important to note that the functions of venous and arterial blood differ, therefore arterial blood is recommended to evaluate all the blood gases because the CO_2 and oxygen exchange from peripheral tissues can be assessed. However, this method can be more strenuous on the animal and not as practical. Due to the nature of veins, in which they carry oxygen depleted blood to the heart, measuring oxygen levels to determine acid-base status would be inconclusive (Moore, 1969). Many studies have demonstrated that pH, pCO_2 and HCO_3^- from venous blood are adequate to determine acid-base balance when comparing to reference values from venous blood of control animals (Kasari, 1999; Moore 1969; Boyd, 1989). The normal physiological values in venous blood in a calf immediately postpartum for pH are 7.22 ± 0.05 to 7.24 ± 0.08; pCO_2, 41.0 ± 5.9 to 67.4 ± 7.2 mmHg; HCO_3^-, 24.2 ± 2.7 to 28.2 ± 4.4 mmol/L (Szenci, 1985). Most calves experience a partially compensated metabolic acidosis when

the pH values are from 7.20 to 7.44, pCO_2 from 38.5 to 88.5 mmHg and bicarbonate from 21.3 to 48 mmol/L (Boyd, 1989). Boyd (1989) observed a positive correlation between the age and plasma pH, pCO_2, and HCO_3^-. Analyzing the parameters to detect the acid-base status are essential because there is a strong correlation between perinatal mortality in the calf caused by metabolic or respiratory acidosis (Szenci, 1985).

Acid-Base Imbalances

An acid-base imbalance, acidosis or alkalosis, can develop from disturbances in respiratory or metabolic control mechanisms (Carlson, 1997). Acidosis is commonly seen at birth, normally caused by the accumulation of excess acid or the removal of base from the extracellular fluid, while alkalosis, not commonly seen at birth, is an imbalance caused by excess base or loss of acid (Kasari, 1999). There are four general classifications of acid-base disturbances: metabolic acidosis, metabolic alkalosis, respiratory acidosis and respiratory alkalosis. Acid-base abnormalities in the blood or urine are quantified by pH, which is defined as the negative logarithm to the base 10 of hydrogen ion concentration in a solution and, therefore, pH is inversely related to hydrogen ion concentration (Butler et al., 1971). Solutions that have H^+ ions greater than 10^{-7} are acidic and those having H^+ ion activity of less than 10^{-7} are alkalotic (Kasari, 1999). When an animal develops metabolic acidosis, their pH is below normal levels because of a decrease in HCO_3^-, normally caused by impaired function of the kidneys, excessive intestinal loss of HCO_3^- during diarrhea, or overproduction of acid in the blood. In compensated metabolic acidosis, there is an increase in ventilation forcing CO_2 to be released, causing the hydration equation ($CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$) to shift to the left, thus increasing pH (Sherwood, 2012). Compensation is the result of another acid-base disturbance used to correct the original pH abnormality (Kasari, 1999). During uncompensated metabolic acidosis, the pH is low and the HCO_3^- concentrations are decreased while the CO_2 concentrations are increased. Animals experiencing acidotic conditions

resulting from increased CO_2 in the blood, is referred to as respiratory acidosis because the respiratory component, CO_2, and not the metabolic component, HCO3-, is the factor altering pH (Quigley and Drewry, 1998).

Acid-Base Imbalances During the Neonatal Period

In a growing infant, acid-base balance is partly maintained by mother's milk while the infant's kidney function to excrete acid (Quigley and Baum, 2004). The neonatal period is a dynamic state involving variability in oxygen and CO_2 concentrations, colostral consumption, and changes between fetal hemoglobin and adult hemoglobin concentrations (Kasari, 1994). Because hemoglobin and erythrocytes play an important role in maintaining the normal acid-base balance, these variations could possibly impact the acid-base status of the calf during the neonatal period. Furthermore, imbalances in the acid-base status can be caused by temporary anaerobic glycolysis initiated by poor perfused tissues attempting to maintain active energy metabolism during the transition between loss of maternal blood supply and establishment of respiratory function (Szenci, 1985). Diarrhea is one of the most recognized causes of metabolic acidosis in calves because it results in intestinal loss of the HCO_3^-, which reduces buffering capacity in the extracellular fluid to counteract the production of organic acids, particularly lactic acid (Kasari, 1999). Particularly during the neonatal period, diarrhea can be caused by viral, bacterial or protozoal organisms in addition to noninfectious conditions including consumption of poor-quality milk, all of which lead to predictable physiological and metabolic events (Kasari, 1999). The cause of acidosis during the neonatal period varies and may be easily influenced by nutrition, infection or simply physiological changes that are disturbed during parturition. Acidosis can lead to dysfunctions in the calves' metabolism. In newborn calves and lambs, severe respiratory and metabolic acidosis may need to be treated or can lead to negative long-term effects in health such as hypoxic-ischemic encephalopathy (Gardiner, 1980). Besser et al. (1990) found that calves with respiratory acidosis

had decreased IgG concentrations in the blood. Also, calves with postnatal respiratory acidosis had prolonged acidosis (> 8 hours) compared with calves diagnosed with postnatal metabolic acidosis (< 4 hours) which resulted in impaired IgG consumption in those calves (Besser et al., 1990). Boyd (1989) found a negative correlation between severely acidotic calves and colostrum intake which led those calves to have decreased blood IgG concentrations, 24.5 g/L. On the other hand, in moderately acidotic calves, with a blood pH greater than 7.20, the correlation between colostral absorption and blood pH was not significant, which resulted in calves with increased blood IgG concentrations, 37.9 g/L (Boyd, 1989). Persistent acidosis can be detrimental to pulmonary function because the pulmonary arterioles remain constricted; without correction, this can lead to death (Kasari, 1994).

Diet-induced Acidosis

Acid-induced diets causing acid-base disequilibrium have been shown to modulate molecular activity including adrenal glucocorticoid, insulin-like growth factor (IGF-1), adipocyte cytokine signaling, dysregulated cellular mechanism, and osteoclast activation (Robey, 2012). Studies in rodents have reported that cortisol concentrations in blood are enhanced by a transiently induced metabolic acidosis, suggesting that acidosis mediates cortisol activity through the pituitary-adrenal cortex renal glutaminase axis, possibly in response to lower bicarbonate concentrations (Welbourne, 1976). Epidemiological studies in humans have correlated obesity with decreased cellular pH, along with the incidence of hypertension, insulin resistance and diabetes (Berkemeyer, 2009). Metabolic acidosis can lead to insulin resistance possibly because metabolic acidosis increases circulating glucose and adiponectin, a hormone secreted from mature adipocytes responsible for insulin-sensitizing and anti-inflammatory properties (Disthabanchong et al., 2011). Additionally, chronic metabolic acidosis has been shown to increase glucocorticoid response in humans (Sicuro et al., 1998) and in rodents (May et al., 1986). It was observed that

humans with a diet-induced metabolic acidosis had increased higher plasma cortisol concentrations and cortisol secretion; however, no change was observed in adrenocorticotrophic hormone (Maurer et al., 2003). Acidogenic diets consumed by humans result in a mild acidosis linked with excess cortisol that might play a role in bone turnover (Maurer et al., 2003). Osteoclast resorption and blood pH are negatively correlated because during acidification there is increased activity of carbonic anhydrase II (Biskobing and Fan, 2000), the pumping of protons that solubilize bone mineral in osetoclasts (Nordstrom et al., 1997), and increased enzymes for organic matrix degradation (Brandao-Burch, et al., 2003). Although acidosis-induced diets present complications in human literature, in the dairy industry the use of acidosis-induced diets to mitigate hypocalcemia, specifically, are a widely-adopted management practice that properly administered can result in benefits to cow health.

Transition Period in Dairy Cows

In dairy cows, the transition period is defined as the three weeks before to three weeks after parturition. Thus, this period is challenging and critical in the dairy cow's lactation cycle because of the major physiological changes that occur from involution of the mammary gland and supporting a fetus to producing colostrum and large quantities of milk. The onset of lactation is characterized by the most substantial endocrine and metabolic changes during the lactation cycle of a cow. Cows need to adjust their metabolism to the dramatic increase in energy and nutrient requirements for lactation. Maternal tissues, particularly the liver, adipose tissue, the mammary gland and the bones, undergo numerous adaptations to support milk synthesis (Kovacs and Kronenberg, 1997), which may result in some cows having inability to adjust the homeorhetic mechanisms to maintain homeostasis, which can lead to diseases.. Disturbances in one or more metabolic processes is known as a metabolic disorder and is manifested when the cow cannot meet the metabolic demands (Ametaj, 2010). Approximately 75% of disease in dairy cows occurs

during the first month after calving and are rooted with the impaired physiological immune functions and reduced feed intake during the 2 to 3 weeks prior to calving (LeBlanc et al., 2006).

Peri-partum Paresis Hypocalcemia

Hypocalcemia is a metabolic disorder that can occur in high producing dairy cows, primarily at the onset of lactation. Colostrum contains approximately 2.3 grams (g) of calcium per kilogram (kg), therefore, approximately 23 g of calcium are needed to produce 10 kg of colostrum (Goff, 2008). In addition, calcium is also sequestered in the mammary gland before colostrum milking, which likely increases the calcium requirements with the onset of lactation. Mature milk requires 1.1 g of calcium per kg, therefore between early and mid-lactation the high-producing dairy cow loses about 30 to 50 g of calcium per day (DeGaris and Lean, 2008). The increased demand of calcium for milk synthesis from the cow's blood and extracellular fluid causes to a sudden decrease in blood calcium leading to the susceptibility of hypocalcemia (Goff, 2008). Nearly 5 to 7% of the 9.2 billion dairy cow population in the U.S., will develop clinical hypocalcemia. Clinical hypocalcemia is characterized by observable clinical signs, such as tremors, hypersensitivity, and recumbency when total calcium concentrations fall below 1.4 m*M* (Goff, 2008). Approximately 25% of periparturient primiparous cows and 50% of periparturient multiparous cows will suffer from subclinical hypocalcemia (USDA NASS, 2012; Reinhardt et al., 2011), when total circulating calcium concentrations range between 1.4 and 2 m*M* (Goff, 2008). There are no visible signs of subclinical hypocalcemia making it challenging to diagnose. The incidence of hypocalcemia increases with the age of the cow and the risk of milk fever increases by 9% per lactation (Lean et al., 2006).

Total calcium in blood is 50% bound to albumin, primarily, but also with salts of phosphate and lactate, and 50% in the ionized form. Normal circulating total calcium concentrations are within 2.1 to 2.8 mM in dairy cows (Reinhardt et al., 2011). Calcium is one of the most widespread

and ubiquitous second messenger ions (Parekh, 2006). Proper cell function requires calcium homeostasis to be a highly-regulated process because of its versatility and critical role in biological processes such as cell signaling, blood coagulation, enzyme activity, membrane permeability and muscle contraction. The occurrence of clinical hypocalcemia has been shown to reduce muscle activity leading to increased susceptibility of dystocia, retained placenta and mastitis in Holstein dairy cows (Curtis et al., 1983). Despite the lack of clinical signs of subclinical hypocalcemia, it has been shown that it can reduce rumen contractions (Larsen et al., 2001), increase lipid accumulation in hepatocytes (Chamberlin et al., 2013) and present an increased risk of displaced abomasum compared with normocalcemic cows (Massey et al., 1993). The variety of other diseases linked to clinical and subclinical hypocalcemia can lead to economic losses given the cost of treatments, culling and reduced productivity. To put it in perspective, treatment costs for clinical hypocalcemia are estimated to be $334 per cow and $125 per cow for subclinical hypocalcemia (Goff, 2008; Reinhardt et al., 2011).

Strategies to Prevent Hypocalcemia in Dairy Cows

At parturition, the cow must increase the pool of plasma calcium to about 30 g of calcium per day to replace for the loss of calcium in colostrum through bone resorption and intestinal absorption of dietary calcium (Horst et al., 1997). When the parathyroid gland senses a decrease in circulating iCa, it secretes parathyroid hormone (PTH) to increase mobilization of calcium through indirectly activating vitamin D_3 in the kidneys. The active vitamin D_3 will stimulate active transport of calcium across the intestinal epithelium and PTH increases renal tubular calcium reabsorption, in addition, continued secretion of PTH will initiate calcium mobilization from the bone. Different strategies have been developed to mitigate the incidence of hypocalcemia. Large doses of vitamin D supplementation can be given to cows about 7 days before calving; however, this presents an issue because calving dates vary and increased doses of vitamin D can lead to

toxicity (Kahn, 2005). Feeding low calcium diets can be implemented into the prepartum diet to increase calcium absorption and bone resorption, but low calcium diets are difficult to formulate because most feeds contain calcium concentrations that result in positive calcium balance, therefore, precluding the activation of homeorhetic mechanisms for maintaining blood calcium in absence of adequate intake. For instance, alfalfa, a common ingredient in formulating dairy feed, could not be used in the prepartum rations if a negative calcium balance is desired (Bethard et al., 1998). The negative dietary cation-anion difference (DCAD) is another strategy in which acidogenic salts are added to the prepartum diet to induce a compensated metabolic acidosis. Even though it is recommended for 21 d, the optimal duration to mitigate hypocalcemia is uncertain. One disadvantage of feeding diets with negative DCAD is that the metabolic acidosis itself or the excess of unpalatable salts might reduce feed intake during a crucial period for the dairy cow (Oetzel and Barmore, 1993).

Dietary Cation-Anion Difference

The negative DCAD is formulated by reducing strong cations, sodium and potassium, while adding more strong anions, chloride and sulfur. Ender et al. (1971) proposed the first equation to compute the DCAD for diet formulation as [mEq of Na^+ + mEq of K^+] – [mEq of Cl^- - mEq of S^{2-}]. The goal of feeding diets with a negative DCAD is to manipulate the acid-base status of the cow by inducing a compensated metabolic acidosis. In cattle, blood pH below 7.4 is considered acidic and may have been caused by accumulation of noncarbonic acids in the blood or decreased HCO_3^- from the kidneys (Reece, 2009). The normal blood pH in cattle ranges from 7.4 to 7.50 (Reece, 2009). Abu Damir et al. (1994) fed prepartum cows either an alkalogenic or an acidogenic diet and estimated the fractional calcium absorption after correcting for endogenous calcium loss. The authors showed that the negative DCAD diet increased calcium balance from 0.436 mol/day (17.4 g/day) to 0.65 mol/day (26.0 g/day), which resulted in an increase in the

estimated fractional calcium absorption from 0.25 to 0.35. Nevertheless, the estimated increased intestinal absorption of calcium with acidogenic diets need to be confirmed with radiolabeled Ca because mobilization from bone could have influenced those results.

The recommended level of negative DCAD to induce a compensated metabolic acidosis range between -100 to -50 mEq/kg and should be fed for a recommended duration of 21 days prepartum (Oetzel, 2000). Feeding prepartum diets with negative DCAD effectively reduces the incidence of clinical and subclinical hypocalcemia from 15% to approximately 4 or 5% when changing the DCAD from +200 to -100 mEq/kg (Charbonneau et al., 2006; Oetzel, 2000). There is still a high percentage of dairy cows that suffer from hypocalcemia and therefore the optimal combination of feeding duration of negative DCAD and the level of the negative DCAD in the prepartum diet are uncertain. During the close up dry period dairy cows reduced their dry matter intake and it is possible that increased feeding of anionic salts can further reduce feed intake because of unpalatability or acidosis. Having a single prepartum ration for the entire dry period will void the uncertainty in calving date and provide flexibility in management strategies. Current research is investigating the optimal level and duration of negative DCAD feeding.

Maternal Metabolic Acidosis in Dairy Cows and Effects on Offspring

It is possible that the maternal metabolic acidosis induced by feeding acidogenic salts prepartum to the gestating dam could impact the neonate *in utero* because of the increased highly-vascularized nutrient transfer system that occurs during the last trimester. Only two studies have evaluated the impact of maternal DCAD on the calf. Morrill et al. (2010) found that the efficiency of immunoglobulin absorption and the blood concentrations of immunoglobulins of calves born to dams fed a negative DCAD (-100 mEq/kg) were not different compared with calves born to dams fed a positive DCAD (+77 mEq/kg). These authors fed a diet with negative DCAD for the last 21 days prepartum. On the other hand, Weich et al. (2013) found no differences in calf birth BW

when they compared two durations of -160 mEq/kg level of DCAD, fed the last 21 or 42 days prepartum, or when compared with a control group fed a positive DCAD of +120 mEq/kg for the entire dry period. It is likely that maternal negative DCAD evaluated in these studies might impact the offspring beyond their birth BW and absorption of immunoglobulins. The effect that diets with negative DCAD exert on the cow's mineral metabolism and acid-base balance have been reported extensively (Charbonneau et al., 2006; Horst et al., 1997; Goff, 2008); however, studies describing the potential carry-over effects on the acid-base balance, mineral and energy metabolism and performance of their offspring are limited.

Summary

There are limited data in dairy cows exploring the impact of the use of anionic salts, for the prevention of hypocalcemia, on the programming of the fetus and the offspring postnatally. Animal scientists and cattle producers are beginning to acknowledge that the management and nutritional decision we make at the farm level during gestation (i.e. late gestation) can impact the future generations of dairy cows. This, together with the urgent need to investigate the optimal DCAD level and duration during late gestation, to more effectively mitigate hypocalcemia in dairy cows, motivated us to pursue this study. The objectives of the experiment are to evaluate different parameters such as growth, immunity, acid-base balance, mineral and energy metabolism, in calves born to cows fed two different negative DCAD levels, -70 vs. -180 mEq/kg, in the diet for two feeding durations, 21 and 42 d. It is hypothesized that growth, immunity and energy metabolism will not be greatly impacted, whereas acid-base balance and mineral metabolism could be influenced by feeding cows the -180 mEq/kg for an extended feeding duration to 42 d. This thesis will contribute to the knowledge in this area and will directly benefit dairy producers by assisting them to make integral decisions taking into account the health and wellbeing of both the cow and the offspring.

CHAPTER 2
EFFECTS OF THE LEVEL AND DURATION OF MATERNAL DIETS WITH NEGATIVE DCAD PREPARTUM ON CALF GROWTH, IMMUNITY, MINERAL AND ENERGY METABOLISM

Summary

The objectives were to investigate the effects that maternal diets containing negative dietary cation-anion differences (DCAD) fed prepartum may have on the acid-base status, hematology, mineral and energy metabolism, growth and health of their calves postnatally. The experiment was a randomized block design with a 2 x 2 factorial arrangement of treatments, which consisted of two levels of negative DCAD, -70 or -180 mEq/kg; and two feeding durations, the last 21 d (short, S) or the last 42 d (long, L) prepartum. A total of sixty calves born to these dams were fed 3.8 L of pooled colostrum for their first feeding. Calf BW was recorded at birth and 21, 42, and 62 ± 3 d of age. Blood was collected at birth, before colostrum feeding, and at 1, 2, 3, 21 and 42 d of age. Data was analyzed by ANOVA fitting mixed models. Calves born to L dams weighed 2.8 kg and 4.8 kg less at birth and 62 d, respectively, compared with calves born to S dams, however, at 3 and 6 months of age BW did not differ with treatments. Calves born to -180 DCAD dams had increased blood concentrations of ionized calcium from birth to 3 d of age compared with calves born to -70 DCAD dams. At birth, calves born to -180 DCAD dams experienced a subtle and transient metabolic acidosis (pH = 7.28 ± 0.02; pCO_2 = 59.2 ± 1.7 mmHg; HCO_3^- = 27.8 ± 0.5 mmol/L) compared with calves born to -70 DCAD cows (pH = 7.33 ± 0.02; pCO_2 = 52.9 ± 1.7 mmHg; HCO_3^- = 27.6 ± 0.5 mmol/L). Calves born to -180 DCAD dams had smaller concentrations beta-hydroxybutric acid and non-esterified fatty acids compared with calves born to -70 DCAD dams. Calf passive transfer of immunity and immunoglobulin G concentrations were not different between maternal treatments. Percentage of lymphocytes and neutrophils were altered by maternal treatments; however, health of the calf was not negatively impacted by maternal dietary treatments.

Extending the duration or exacerbating the level of maternal negative DCAD diets exerted a transient metabolic acidosis in the calves and slightly impacted measures of mineral, energy metabolism and growth.

Introductory Remarks

Approximately 25% of periparturient primiparous cows and 50% of periparturient multiparous cows in the U. S. suffer from subclinical hypocalcemia, and 5 to 7% of cows in the U.S. will develop clinical hypocalcemia (Reinhardt et al., 2011). Hypocalcemia is a metabolic disorder that occurs when there are reduced circulating calcium concentrations in the blood. Hypocalcemia in Holstein dairy cows can lead to other disorders or diseases such as mastitis and displaced abomasum. Many dairy farms implement a diet with a negative DCAD to reduce the incidence of hypocalcemia. The typical recommendation is for the last 21 d prepartum and the recommended DCAD typically ranges from -50 to -100 mEq/kg. In some situations, dairy producers might prefer a single prepartum diet which would result in feeding dairy cows a diet with a negative DCAD for more than 21 d prepartum. The effects of extending a diet with a negative DCAD remain to be determined. Additionally, there is a need to investigate the proper level of negative DCAD in combination with the feeding duration to further reduce the incidence of hypocalcemia.

A diet with a negative DCAD prepartum induces a compensated metabolic acidosis in dairy cows which decreases the blood pH in the dairy cows and has been correlated with increased blood calcium concentrations. The effects anionic salts fed to dairy cows during late gestation may have on the developing offspring are uncertain. However, it has been demonstrated that nutrient manipulations during the third trimester of gestation can influence fetal and postnatal development of the offspring such as maturation of organs, adipose accretion, muscle and skeletal development (Corah et al., 1975; Du et al., 2010; Gao et al., 2012). Acidosis in neonates can occur as the result

of dystocia and hypoxia during the first hours after birth (Quigley and Baum, 2004). Postnatal respiratory or metabolic acidosis has been linked to reduced colostral immunoglobulin absorption leading to increased risk of mortality in dairy calves (Besser et al., 1990). It is possible that maternal metabolic acidosis induced by feeding anionic salts prepartum could impact the neonate *in utero* because of the highly-vascularized nutrient transfer system that occurs during the last trimester. Limited research has explored whether the induced maternal compensated metabolic acidosis might influence the physiology of the calf postnatally. Morrill et al. (2010) found that the efficiency of absorption and the concentrations of immunoglobulins in calves born to dams fed a diet with DCAD of -100 mEq/kg did not differ from that of calves born to dams fed a diet with a DCAD of +77 mEq/kg during the last 21 d prepartum. Weich et al. (2013) observed that reducing the DCAD from +120 to -160 mEq/kg, or extending the feeding the -160 mEq/kg from 21 to 42 d prepartum did not influence colostrum yield or calf birth BW. The effect that negative DCAD exerts on the cow's mineral metabolism and acid-base balance has been extensively reported (Charbonneau et al., 2006; Horst et al., 1997; Goff, 2008); however, the effects of such dietary manipulations exert on the acid-base balance, mineral and energy metabolism and performance of the offspring remains mostly unknown.

The objectives of this experiment are to evaluate parameters of immunity, acid-base balance, mineral and energy metabolism in calves born to cows fed two different levels of negative DCAD, -70 or -180 mEq/kg, for two durations, 21 or 42 d prepartum, and to determine the impact of these diets on their postnatal growth and health. It is hypothesized that growth, immunity and energy metabolism will not be greatly impacted, whereas acid-base balance and mineral metabolism could be influenced by feeding cows the -180 mEq/kg for an extended feeding duration to 42 d.

Materials and Methods

Animals and Experimental Design

All procedures involving animals were approved by the University of Florida and Institutional Animal Care and Use Committee (protocol number 201509133). The experiment was conducted from January to June 2016 at the Dairy and Calf Research Units of the University of Florida (Alachua, FL). The experiment was a randomized block design with a 2 x 2 factorial arrangement of treatments. Parous Holsteins cows were used in the experiment. Weekly cohort of cows were blocked by parity and 305-d milk yield and, within each block, they were randomly assigned to one of the four treatments with two levels of negative DCAD, -70 mEq/kg (-70) or -180 mEq/kg (-180) fed for two durations, the last 21 d of gestation which was designated as short (S) or the last 42 d of gestation which was designated as long period of feeding (L).

Cows, Housing and Prepartum Diets

Dams at 230 ± 3 d of gestation were moved to a barn with individual feeding gates for treatment administration. Cows were trained for 2 d and treatments started at 232 ± 3 d of gestation. Description of the diets is presented in Table 2-1. Cows in S were fed a diet with positive DCAD from 232 ± 3 to 255 d of gestation, and then they were switched to diets containing negative DCAD starting at 255 d of gestation until calving. Cows in L were fed the respective negative DCAD treatments from 232 ± 3 to calving. Diets were isonitrogenous and isocaloric and were formulated to differ in the concentrations of strong ions to manipulate the DCAD to achieve -70 or -180 mEq/kg. Samples of forages and concentrates were collected weekly and analyzed for their chemical composition to assure desired negative DCAD levels. Details of diet sampling and analyses is presented elsewhere (Lopera et al., 2015).

Calf Management

Sixty calves born to dams fed -70 S (n = 9 heifers, n = 5 bulls), -70 L (n = 12 heifers, n = 3 bulls), -180 L (n = 11 heifers, n = 4 bulls) or -180 S (n = 12 heifers, n = 4 bulls) were used in the experiment. Twins and stillbirths were not included and one dystocia case occurred. Gestation length of dams was calculated and BW of calves was recorded at birth. Day of birth was considered experiment d 0. Calves were separated from their dams, had their navels dipped with 2% iodine to prevent infection, and were fed 3.8 liters of pooled colostrum. Samples of the pooled colostrum fed to the calves were collected three times per week and placed at -20°C until analysis. All calves were transported to the University of Florida Calf Unit and housed in individual hutches and calves received ad libitum calf starter grain and water, and the vaccination was according to the University of Florida standard operating procedures. Calves were fed pasteurized milk in two meals, 6:00 A.M. and 6:00 P.M. until 42 d, by bucket. In the first 21 d of life, calves were given 6 L of milk/d and then, from 21 to 42 d of life, 8 L/d. Milk allotment was reduced to 3 L/d before complete weaning at 49 d. Calves were moved to a group pen at 62 ± 3 d of age.

Growth and Health Parameters

Calf BW, hip height, respiration rate and rectal temperature were recorded at 21 and 42 d of calf age. Additionally, BW at 62 ± 3 d and at 3 and 6 months of age were recorded. Average daily gain from birth to 62 ± 3 d was calculated. Health scores were determined using the University of Wisconsin-Madison's Calf Health Scoring Sheet (found at: http://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring_chart.pdf) to assess the physical health status of the calves at 21 and 42 d. Scores were determined by the same person throughout the experiment. Scores were assigned for the presence and severity of nasal and ocular discharge (0 to 3; 0 = normal and 3 = heavy discharge) and cough (0 to 3; 0 = none, 1 = induced and 3 = spontaneous cough). The total respiratory scores were calculated by adding the nasal,

cough and ocular scores; if total respiratory score exceeded 4 then calves required treatment. Calves that were treated for scours were documented on AfiFarm Dairy Farm Management Software (AfiMilk Ltd, Israel) and assigned a score of 1 if treated and a score of 0 if untreated.

Blood Minerals and Acid-Base Balance

Blood samples were collected from calves via venipuncture of the jugular vein into 10 mL BD Vacutainer sodium heparin plasma tubes (Franklin Lakes, NJ, USA) at birth, before colostrum feeding, and at 1 (24 ± 3 h), 2, 3, 21, and 42 d of age. Within 20 min of collection, plasma was separated by centrifugation at 2,800 rpm for 20 min and stored at -20 °C until laboratory analysis. Additional blood samples were collected at birth and 3 d and analyzed, within 5 min of collection, for pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), bicarbonate (HCO^-_3), sodium (Na), potassium (K) and ionized calcium (iCa) concentrations using a handheld biochemical analyzer (CG8+; VetScan iSTAT, Abaxis, Union City, CA). Plasma samples were analyzed for total calcium (tCa) and magnesium (Mg) concentrations using an atomic absorption spectrophotometer (AAnalyst 200, Perkin-Elmer Inc. Waltham, MA) according to procedures previously described by Martinez et al. (2012). Intra-assay coefficient variations were 9.9% for tCa and 4.8% for Mg.

Energy Metabolism

Plasma concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutric acid (BHBA) were measured by colorimetric and enzymatic methods, respectively (kit no. FA115 and RB1007, Randox Laboratories Ltd, UK). The inter-assay coefficient of variations for NEFA and BHBA were 8.5% and 8.3%, respectively. A Technicon Autoanalyzer (Technicon Instruments Corp., Chauncey, NY) was used to measure concentrations of plasma glucose (Bran and Luebbe Industrial Method 339-19; Gochman and Schmitz, 1972). The intra- and inter-assay coefficient of variations were 2% and 13%, respectively.

Efficiency of Immunoglobulin Absorption

Pooled colostrum samples and plasma samples collected 1 d after birth were used to measure total immunoglobulin (IgG) concentrations by radial immunodiffusion assay (Triple J Farms, Bellingham, WA) per manufacturer's protocol. Briefly, plasma and colostrum samples were diluted 1:2 and 1:5, respectively, in 0.9% saline to fall within the linear range of the standard curve. The diluted samples were pipetted into the bovine anti-bovine IgG antibody plate, and incubated for 27 h in a flat surface protected from light. The diameter of the precipitin ring was measured using a 7x scale lupe (Peak, nº. 1975) and used to calculate the IgG concentrations. The inter-assay CV of the radial immunodiffusion assay was 11%. To calculate the percentage of apparent efficiency of IgG absorption of the calves at 1 d of age (24 ± 3 h) we used the equation described by Quigley (1998). Briefly, the calf's IgG concentration at 1 d of age in plasma was multiplied by birth BW in kg and by 0.091, assuming the plasma volume is consistently 9.1% of the birth BW, then divided by IgG intake. Colostrum IgG concentrations in grams were used to determine IgG intake. Plasma total protein was assessed using a digital refractometer (Milwaukee Instruments; Rocky Mount, NC).

Hematology Analysis

For the assessment of blood hematology, whole blood was collected via venipuncture of the jugular vein from calves into BD Vacutainer K_2EDTA Tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at birth and before colostrum feeding, and at 1, 2, 3, 21 and 42 d of age. Samples were carefully mixed, placed on ice and transported to the laboratory within 2 h of collection to be analyzed using the IDEXX ProCyte Dx® analyzer (IDEXX Laboratories, Inc., Westbrook, Maine, U.S.A.). The ProCyte Dx analyzer employs laser flow cytometry, optical fluorescence and laminar flow impedance technologies in combination with chemical reagents that lyse or alter the blood cells to enable the measurement of the complete blood count. The complete

blood count parameters analyzed were red blood cell count, hematocrit, hemoglobin, percentage of reticulocyte and their counts, platelet count, white blood cell count, percentage of monocytes and their count, percentage of lymphocytes and their counts, percentage of neutrophils and their counts, percentage of eosinophils and their counts, and percentage of basophils and their counts.

Statistical Analysis

Continuous data were analyzed by ANOVA with mixed models using the MIXED procedure of SAS (SAS ver. 9.4, SAS Institute Inc., Cary, NC). The model included the fixed effects of level of DCAD, -70 or -180 mEq/kg, the feeding durations, S or L, the sampling time or age of calf (age) and their interactions. The random effect was calf nested within DCAD level and duration. Body weight at birth, 62 ± 3 d and average daily gain were analyzed using the same model without repeated measures. The Kenward-Roger method was used to calculate the denominator degrees of freedom for the F tests in the mixed models and the AR (1) or SP (POW) covariance structure was used as the covariate structure, depending on the variable analyzed. Data from bull calves was only included for the analysis of birth BW and gestation length. Normality of residuals and homogeneity of variance was assessed in all models before final analyses. Whenever appropriate, transformation of data was performed to achieve normality or non-parametric tests were performed. Categorical variables were analyzed by logistic regression using the GLIMMIX procedure of SAS fitting binary distribution. All results reported are least squares means (LSM) ± SEM. Differences with $P \leq 0.05$ were considered statistically significant and between $0.05 < P \leq 0.10$ tending towards significance.

Results

Growth and Health Parameters

Dams fed DCAD for L duration had shorter ($P = 0.02$) gestation lengths relative to S dams (274 vs. 277 ± 0.85 d, respectively). Consequently, calves born to dams supplemented negative

DCAD prepartum for L duration weighed less (P = 0.001) compared with those born to S dams (40 vs. 42.8 ± 0.8 kg, respectively; Table 2-2). When gestation length was included as a covariate in the statistical model for birth BW, there was still a significant difference between S and L DCAD duration, in which calves born to L dams weighed less (P = 0.04) compared with calves born to S dams (40.6 vs. 42.3 ± 0.7 kg, respectively). As expected, males weighed more (P = 0.01) than females at birth (42.9 vs. 39.7 ± 0.81 kg, respectively), but there were no interactions between gender of the calf, DCAD level or duration. There was a significant interaction between duration and age of the calves for BW at 21, 42 and 62 d of age. Calves born to L dams weighed less (P = 0.01) at 62 d compared with calves born to S dams (76.7 vs. 81.5 ± 1.8 kg, respectively; Table 2-2). There were no differences between DCAD level, duration or their interactions for BW at 3 and 6 months of age and average daily gain from birth to 62 d of age. There was a tendency for the interaction between DCAD level and duration for hip height. Calves born to dams fed a -180 DCAD for an S duration tended to be taller (P = 0.08) than calves born to dams fed a -70 DCAD for a L duration (Table 2-2). The total respiratory scores were not affected by treatments and no calves exceeded a total respiratory score of 4. The number of calves treated for scours was not different between maternal treatments. There were three instances of respiratory problems, however, these were not attributed to maternal DCAD treatments prepartum. There were no differences between total respiratory scores (P > 0.9), and therefore calves were considered healthy throughout the experiment.

Mineral Metabolism

Circulating concentration of K and Na were not affected by DCAD level, duration or their interactions (Table 2-3). There was a significant effect of age (P < 0.001) of the calves for both minerals, in which K concentrations increased and Na concentrations decreased from birth to 3 d (Table 2-3). Circulating Mg concentrations were not affected by DCAD level, duration or their

interactions, but there was a significant effect of age, in which Mg concentrations were elevated at birth and at 1 d of age and decreased thereafter ($P < 0.001$, Table 2-3). There was a significant interaction ($P = 0.05$) between DCAD level and age of the calves for iCa, in which calves born to -180 dams had increased iCa concentrations compared with calves born to -70 dams at 3 d (Table 2-3). Calves born to -180 dams tended ($P = 0.10$) to have greater tCa concentrations compared with calves born to -70 dams (Table 2-3). Additionally, tCa concentrations were greater ($P = 0.002$) on 0 d relative to 42 d of age (Table 2-3).

Acid-Base Balance

Blood pH was significantly affected by the interaction ($P = 0.01$) between DCAD level and age of the calves (Table 2-4). Calves born to -180 dams had a less acidic blood pH at birth compared with calves born to -70 dams (7.33 vs. 7.28, respectively); however, at 3 d of age blood pH did not differ among treatments. There was a significant effect of age ($P < 0.001$) of the calves for HCO_3^- concentrations (Table 2-4), in which concentrations of HCO_3^- increased from birth to 3 d of age (27.8 vs. 34.1 mmol/L). Similar to pH, there was a significant interaction ($P = 0.01$) between DCAD level and age of the calves for pCO_2 (Table 2-4). At birth, calves born to -180 dams had less pCO_2 compared with calves born to -70 dams, but at 3 d pCO_2 levels were not different DCAD levels.

Energy Metabolism

There was a significant interaction ($P = 0.04$) between DCAD level and age of the calves for BHBA concentrations (Figure 2-1 A). Calves born to -180 dams had lower BHBA concentrations compared with calves born to -70 dams, specifically at 1 and 42 d of age. Plasma concentration of NEFA tended to be less ($P = 0.07$) in calves born to -180 dams compared with those born from -70 dams (Figure 2-1 B). At birth, calves had greater ($P < 0.001$) plasma NEFA

concentrations compared with later days, possibly because they were not fed until after the first blood sample was collected (Figure 2-1 B). Glucose concentrations did not differ between treatments, however, there was a significant effect of age of the calves ($P < 0.001$) in which glucose concentrations increased markedly from birth to 2 d of age (80 to 140 mg/dL, respectively), then decreased and remained steady thereafter (106.9 ± 6.1 mg/dL; Figure 2-1 C).

Efficiency of Immunoglobulin Absorption

Plasma IgG concentrations at 1 d of age (24 ± 3 hours) did not differ between DCAD level or duration and the average IgG concentrations was 24.8 ± 2.7 g/L (Table 2-5). Treatments or the interaction between treatment and age did not influence efficiency of IgG absorption, which averaged 33% (Table 2-5). Total protein concentrations were not affected by level of maternal DCAD, however, at 3 d of age calves born to L dams had decreased ($P = 0.04$) total protein compared with calves born to S dams and concentrations were not statistically different thereafter (9.3 vs. 9.7 ± 0.15, respectively; Figure 2-1 D).

Hematology Analysis

There were no differences ($P > 0.11$) between DCAD level, duration or their interactions for red blood cells and hematocrit (Table 2-6). There was a significant effect ($P < 0.001$) of age of the calves for hematocrit and red blood cells, in which both parameters decreased from birth to 3 d, and then increased at 21 and 42 d (Table 2-6). Hemoglobin concentrations did not differ ($P > 0.12$) between maternal treatments (Table 2-6). Reticulocyte counts peaked at 3 d of age and then drastically decreased ($P < 0.001$) at 21 and 42 d, but there were no differences between treatments (Table 2-6). There was a significant interaction ($P = 0.05$) between DCAD duration and age of the calf for platelet counts, in which calves born to L dams had decreased platelet counts compared with calves born to S dams, specifically at birth (Table 2-6).

Overall, white blood cell counts increased ($P < 0.001$) from birth to 42 d of age, but there were no differences ($P > 0.39$) between DCAD level, duration or their interactions. There was only an age effect for lymphocyte counts in which calves at 1, 2 and 3 d of age had less counts compared with calves at 21 and 42 d of age ($P < 0.001$). However, calves born to L dams had increased ($P = 0.02$) percentage of lymphocytes at birth and at 1 d of calf age compared with calves born to S dams (Table 2-7). Neutrophil counts were not affected ($P > 0.34$) by DCAD level, duration or their interactions. However, there was an interaction ($P = 0.03$) between DCAD duration and age for the percentage of neutrophils, in which calves born to L dams had less at birth and at 1 d of age compared with calves born to S dams (Table 2-7). There were no differences between DCAD level, duration or their interactions for monocytes, basophils, and eosinophils counts. Similarly, there were no differences between DCAD level, duration or their interactions for the percentage of monocytes and the percentage of basophils. There was a tendency ($P = 0.10$) for an interaction between DCAD level and duration for percentage of eosinophils (Table 2-7), in which calves born to dams fed -70 DCAD for an S duration had a decreased percentage of eosinophils relative to calves born to dams fed -180 DCAD for an S duration.

Discussion

The nutritional management of dairy cows during gestation not only influences cow productivity but can also influence offspring health and productivity. Several studies have shown that maternal nutrition manipulations during the last trimester in cattle can have long-term impacts on the developing offspring. It is well established that when intrauterine conditions are poor, or not optimal, the progeny can experience complications later in life (Barker et al., 2002). Integral dairy cow and calf management decisions are needed in order to assure a healthy herd. There is limited data investigating the effects that maternal nutrition manipulation might have on the offspring postnatally. The use of diets with negative DCAD is a recommended dietary intervention and

widely-adopted management practice in dairy farms to prevent the occurrence of hypocalcemia during early lactation (Reinhardt et al., 2011). Lowering the level of negative DCAD in the diet and supplying it for 21 days prepartum have proved to reduce the incidences of hypocalcemia by inducing a compensated metabolic acidosis, but the optimal duration and level are still under investigation. The main objective of the present study was to examine whether extending the duration and exacerbating the level of the maternal negative DCAD prepartum may impact the metabolism, health and performance of the offspring postnatally.

Our results show that extending the duration of supplemented DCAD from the recommended 21 d to 42 d in the dam reduced the calf's BW at birth from 42.9 to 40 kg. The normal Holstein calf birth weight ranges from 38.2 to 41.7 kg and weights outside this range can lead to perinatal mortality (Johanson and Berger, 2003). Some factors shown to increase perinatal mortality in dairy calves are birth weights greater than 42 kg, gestation lengths shorter than 275 d, and higher ratio of dam weight to calf birth BW (Johanson and Berger, 2003). Contrary to my experiment, Weich et al. (2013) did not find differences in birth weight when -160 mEq/kg DCAD was extended from 21 to 42 d prepartum and compared with a control group fed +120 mEq/kg for 42 d. Calf BW can be a predictor of calving ease and perinatal mortality, and weights below or above normal can present complications for both the offspring and dam, such as dystocia and retained placenta (Johanson and Berger, 2003). In our study, there were no cases of perinatal mortality even though calves born to dams given negative DCAD for a shorter duration had increased birth weights (42.9 kg) relative to calves born to dams with an extended duration of negative DCAD (40 kg). Notably, calves born to dams fed a negative DCAD for a shorter duration continued to have greater BWs at weaning compared with calves born to dams with an extended negative DCAD duration. However, at 3 and 6 month of age the BW of the heifers was not different between DCAD durations. Although calf BW is highly correlated with gestation length,

maternal nutrition is a major influence on fetal growth and may trigger parturition (Warnes et al., 1998; Tudor, 1972). In our present study, extending the negative DCAD significantly reduced gestation length compared with cows given a shorter duration by approximately 3 days. The 3-day difference may seem negligible but it has been shown that gestation lengths of 268, 273, 284 and 290 d yield probabilities of calf mortality of 5.5, 3.9, 3.1, 3.1 and 3.6%, respectively (Johanson and Berger, 2003). Changes in circulating hormone levels in both the maternal and the fetal circulations at the end of pregnancy impacts the timing of parturition (Kota et al., 2006). It could be speculated that the prolonged acidosis of the dam stimulated fetal hormones of the hypothalamic-pituitary-adrenal axis resulting in shorter gestation lengths. Regardless of the shorter gestation lengths and the reduced birth BW, extending the maternal negative DCAD did not detrimentally impact calf BW after weaning. The duration of maternal DCAD diets, but not the negative level of maternal DCAD, seem to have a significant impact on calf BW and growth during early life.

The primary goal of the implementation of a diet with negative DCAD prepartum in dairy cows is to induce dam's calcium mobilization before the onset of lactation to prevent the sudden and steep decrease in circulating calcium at calving. It has been suggested that calcium ions are actively pumped across the placenta from mother to fetus but that the fetus is capable of independently controlling its own calcium homeostasis (Delivoria-Papadopoulos et al., 1967; Care, 1989). Specifically, in sheep, pigs and guinea pigs, it has been shown that increased calcium in the pregnant dam does not have a significant effect on fetal plasma calcium levels (Bawden and Wolkoff, 1967; Abbas et al., 1987; Greeson et al., 1968). In our study, this could potentially explain the lack of difference in circulating iCa concentrations in the calves immediately after birth, despite the increased iCa concentrations in the dams fed an exacerbated negative DCAD prepartum (data not shown). Interestingly, we observed that at 3 d of age calves born to -180

DCAD fed dams had higher iCa compared with calves born to -70 DCAD fed dams. Calves begin to depend on absorption of calcium through the intestines and skeletal calcium stores at approximately 3 d of age (Kovacs and Kronenberg, 1997). Whether the origin of the increased iCa in calves born to -180 DCAD fed dams was a result of increased absorption efficiency or an increase in feed intake is unknown because colostrum intake was not recorded after the first feeding. Additionally, milk and grain intake was not recorded. An *in vitro* study observed that an acidic environment, decreases calcium binding to proteins, mainly albumin, and increases iCa (Wang et al., 2002). This could possibly explain the decrease in circulating tCa in calves born to dams fed that -70 DCAD fed dams compared with calves born to dams fed that exacerbated negative DCAD.

It is known that feeding negative DCAD prepartum induces a compensated metabolic acidosis in cows (Goff, 2008), but it is unknown whether the maternal acid-base status can influence the developing offspring postnatally. Metabolic acidosis is characterized by imbalances in the HCO_3^- buffer system, while respiratory acidosis is characterized by imbalances in pCO_2 and both imbalances can cause blood pH to fall below a normal range of 7.35 to 7.5, in cows. These imbalances in HCO_3^- and pCO_2, reflect kidney and lung function (Kasari, 1999). During compensated metabolic acidosis, the lungs assist in removing the excess acid in the blood by increasing respiration rate, thus, increasing pH to be within the normal range. In the present study, the acid-base status of the calves was determined by measuring the pH, pCO_2 and HCO_3^- from their venous blood. According to Boyd (1989), calves with a mean pH of 7.24, mean pCO_2 of 67.4 mmHg and mean HCO_3^- of 28.3 mmol/L are diagnosed with mixed (respiratory or metabolic) acidosis. If the mixed acidosis persisted 24 h after birth, in addition to reduced pCO_2, then calves are diagnosed with respiratory acidosis. However, if the calves recovered after 24 h they are diagnosed with metabolic acidosis (Boyd, 1989). Here, calves born to -70 DCAD dams had a more

evident metabolic acidosis because they had decreased pH and their pCO_2 was greater compared with calves born to dams fed the exacerbated negative DCAD. It is worth mentioning that all the calves had some degree of metabolic acidosis at birth. It has been reported that acidosis may be common at birth when blood pH and plasma HCO_3^- concentrations are lower and the pCO_2 are greater; then with increasing age pCO_2 decreases and blood pH increases as a result of improved respiratory function (Moore, 1969). By 3 d of age, all the calves from the present study achieved pH, pCO_2 and HCO_3^- values that no longer reflected metabolic acidosis. Overall, neither the level nor the duration of maternal negative DCAD fed prepartum, induced a noticeable uncompensated metabolic acidosis in their calves.

Scours have been linked to metabolic acidosis (Kasari, 1999), however, maternal dietary treatments did not affect the number of calves that had to be treated for diarrhea during the pre-weaning period. Furthermore, we did not observe differences in calves' blood electrolytes, Na and K, which could be indicative of gastrointestinal tract inflammation and loss of buffers (Sobiech et al., 2013). There were only two cases of respiratory problems and one case of pneumonia, but none of them were associated with the maternal negative DCAD treatments. Additionally, regardless of the transient acidosis observed at birth in calves born to dams fed the exacerbated negative DCAD, the plasma concentrations of IgG and the apparent efficiency of IgG absorption in the calves at 1 d of age did not differ. Boyd (1989) reported a negative correlation between extremely acidotic calves (with blood pH < 7.15) and reduced colostrum intake which led to decreased IgG concentrations in the calves, however they did not find any correlations between moderately or normal calves and their IgG concentrations (Boyd, 1989). Calves are born agammaglobunemic and therefore rely on colostrum supply to provide vital IgG in addition to other immune proteins and nutrients that support the newborn calf while transitioning from its naïve state to acquired immune system. Here, extending the duration or exacerbating the level of negative DCAD fed to the dams

prepartum did not impact the calf's intrinsic ability to absorb IgG. It is still unknown whether the extended duration of negative DCAD or exacerbated negative DCAD affects the quality and/or the quantity of colostrum produced by the dam, in this study we feed the newborn calves with pooled colostrum to avoid any confounding effects of maternal colostrum.

It is possible that slight alterations of the calves' acid-base balance and mineral metabolism due to the maternal diets could have altered the intracellular signaling of cells (i.e. adipocytes). Parameters such as glucose, NEFA and BHBA are commonly used as indicators of energy balance in dairy cows, thus, we set out to determine if maternal DCAD treatments could have affected the calf's energy metabolism. The exacerbated negative DCAD level slightly affected the energy metabolism of the calves by decreasing circulating concentrations of both BHBA and NEFA without alterations in circulating glucose concentrations during the preweaning period. It is important to note that in the present experiment we did not evaluate measures of intracellular signaling to confirm this. In a young calf, BHBA concentrations are indicators of rumen development with increasing calf starter or grain intake (Quigley et al., 1991). Decreased NEFA concentrations are associated with increased nutrient consumption. It could be speculated that the decrease in both NEFA and BHBA concentrations, in calves born to -70 dams, could have been a response to increased feed consumption or increased efficiency absorption of nutrients. Despite subtle differences seen in BHBA and NEFA concentrations, the energy metabolism of the calves' was within normal range. Moreover, glucose concentrations did not differ between maternal treatments and the patterns of glucose concentrations in calves varied with their age and are similar to previous studies (Lents et al., 1998; Knowles et al., 2000).

The complete blood count of the calves was not greatly impacted by maternal dietary treatments fed prepartum and no hematologic abnormalities were detected. However, as expected, both the erythrocytes and leukocytes were impacted by the age of the calf. This is not surprising

given the young calf is actively developing immunological maturation (i.e. increased erythropoiesis, replacing fetal hemoglobin with adult hemoglobin and increased B cells and T-helper cells due to the introduction of environmental antigens during the first month of life; Reece, 2009). Calves born to dams fed the negative DCAD for an extended duration had less platelet counts compared with calves born to dams fed the negative DCAD for a shorter duration. Platelets are necessary for the coagulation process; therefore, decreased platelets are associated with failure of clot retraction; however, platelet blood indices such as mean platelet volume and platelet hematocrit did not suggest susceptibility of illness in the calves with less platelet counts. When the negative DCAD duration was extended the percentage of neutrophils decreased while the percentage of lymphocytes increased. In addition, monocyte, basophil, eosinophil counts and their respective percentages were expected to change as the calves undergo immunological maturation, but similarly, these parameters were not impacted by the duration or level of maternal DCAD. Regardless of these hematological differences, the health of the calves was not compromised.

Conclusions

Extending the duration of feeding, from the recommended 21 d to 42 d, and exacerbating the level of negative DCAD, from -70 to -180 mEq/kg, fed prepartum impacted the offspring's growth and energy metabolism, which does not support our hypothesis. However, the acid-base status and mineral metabolism during the first 3 d of life and the pre-weaning period were impacted by the more negative DCAD in the diet. Gestation length and the growth of the calves was affected primarily by the extended duration of maternal DCAD and not by the level of the negative DCAD. Despite the transient acidosis observed in calves born to -70 DCAD fed dams, these calves were able to recover completely by 3 d of age, suggesting that it was a compensated acidosis. Regardless of the subtle differences in measures of innate immunity observed in this study, the hematology parameters were all within the normal ranges of healthy calves. In fact,

there was a sudden death of unknown cause and instances of scours and respiratory acidosis observed herein, but overall all the heifers were healthy and the lag in the growth of the calves born to -180 DCAD dams was compensated by 3 and 6 months of age. It is important to note that we did not gather data from calves that were born to dams fed an alkalogenic diet, therefore we only investigated the effects a maternal diet with negative DCAD may have on the calves. However, the results obtained from this study are valuable for dairy producers because it would allow them to have more flexibility on the feeding management of anionic salts prepartum to prevent hypocalcemia, without compromising the health and performance of the offspring postnatally. Further research is needed to evaluate the long-term performance of the heifers during their first lactation, when the mineral and energy metabolism are greatly challenged.

Table 2-1. Ingredient composition and nutrient profile of diets fed to cows

		Diet	
Ingredient, % dry matter	Positive DCAD	-70 mEq/kg	-180 mEq/kg
Corn silage	34.2	34.2	34.2
Triticale silage	20.4	20.4	20.4
Bermuda hay	6.7	6.7	6.7
Straw	13.8	13.8	13.8
Citrus pulp	7.7	7.1	6.7
Soybean meal	13.1	8.5	5.8
Prepartum mineral	4.2	4.2	4.2
Bio-Chlor[1]	0	5.2	8.3
Nutrient content, mean ± SD			
Crude protein, %	14.9 ± 0.8	14.7 ± 0.4	14.6 ± 0.6
Acid detergent fiber, %	29.4 ± 1.4	28.9 ± 1.2	29.1 ± 1.1
Neutral detergent fiber, %	43.1 ± 1.7	43.7 ± 1.5	43.8 ± 1.5
Forage neutral detergent fiber, %	39.3 ± 1.7	39.3 ± 1.7	39.3 ± 1.7
Nonfibrous carbohydrates, %	31.7 ± 1.3	31.1 ± 1.6	31.1 ± 1.9
Starch, %	12.3 ± 0.4	12.6 ± 0.5	12.9 ± 0.6
Fat, %	2.8 ± 0.2	2.8 ± 0.1	2.8 ± 0.1
Calcium, %	0.67 ± 0.07	0.64 ± 0.05	0.62 ± 0.05
Phosphorus, %	0.33 ± 0.01	0.33 ±0.02	0.33 ± 0.03
Magnesium, %	0.44 ± 0.06	0.47 ± 0.06	0.48 ± 0.03
Potassium, %	1.54 ± 0.10	1.49 ± 0.09	1.46 ± 0.09
Sulfur, %	0.29 ± 0.03	0.40 ± 0.03	0.47 ± 0.03
Sodium, %	0.08 ± 0.03	0.11 ± 0.03	0.13 ± 0.04
Chloride, %	0.50 ± 0.07	0.86 ± 0.07	1.11 ± 0.03
DCAD[2], mEq/kg	+109 ± 35	-66 ± 17	-176 ± 20

[1]Bio-Chlor® anion source (Arm & Hammer Animal Nutrition, Inc.).

[2]DCAD = dietary cation anion difference and calculated as follows: DCAD = [(mEq of K) + (mEq of Na)] – [(mEq of Cl) + (mEq of S)].

Table 2-2. Body weight (BW) at birth, 21, 42 and 62 d and calf hip height at 21, 42, and 62 d from calves born to Holstein dams fed two different levels of negative DCAD, -70 or -180 mEq/kg, for two different durations, the last 21 d (short, S) or the last 42 d (long, L) prepartum; (n = 9 to 12 calves per treatment)

	DCAD level		DCAD duration			*P*-values					
Item	-70	-180	S	L	SEM	Level	Duration	Age	Lev*Dur	Lev*Age	Dur*Age
BW, kg											
Birth[§]	41.6	41.3	42.9	40.0	0.81	0.80	0.001		0.31		
21 d	53.6	51.9	54.0	51.5	1.8	0.47	0.38	<0.001	0.29	0.95	0.01
42 d	69.5	67.9	67.6	69.7							
62 d	79.6	78.7	81.5	76.7							
Hip height, cm											
21 d	33.6	33.6	33.8	33.4	0.29	0.74	0.77	<0.001	0.08	0.83	0.48
42 d	35.7	35.8	35.7	35.8							
62 d	36.9	37.2	37.1	37.1							

Data is presented as least squares means ± SEM; $P \leq 0.05$ indicates statistical difference.
Age = 21, 42 and 62 days (d) after birth DCAD = dietary cation anion difference. Lev = DCAD level. Dur = DCAD duration.
The triple interaction DCAD level*duration*age, was not significant for any of the parameters estimated (P > 0.10).
[§]Birth BW includes data from bulls and heifers.

Table 2-3. Ionized calcium (iCa), sodium (Na), potassium (K), total calcium (tCa) and magnesium (Mg) concentrations of calves born to Holstein dams fed two different levels of negative DCAD, -70 or -180 mEq/kg, for two different durations, the last 21 d (short, S) or the last 42 d (long, L) prepartum; (n = 9 to 12 calves per treatment)

		DCAD level		DCAD duration			*P*-value					
Item	Age, d	-70	-180	S	L	SEM	Level	Duration	Age	Lev*Dur	Lev*Age	Dur*Age
iCa, mmol/L	0	1.30	1.28	1.28	1.30	0.02	0.62	0.63	0.25	0.53	0.05	0.66
	3	1.29	1.33	1.31	1.31							
Na, mmol/L	0	139.4	139.7	139.9	139.2	0.31	0.47	0.85	<0.001	0.51	0.96	0.47
	3	136.2	136.5	136.4	136.3							
K, mmol/L	0	4.42	4.28	4.33	4.38	0.06	0.53	0.63	<0.001	0.36	0.30	0.95
	3	4.77	4.80	4.76	4.80							
tCa, mmol/L	0	3.00	3.05	2.98	3.07	0.07	0.10	0.25	0.002	0.28	0.84	0.31
	1	2.91	3.03	2.88	3.05							
	2	2.99	2.99	3.01	2.96							
	3	3.12	3.17	3.05	3.24							
	21	2.78	2.97	2.91	2.84							
	42	2.83	2.91	2.86	2.88							
Mg, mmol/L	0	0.65	0.63	0.63	0.64	0.02	0.50	0.81	<0.001	0.30	0.88	0.44
	1	0.69	0.69	0.69	0.69							
	2	0.57	0.57	0.57	0.56							
	3	0.54	0.55	0.54	0.55							
	21	0.54	0.58	0.56	0.55							
	42	0.54	0.55	0.56	0.54							

Data is presented as least squares means ± SEM; $P \leq 0.05$ indicates statistical difference.
Age = 0, 1, 2, 3, 21, 42 days (d) after birth. DCAD = dietary cation anion difference. Lev = DCAD level. Dur = DCAD duration.
The triple interaction DCAD level*duration*age was not significant for any of the parameters estimated (P > 0.10).

Table 2-4. Bicarbonate (HCO_3^-), pH, and partial pressure of carbon dioxide (pCO_2) in calves born to Holstein dams fed two different levels of negative DCAD, -70 or -180 mEq/kg, for two different durations, the last 21 d (short, S) or the last 42 d (long, L) prepartum; (n = 9 to 12 calves per treatment)

		DCAD level		DCAD duration			*P*-value					
Item	Age, d	-70	-180	S	L	SEM	Level	Duration	Age	Lev*Dur	Lev*Age	Dur*Age
pH	0	7.28	7.33	7.32	7.29	0.02	0.62	0.20	<0.001	0.31	0.01	0.45
	3	7.53	7.50	7.52	7.50							
HCO_3^-, mmol/L	0	27.8	27.6	28.0	27.4	0.53	0.36	0.76	<0.001	0.12	0.56	0.44
	3	34.4	33.7	34.0	34.3							
pCO_2, mmHg	0	59.2	52.9	54.7	57.5	1.74	0.23	0.26	<0.001	0.85	0.01	0.61
	3	41.8	43.9	42.2	43.4							

Data is presented least squares means ± SEM; $P \leq 0.05$ indicates statistical difference.
Age = 0 and 3 days (d) after birth. DCAD = dietary cation-anion difference. Lev = DCAD level. Dur = DCAD duration.
The triple interaction DCAD level*duration*age was not significant for any of the parameters estimated (P > 0.10).

Table 2-5. Immunoglobulin G and apparent efficiency of absorption in calves born to Holstein dams fed two different levels of negative DCAD, -70 or -180 mEq/kg, for two different durations, the last 21 d (short, S) or the last 42 d (long, L) prepartum; (n = 9 to 12 calves per treatment)

	DCAD Level		DCAD duration			*P*-value		
Item	-70	-180	S	L	SEM	Level	Duration	Lev*Dur
Immunoglobulin G, g/L	24.82	24.75	24.89	24.68	2.67	0.99	0.96	0.94
Apparent efficiency of absorption, %	35.90	29.40	36.70	28.60	0.04	0.24	0.15	0.70

Data is presented least squares means ± SEM; $P \leq 0.05$ indicates statistical difference.
DCAD = dietary cation-anion difference. Lev = DCAD level. Dur = DCAD duration.

Table 2-6. Hematology parameters in calves born to Holstein dams fed two different levels of negative DCAD, -70 or -180 mEq/kg, for two different durations, the last 21 d (short, S) or the last 42 d (long, L) prepartum; (n = 9 to 12 calves per treatment)

		DCAD level		DCAD duration			*P*-value					
Item	Age, d	-70	-180	S	L	SEM	Level	Duration	Age	Lev*Dur	Lev*Age	Dur*Age
RBC, M/µL	0	8.1	8.4	8.2	8.4	0.25	0.15	0.47	<0.001	0.62	0.17	0.82
	1	7.1	7.4	7.1	7.4							
	2	6.7	6.9	6.8	6.8							
	3	6.3	6.9	6.6	6.6							
	21	7.4	8.2	7.7	8.0							
	42	8.4	8.7	8.4	8.8							
Hematocrit, %	0	35.9	37.2	35.4	37.8	1.24	0.11	0.24	<0.001	0.61	0.28	0.97
	1	29.3	31.3	29.3	31.3							
	2	26.9	27.8	26.7	28.0							
	3	23.7	26.5	24.5	25.7							
	21	25.9	30.1	27.3	28.7							
	42	28.4	30.5	28.7	30.2							
Hemoglobin, g/dL	0	3.8	3.7	3.6	3.9	0.22	0.30	0.19	0.002	0.74	0.12	0.99
	1	3.4	3.1	3.1	3.4							
	2	3.1	3.6	3.2	3.4							
	3	3.0	3.2	3.0	3.2							
	21	2.7	3.2	2.8	3.1							
	42	3.0	3.5	3.1	3.4							
Reticulocyte, K/µL	0	6.8	3.2	5.4	4.6	1.26	0.98	0.47	<0.001	0.99	0.96	0.99
	1	5.9	4.0	4.6	5.4							
	2	5.1	4.4	4.5	4.9							
	3	16.8	11.8	12.9	15.8							
	21	0.7	1.0	0.7	1.0							
	42	0.5	0.7	0.4	0.8							
Platelet, K/µL	0	331.4	314.7	388.2	257.9	30.50	0.34	0.24	<0.001	0.97	0.35	0.05
	1	266.6	339.4	295.0	311.0							
	2	247.4	285.9	273.6	259.7							
	3	302.4	331.9	304.2	330.0							
	21	561.1	535.9	575.2	521.9							
	42	462.3	515.7	505.5	472.5							

Data is presented as least squares means ± SEM; $P \leq 0.05$ indicates statistical difference.
RBC = red blood cells = RBC. Age = 0 and 3 days (d) after birth. DCAD = dietary cation-anion difference. Lev = DCAD level. Dur = DCAD duration.
The triple interaction DCAD level*duration*age was not significant for any of the parameters estimated (P > 0.10).

Table 2-7. Neutrophil percentage and count, lymphocyte percentage and count, monocyte percentage and count, basophil percentage and count, and eosinophil percentage and count of their calves born to Holstein dams fed two different levels of negative DCAD, -70 or -180 mEq/kg, for two different durations, the last 21 d (short, S) or the last 42 d (long, L) prepartum; (n = 9 to 12 calves per treatment)

		DCAD level		DCAD duration			*P*-value					
Item	Age, d	-70	-180	S	L	SEM	Level	Duration	Age	Lev*Dur	Lev*Age	Dur*Age
Neutrophil, %	0	54.5	54.5	58.5	50.5	2.81	0.56	0.03	<0.001	0.65	0.37	0.92
	1	65.0	62.4	67.0	60.4							
	2	59.9	66.2	64.6	61.5							
	3	50.0	54.7	54.9	49.9							
	21	39.3	41.0	42.5	37.8							
	42	39.1	36.9	39.4	36.6							
Neutrophil, K/µL	0	4.3	5.1	5.5	3.9	0.66	0.72	0.35	0.08	0.68	0.63	0.80
	1	5.6	6.4	6.2	5.8							
	2	5.3	4.9	5.4	4.9							
	3	4.5	4.8	4.7	4.6							
	21	4.7	5.3	5.3	4.7							
	42	4.9	4.3	4.8	4.4							
Lymphocyte, %	0	44.3	42.2	38.5	48.1	2.80	0.66	0.02	<0.001	0.87	0.31	0.75
	1	33.7	35.5	31.0	38.2							
	2	35.5	31.1	32.2	34.4							
	3	46.8	41.5	41.6	46.7							
	21	54.3	53.7	51.4	56.6							
	42	50.7	55.2	51.3	54.6							
Lymphocyte, K/µL	0	3.5	3.5	3.6	3.4	0.19	0.35	0.44	<0.001	0.98	0.29	0.48
	1	2.7	3.5	2.7	3.5							
	2	2.7	2.3	2.6	2.4							
	3	3.5	3.4	3.3	3.6							
	21	5.9	6.6	6.0	6.5							
	42	5.7	6.0	5.8	5.8							
Monocyte, %	0	0.15	0.22	0.20	0.17	0.74	0.51	0.21	<0.001	0.56	0.65	0.80
	1	0.21	0.35	0.22	0.34							
	2	0.03	0.16	0.18	.0001							
	3	0.58	0.83	1.33	0.08							
	21	3.27	1.24	2.94	1.57							
	42	8.00	6.97	8.25	6.73							

Table 2-7. Continued

Item	Age, d	DCAD level -70	DCAD level -180	DCAD duration S	DCAD duration L	SEM	*P*-value Level	*P*-value Duration	*P*-value Age	*P*-value Lev*Dur	*P*-value Lev*Age	*P*-value Dur*Age
Monocyte, K/µL	0	0.0005	0.033	0.029	0.009	0.10	0.47	0.21	<0.001	0.69	0.76	0.83
	1	0.008	0.047	0.037	0.018							
	2	0.004	0.031	0.035	-0.001							
	3	0.07	0.05	0.11	0.005							
	21	0.22	0.18	0.22	0.18							
	42	0.98	0.76	1.01	0.73							
Basophil, %	0	0.63	1.56	0.87	1.32	0.76	0.94	0.45	<0.001	0.63	0.43	0.79
	1	0.66	0.81	0.47	0.99							
	2	4.30	2.54	4.02	2.81							
	3	1.98	2.50	2.70	1.78							
	21	3.18	3.73	4.05	2.86							
	42	0.37	0.24	0.33	0.28							
Basophil, K/µL	0	0.054	0.134	0.12	0.07	0.08	0.57	0.90	<0.001	0.60	0.59	0.58
	1	0.045	0.087	0.09	0.04							
	2	0.36	0.18	0.23	0.31							
	3	0.26	0.26	0.20	0.32							
	21	0.36	0.48	0.39	0.46							
	42	0.03	0.03	0.03	0.03							
Eosinophil, %	0	0.75	1.53	0.78	1.50	0.42	0.97	0.5	<0.001	0.10	0.4	0.35
	1	0.46	1.29	0.62	1.13							
	2	0.23	0.40	0.29	0.34							
	3	0.52	0.71	0.67	0.56							
	21	0.20	0.19	0.21	0.18							
	42	1.66	0.82	1.76	0.73							
Eosionphils, K/µL	0	0.06	0.11	0.12	0.05	0.08	0.52	0.23	<0.001	0.17	0.43	0.75
	1	0.04	0.13	0.12	0.05							
	2	0.02	0.02	0.02	0.03							
	3	0.02	0.06	0.05	0.04							
	21	0.02	0.02	0.02	0.02							
	42	0.32	0.08	0.05	0.32							

Data is presented as least squares means ± SEM; $P \leq 0.05$ indicates statistical difference.
DCAD = Dietary cation-anion difference
The triple interaction DCAD level*duration*age, was not significant for any of the parameters estimated ($P > 0.10$).

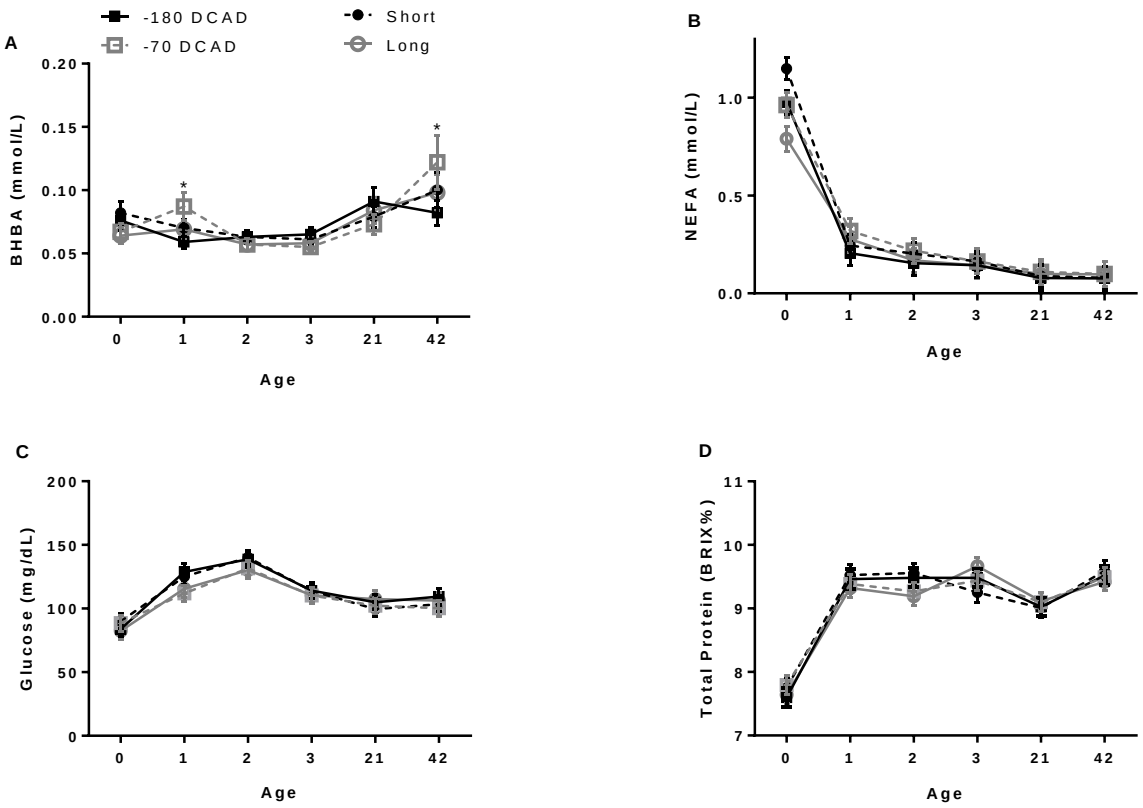

Figure 2-1. Effects of exacerbating the level (Lev; -70 *vs.* -180 mEq/kg) and extending the duration (Dur; 21 d, Short *vs.* 42 d, Long) of maternal negative dietary cation-anion difference (DCAD) during late gestation on circulating β-hydroxybutyric acid (BHBA), non-esterified fatty acids (NEFA), glucose and total protein (TP) of their calves (n = 9 to 12 per treatment) at birth (0 h), 24 h, 48 h, 72 h, 3 and 6 weeks after birth. Calves born to dams fed the -180 DCAD had decreased BHBA concentrations compared to calves born to dams fed -70 DCAD, specifically at 1 and 42 d of age (0.059 and 0.082 ± 0.008 mmol/L vs. 0.087 and 0.122 ± 0.008 mmol/L, respectively; P = 0.01). Calves born to dams fed the -180 DCAD tended to have decreased NEFA concentrations compared with calves born to -70 DCAD fed dams (0.27 vs. 0.31 ± 0.03 mmol/L, respectively; P = 0.07). Furthermore, there was an age effect for NEFA concentrations (P < 0.001). There no treatment effect for glucose and total protein (P > 0.13); only an age effect both for glucose (P < 0.001) and total protein (P < 0.001). Triple interactions were not significant (P > 0.10). *denotes statistical difference, $P \leq 0.05$.

CHAPTER 3
GENERAL DISCUSSION AND SUMMARY

It is well recognized that maternal nutrient restriction at different time periods of gestation may lead to permanent alternations in development possibly resulting in adult diseases. However, consumption of excess supplementation of nutrients such as minerals, energy-rich nutrients and proteins can either negatively or positively influence or "program" offspring growth, immunity, energy metabolism and reproduction. There are limited data that explore fetal programming in dairy cows, specifically the effects of negative DCAD for the prevention of hypocalcemia. The negative DCAD diet has been shown to mitigate hypocalcemia, a metabolic disorder that will affect nearly 5 to 7% of U.S. dairy cows (Reinhardt, 2011). Fetal programming in dairy cattle will help researchers understand the relationships between maternal nutrition during late gestation and postnatal calf health. This knowledge will improve management and nutritional strategies of future generations of high producing dairy cows, thus, improving the performance and health of the dairy herd.

Calves born to the cows that were given the negative DCAD for an extended duration had reduced birth BW. Birth BW above or below 40.3 kg can lead to dystocia, which increases the risk of neonatal mortality, defined as death within 28 d after birth (Johanson and Berger, 2003). In the present study, we observed a reduction of 3 d in the gestation lengths of the cows that were given the negative DCAD for an extended duration. Human studies have shown that a gestation length of 40 weeks is essential for the maturation of fetal organs and tissues. For instance, preterm infants born at 37 weeks have an increased risk of neonatal mortality and increased respiration (McIntire and Leveno, 2008). Additionally, in Holstein dairy cows it has been reported that heifers born to cows with normal gestation length ranging from 270 to 282 days lived longer and had improved reproductive performance compared with heifers born to dams

with a shorter or longer gestation length compared to the average (Vieira-Neto et al., 2017). The fetus initiates the act of parturition through a cascade of endocrine signaling from the hypothalamus-pituitary-adrenal axis (Mcmillen et al., 1995), but maternal factors such as nutrition and stressors may influence the timing of parturition. Limited studies have investigated maternal factors that may alter mechanisms in the fetal hypothalamus-pituitary-adrenal axis and endocrine signaling. In the current study, there were no evaluations measuring endocrine signaling or concentration differences between the two groups, however it would be worthwhile to further investigate the impacts over-acidification may have on gestation length and the potential it has to "program" the offspring's endocrine system. Moreover, exploring the conception rate of calves born to dams fed the negative DCAD for an extended duration compared with the shorter duration would provide insight on alterations that may have occurred *in utero*.

The physiological changes that occur during parturition may lead the newborn calf into a respiratory or metabolic acidosis. In the present study, all the calves experienced some degree of metabolic acidosis, but the calves born to -70 dams had a more defined case. Notably, by 3 d of age all the calves were able to recover from metabolic acidosis, therefore the maternal DCAD did not have long-lasting negative effects. There was a noticeable difference in the calf's iCa concentration at 3 d of age, in addition to differences in NEFA and BHBA concentrations. In addition, the rumen development in a calf is dependent on the initiation and amount of consumption of grain, therefore the ability to absorb nutrients may vary depending on the grain consumption of each individual calf (Meyer and Canton, 2016). As previously discussed, the mechanism explaining these differences are uncertain, future studies measuring the feed intake of the calves may provide more insights into these physiological responses.

In summary, this thesis demonstrated that extending the duration or exacerbating the level of maternal DCAD during late gestation impact the offspring's growth, as well as their acid-base status, mineral and energy metabolism during early life, and that these effects are not long-lasting. Our results indicate that regardless of subtle differences in measures of innate immunity, the health of the calves born to cows consuming different combinations of DCAD levels and durations was not greatly impacted.

LIST OF REFERENCES

Abbas, S. K., I. W. Caple, A. D. Care, N. Loveridge, T. J. Martin, D. W. Pickard, and Rodda, C. 1987. The role of the parathyroid-glands in fetal calcium homeostasis in the sheep. In J. Phys.-London. 386: P27-P27.

Abdelrahman, M. M. and R. L. Kincaid. 1995. Effect of selenium supplementation of cows on maternal transfer of selenium to fetal and newborn calves. J. Dairy Sci. 78:625–630.

Abu Damir, H., M. Phillippo, B. H. Thorp, J. S. Milne, L. Dick, and I. M. Inevison. 1994. Effects of dietary acidity on calcium balance and mobilisation, bone morphology and 1,25 dihydroxyvitamin D in prepartal dairy cows. Res. Vet. Sci. 56:310–318.

Ametaj, B. N. 2010. Metabolic disorders of dairy cattle. In Hudson, R. J., Nielsen, O., Bellamy, J., Stephan, Craig (Eds.). Veterinary Science. pp 24:56.

Banos, G., S. Brotherstone, and M. P. Coffey. 2007. Prenatal maternal effects on body condition score, female fertility, and milk yield of dairy cows. J. Dairy Sci. 90:3490-3499.

Barker, D. J., C. Osmond, P. D. Winter, B. Margetts, and S. J. Simmonds. 1989. Weight in infancy and death from ischaemic heart disease. The Lancet. 334:577-580.

Barker, D. J., C. Osmond, S. J. Simmonds, and G. A. Wield. 1993. The relation of small head circumference and thinness at birth to death from cardiovascular disease in adult life. Bmj, 306:422-426.

Barker, D., J. Eriksson, T. Forsen, and C. Osmond. 2002. Fetal origins of adult disease: strength of effects and biological basis. Int. J. Epidemiol. 31:1235–1239.

Bauman, D. E., J. H. Eisemann, and W. B. Currie. 1982. Hormonal effects on partitioning of nutrients for tissue growth: role of growth hormone and prolactin. In Federation Proceedings. 41:2538-2544.

Bawden, J. W. and A. S. Wolkoff. 1967. Fetal blood calcium responses to maternal calcium infusion in sheep. Am. J. Obs. and Gyn. 99(1):55-60.

Bell, A. W. 1995. Early lactation Regulation of Organic Nutrient Late Pregnancy Metabolism During Transition from to Early. J. Anim. Sci. 73:2804–2819.

Berger, P. J. and C. L. Meyer. 2004. Perinatal Mortality in Holsteins. Animal Industry Report. 650(1): 57.

Berkemeyer, S. 2009. Acid-base balance and weight gain: Are there crucial links via protein and organic acids in understanding obesity? Medical Hypotheses. 73:347–356.

Besser, T. E., O. Szenci, and C. C. Gay. 1990. Decreased colostral immunoglobulin absorption in calves with postnatal respiratory acidosis. J. Am. Vet. Med. Assoc. 196:1239-1243.

Besser, T. E., and C. C. Gay. 1994. The importance of colostrum to the health of the neonatal calf. Veterinary Clinics of North America: Food Animal Practice. 10(1): 107-117.

Bethard, G., R. Verbeck, and J. F. Smith. 1998. Technical Report 31 - Controlling Milk Fever and Hypocalcemia in Dairy Cattle: Use of Dietary Cation-Anion Difference (DCAD) in Formulating Dry Cow Rations. 1–5.

Biskobing, D. M., and D. Fan. 2000. Acid pH increases carbonic anhydrase II and calcitonin receptor mRNA expression in mature osteoclasts. Calc. Tiss. Internat. 67:178-183.

Boyd, J. W. 1989. Relationships between acid-base balance, serum composition and colostrum absorption in newborn calves. Br. Vet. J. 145:249-256.

Brandao-Burch, A., S. Meghji, and T.R. Arnett. 2003. Acidosis strongly upregulates mRNA for cathepsin K, TRAP and TRAF-6 in bone. Calc. Tiss. Internat. 72:364.

Butler, D. G., R. A. Willoughby, and B. J. McSherry. 1971. Studies on diarrhea in neonatal calves. 3. Acid-base and serum electrolyte values in normal calves from birth to ten days of age. Canadian J. Compar. Med. 35:36–39.

Care, A. D. 1989. 4 – Development of endocrine pathways in the regulation of calcium homeostasis. Baillière's Clin. Endocrinol. and Metab. 3:671–688.

Carlson, G. P. 1997. Fluid, electrolyte, and acid-base balance. In Kaneko, J., Harvey, J., Bruss, M (Eds.). Clinical Biochemical in Domestic Animals. 5th ed. pp 485-516.

Cawley, G. D., and R. Bradley. 1978. Sudden death in calves associated with acute myocardial degeneration and selenium deficiency. Vet. Rec. 103:239-240.

Chamberlin, W. G., J. R. Middleton, J. N. Spain, G. C. Johnson, M. R. Ellersieck, and P. Pithua. 2013. Subclinical hypocalcemia, plasma biochemical parameters, lipid metabolism, postpartum disease, and fertility in postparturient dairy cows. J. Dairy Sci. 96:7001–7013.

Chapinal, N., M. Carson, T. F. Duffield, M. Capel, S. Godden, M. Overton, J. E. P. Santos, and S. J. LeBlanc. 2011. The association of serum metabolites with clinical disease during the transition period. J. Dairy Sci. 94:4897–4903.

Charbonneau, E., D. Pellerin, and G. R. Oetzel. 2006. Impact of Lowering Dietary Cation-Anion Difference in Nonlactating Dairy Cows: A Meta-Analysis. J. Dairy Sci. 89:537–548.

Christakos, S. 2012. Recent advances in our understanding of 1, 25-dihydroxyvitamin D 3 regulation of intestinal calcium absorption. Arch. Biochem. and Biophys. 523:73-76.

Close, W. H., and J. E. Pettigrew. 1989. Mathematical models of sow reproduction. J. Repro. and Fert. 40:83-88.

Comline, R. S. and M. A. R. I. A. N. Silver. 1976. Some aspects of foetal and uteroplacental metabolism in cows with indwelling umbilical and uterine vascular catheters. J. Physiol. 260:571.

Constable, P. D., L. M. Schmall, W. W. Muir 3rd, and G. F. Hoffsis. 1991. Respiratory, renal, hematologic, and serum biochemical effects of hypertonic saline solution in endotoxemic calves. Am. J. Vet. Res. 52:990-998.

Corah, L. R., T. G. Dunn. and C. C. Kaltenbach. 1975. Influence of prepartum nutrition on the reproductive performance of beef females and the performance of their progeny. J. Anim. Sci. 41:819–824.

Curtis, C.R., H. N. Erb, C. J. Sniffen, R. D. Smith, P. A. Powers, M. C. Smith, M. E. White, R. B. Hillman, and E. J. Pearson. 1983. Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. J. Am. Vet. Med. Assoc. 183:559-561.

DeGaris, P. J., amd I. J. Lean. 2008. Milk fever in dairy cows: A review of pathophysiology and control principles. Vet. J. 176:58–69.

Delivoria-Papadopoulos, M., Battaglia, F. C., Bruns, P. D., & Meschia, G. 1967. Total, protein-bound, and ultrafilterable calcium in maternal and fetal plasmas. Am. J. Phys.-Legacy Content. 213(2):363-366.

Detweiler, D. K., and D. H. Riedesel. 1993. Regional and fetal circulations. In Swenson, M. J., Reece, W. O. (Eds). Duke's Physiol. Dom. Anim. pp 11:227.

Disthabanchong, S., K. Niticharoenpong, P. Radinahamed, W. Stitchantrakul, B. Ongphiphadhanakul, and S. Hongeng. 2011. Metabolic acidosis lowers circulating adiponectin through inhibition of adiponectin gene transcription. Nephrology Dialysis Transplantation. 26:592-598.

Du, M., J. Tong, J. Zhao, K. R. Underwood, M. Zhu, S. P. Ford and P. W. Nathanielsz. 2010. Fetal programming of skeletal muscle development in ruminant animals. J. Anim. Sci. 88(13): E51-E60.

Ender, F., I. W. Dishington, and A. Helgebostad. 1971. Calcium balance studies in dairy cows under experimental induction and prevention of hypocalcaemic paresis puerperalis. Zeitschrift fuer Tierphysiologie Tierernaehrung und Futtermittelkunde. 28:233-256.

Espino, L., M. L. Suarez, G. Santamarina, A. Goicoa, and L. E. Fidalgo. 2005. Effects of dietary cation-anion difference on blood cortisol and ACTH levels in reproducing ewes. J. Vet. Med. Series A: Physiol. Path. Clin. Med. 52:8–12.

Fernandez-Twinn, D. S., S. E. Ozanne, S. Ekizoglou, C. Doherty, L. James, B. Gusterson, and C. N. Hales. 2003. The maternal endocrine environment in the low-protein model of intra-uterine growth restriction. Br. J. Nutr. 90:815–822.

Funston, R. N., Larson, D. M., & Vonnahme, K. A. 2010. Effects of maternal nutrition on conceptus growth and offspring performance: Implications for beef cattle production. J. Anim. Sci. 88(13): E205-E215.

Gao, F., Y. C. Liu, Z. H. Zhang, C. Z. Zhang, H. W. Su, ans S. L. Li. 2012. Effect of prepartum maternal energy density on the growth performance, immunity, and antioxidation capability of neonatal calves. J. Dairy Sci. 95:4510–4518.

Gao, L., E. H. Rabbitt, J. C. Condon, N. E. Renthal, J. M. Johnston, M. A. Mitsche, and C. R. Mendelson. 2015. Steroid receptor coactivators 1 and 2 mediate fetal-to-maternal signaling that initiates parturition. J. Clin. Inves. 125:2808–2824.

Gardiner, R. M. 1980. Cerebral blood flow and oxidative metabolism during hypoxia and asphyxia in the new-born calf and lamb. J. Physiol. 305:357.

Gardner, D. S., P. J. Buttery, Z. Daniel, and M. E. Symonds. 2007. Factors affecting birth weight in sheep: Maternal environment. Reproduction. 133:297–307.

Glore, S.R. and D. K. Layman. 1982. Cellular growth of skeletal muscle in weanling rats during dietary restrictions. Growth. 47:403-410.

Gochman, N., and J. M. Schmitz. 1972. Application of a new peroxide indicator reaction to the specific, automated determination of glucose with glucose oxidase. Clini. Chem. 18(9): 943-950.

Godfrey, K.M. and D. J. Barker. 2000. Fetal nutrition and adult disease. Am. J. Clin. Nutr. 71:1344s-1352s.

Goff, J.P. and R. L. Horst. 1997. Effects of the addition of potassium or sodium, but not calcium, to prepartum rations on milk fever in dairy cows 1. J. Dairy Sci. 80:176-186.

Goff, J.P., R. Ruiz, and R. L. Horst. 2004. Relative acidifying activity of anionic salts commonly used to prevent milk fever. J. Dairy Sci. 87:1245-1255.

Goff, J. P. 2008. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. Vet. J. 176:50–57.

Greenwood, P.L., A. S. Hunt, J. W. Hermanson, and A. W. Bell. 2000. Effects of birth weight and postnatal nutrition on neonatal sheep: II. Skeletal muscle growth and development. J. Anim. Sci. 78:50-61.

Greeson, C.D., E. G. Crawford and J. W. Bawden. 1968. Fetal Blood Calcium Response to Maternal Hypercalcemia in Guinea Pigs. J. Dental Res. 47:447–449.

Hawkins, P., C. Steyn, T. Ozaki, T. Saito, D. E. Noakes, and M. A. Hanson. 2000. Effect of maternal undernutrition in early gestation on ovine fetal blood pressure and cardiovascular reflexes. Am. J. Physiol.: Regulatory, Integrative and Comparative, 279:R340–R348.

Horst, R. L., J. P. Goff, T. A. Reinhardt, and D. R. Buxton. 1997. Strategies for preventing milk fever in dairy cattle1, 2. J. Dairy Sci. 80:1269-1280.

Hough, R.L., F. D. McCarthy, H. D. Kent, D. E. Eversole, and M. L. Wahlberg. 1990. Influence of nutritional restriction during late gestation on production measures and passive immunity in beef cattle. J. Anim. Sci. 68:2622-2627.

Johanson, J. M., and P. J. Berger. 2003. Birth weight as a predictor of calving ease and perinatal mortality in Holstein cattle. J. Dairy Sci. 86:3745–3755.

Kahn, C. M. 2005. The Merck Veterinary Manual 9th edition. Merck & CO., Inc. Whitehouse station, NJ. pp. 808.

Kasari, T. R. 1994. Physiologic mechanisms of adaptation in the fetal calf at birth. Veterinary Clinics of North America: Food Animal Practice. 10(1): 127-136.

Kasari, T. R. 1999. Metabolic acidosis in calves. Vet. Clin. N. Am.: Food Anim. Pract. 15:473-486.

Kota, S., K. G. Kotni, S. Jammula, S. K. Kota, S. V.S. Krishna, L. K. Meher, K. D. Modi. 2006. Endocrinology of parturition. Endocrinol. and Metab. Clin. N. Am. 35:173–191.

Kovacs, C. S., and H. M. Kronenberg. 1997. Maternal-fetal calcium and bone metabolism during pregnancy, puerperium, and lactation. Endocrine Reviews. 18:832–872.

Knowles, T. G., J. E. Edwards, K. J. Bazeley, S. N. Brown, A. Butterworth, and R. D. Warriss. 2000. Changes in the blood biochemical and haematological profile of neonatal calves with age. Vet. Rec. 147:593-598.

Langley-Evans, S. C., D. S. Gardner, and A. A. Jackson. 1996. Maternal protein restriction influences the programming of the rat hypothalamic-pituitary-adrenal axis. J. Nutr. 126:1578–1585.

Larsen, T., G. Moller, and R. Bellio. 2001. Evaluation of clinical and clinical chemical parameters in periparturient cows. J. Dairy Sci. 84:1749–1758.

Larson, D. M., J. L. Martin, D. C. Adams, and R. N. Funston. 2009. Winter grazing system and supplementation during late gestation influence performance of beef cows and steer progeny. J. Anim. Sci. 87:1147-1155.

Lean, I. J., P. J. DeGaris, D. M. McNeil, and E. Block. 2006. Hypocalcemia in dairy cows: meta-analysis and dietary cation anion difference theory revisited. J. Dairy Sci. 89:669-684.

LeBlanc, S. J., K. D. Lissemore, D. F. Kelton, T. F. Duffield, and K. E. Leslie. 2006. Major advances in disease prevention in dairy cattle. J. Dairy Sci. 89:1267–1279.

Lents, C. A., M. Wettemann, M. L. Looper, I. Bossis, L. J. Spicer, and J. A. Vizcarra. 1998. Concentrations of GH, IGF-I, insulin, and glucose in postnatal beef calves. Anim. Sci. Res. Rep. Oklahoma: Oklahoma University State. 215-222.

Levitt, N. S., R. S. Lindsay, M. C. Holmes, and J. R. Seckl. 1996. Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. Neuroendocrinology. 64: 412-418.

Lopera, C., R. Zimpel, F. R. Lopes, W. G. Ortiz, B. N. Faria, M. R. Carvalho, A. Viera Neto, M. L. Gambarini, E. Block, C. D. Nelson and J. E. P. Santos. 2016. 1541 Effect of level of dietary cation–anion difference and duration of prepartum feeding on calcium and measures of acid–base status in transition cows. J. Anim. Sci. 94(supplement5): 748-749.

Martin, J. L., K. A. Vonnahme, D. C. Adams, G. P. Lardy, and R. N. Funston. 2007. Effects of dam nutrition on growth and reproductive performance of heifer calves. J. Anim. Sci. 85:841–847.

Martinez, N., C. A. Risco, F. S. Lima, R. S. Bisinotto, L. F. Greco, E. S. Ribeiro, and J. E. P. Santos. 2012. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. J. Dairy Sci. 95:7158–7172.

Massey, C. D., C. H. A. N. G. Z. H. E. N. G. Wang, G. A. Donovan, and D. K. Beede. 1993. Hypocalcemia at parturition as a risk factor for left displacement of the abomasum in dairy cows. J. Am. Vet. Med. Assoc.203:852-853.

Maurer, M., W. Riesen, J. Muser, H. N. Hulter, and R. D. Krapf. 2003. Neutralization of Western diet inhibits bone resorption independently of K intake and reduces cortisol secretion in humans. Am. J. Physiol-Renal Physiol. 284:F32–F40.

May, R. C., R. A. Kelly, and W. E. Mitch. 1986. Metabolic acidosis stimulates protein degradation in rat muscle by a glucocorticoid-dependent mechanism. J. Clin. Inves. 77:614.

Mellor, D. J., and L. Murray. 1982. Effects of long term undernutrition of the ewe on the growth rates of individual fetuses during late pregnancy. Res. Vet. Sci. 32:177-180.

Meyer, A. M. and J. S. Caton. 2016. Role of the Small Intestine in Developmental Programming: Impact of Maternal Nutrition on the Dam and Offspring. Adv. Nutr.: An International Review Journal. 7:169-178.

McIntire, D. D., and K. J. Leveno. 2008. Neonatal mortality and morbidity rates in late preterm births compared with births at term. Obs. and Gyn. 111:35–41.

Mcmillen, I. C., I. D. Phillips, J. T. Ross, J. S. Robinson, and J. A. Owens. 1995. Chronic stress The key to parturition? Repro. Fert.and Develop. 7:499–507.

Moore, W. E. 1969. Acid-base and electrolyte changes in normal calves during the neonatal period. Am. J. Vet. Res. 30:1133-1138.

Morin, D.E., G. C. McCoy, and W. L. Hurley. 1997. Effects of quality, quantity, and timing of colostrum feeding and addition of a dried colostrum supplement on immunoglobulin G1 absorption in Holstein bull calves. J. Dairy Sci. 80:747-753.

Morrill, K. M., S. P. Marston, N. L. Whitehouse, M. E. Van Amburgh, C. G. Schwab, D. M. Haines, and P. S. Erickson. 2010. Anionic salts in the prepartum diet and addition of sodium bicarbonate to colostrum replacer, and their effects on immunoglobulin G absorption in the neonate. J. Dairy Sci. 93:2067-2075.

NASS, U. 2012. CropScape-cropland data layer. US Department of Agriculture, National Agricultural Statistics Service, Washington, http://nassgeodata.gmu.edu/CropScape.

Nogalski, Z., and D. Piwczyński. 2012. Association of length of pregnancy with other reproductive traits in dairy cattle. Asian-Australasian J. Anim. Sci. 25:22–27.

Nordstrom, T., O. D. Shorde, R. Rotestein, T. Romanek, J. N. Goto, M. F. Heerscher, G. F Manolson, S. Brisseau, J. Grinstein. 1997. J. Biol. Chem. 272: 6354-6360.

NRC. 1970. The Nutrient Requirements of Domestic Animals. No. 4. Nutrient Requirements of Beef Cattle. National Research Council, Washington, DC.

NRC. 1985. Nutrient Requirments of Sheep. 6th ed. Natl. Acad. Press, Washington, DC.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th ed. Natl. Acad. Press, Washington, DC.

Oetzel, G. R. 1988. Parturient paresis and hypocalcemia in ruminant livestock. Vet. Clin. North Am. Food Amin. Pract. 4:351-364

Oetzel, G. R. 2000. Management of dry cows for the prevention of milk fever and other mineral disorders. Vet. Clin. N. Am. Food Anim. Pract. 16:369–386

Oetzel, G. R. and J. A. Barmore. 1993. Intake of a Concentrate Mixture Containing Various Anionic Salts Fed to Pregnant, Nonlactating Dairy Cows1. J. Dairy Sci. 76:1617-1623.

Parekh, A. B. 2006. Cracking the calcium entry code. Nature. 441:1220–1223.

Patten, B. M., 1964. Foundations of Embryology. 2nd ed. McGrawHill, New York, NY.

Prior, R.L. and D. B Laster. 1979. Development of the bovine fetus. J. Anim. Sci. 48:1546-1553.

Quigley, J. D., L. A. Caldwell, G. D. Sinks, and R. N. Heitmann. 1991. Changes in blood glucose, nonesterified fatty acids, and ketones in response to weaning and feed intake in young calves. J. Dairy Sci. 74(1): 250-257.

Quigley, J. D., and J. J. Drewry. 1998. Nutrient and immunity transfer from cow to calf pre- and postcalving. J. Dairy Sci. 81:2779–2790.

Quigley, J., J. J. Drewry, and K. R. Martin. 1998. Estimation of plasma volume in Holstein and Jersey calves. J. Dairy Sci. 81(5): 1308-1312.

Quigley, R., and M. Baum. 2004. Neonatal acid base balance and disturbances. Seminars in Perinatol. 28:97–102.

Ravelli, C. J., J. H. P. Vandermeulen, R. P. J. Michels, C. Osmond, D. J. P. Barker, C. N. Hales, and O. P. Bleker. 1998. Glucose-tolerance in adults after prenatal exposure to famine. The Lancet. 351:173–177.

Reece, W. O. 2009. The respiratory system. Physiology of Domestic Animals. 4th ed. pp 269-311.

Reinhardt, T. A., J. D. Lippolis, B. J. McCluskey, J. P. Goff, and R. L. Horst. 2011. Prevalence of subclinical hypocalcemia in dairy herds. Vet. J. 188:122–124.

Robey, I. F. 2012. Examining the relationship between diet-induced acidosis and cancer. Nutr and Metab. 9:72.

Robinson, J.J., I. McDonald, C. Fraser, and R. M. J. Crofts. 1977. Studies on reproduction in prolific ewes. J. Agri. Sci. 88:539-552.

Roseboom, T., S. de Rooij, and R. Painter. 2006. The Dutch famine and its long-term consequences for adult health. Early Human Development 82:485-491.

Ruth, V., and K. Raivio. 1988. Perinatal brain damage: predictive value of metabolic acidosis and the Apgar score. Bmj. 297

Schoonmaker, J., and M. Eastridge. 2013. Effect of maternal nutrition on calf health and growth. 22nd Tri-State Dairy Nutr. 26:63–80.

Sherwood, L., Klandorf, H., and Yancey, P. 2012. Fluid and Acid-Base Balance. Animal Physiology: From Genes to Organisms. 2nd Ed. 612-653.

Sicuro, A., K. Mahlbacher, H. N. Hulter, and R. Krapf. 1998. Effect of growth hormone on renal and systemic acid-base homeostasis in humans. Am. J. Physiology-Renal Physiol. 274:F650-F657.

Sobiech, P., W. Rekawek, M. Ali, R. Targonski, K. Zarczynska, A. Snarska, and A. Stopyra. 2013. Changes in blood acid-base balance parameters and coagulation profile during diarrhea in calves. Pol. J. Vet. Sci. 16:543–549.

Staley, T.E. and L. J. Bush. 1985. Receptor mechanisms of the neonatal intestine and their relationship to immunoglobulin absorption and disease. J. Dairy Sci. 68:184-205.

Stalker, L.A., L. A. Ciminski, D. C. Adams, T. J. Klopfenstein, and R. T. Clark. 2007. Effects of weaning date and prepartum protein supplementation on cow performance and calf growth. Rangeland Ecology and Management. 60:578-587.

Stott, G.H., D. B. Marx, B. E. Menefee, and G. T. Nightengale. 1979. Colostral immunoglobulin transfer in calves I. Period of absorption1. J. Dairy Sci. 62:1632-1638.

Sundrum, A. 2015. Metabolic disorders in the transition period indicate that the dairy cow's ability to adapt is overstressed. Animals. 5:978–1020.

Szenci, O., 1985. Role of acid-base disturbances in perinatal mortality of calves. Acta. Vet. Hung. 33(3-4): 205-220.

Trinder, N., R. J. Hall, and C. P. Renton. 1973. The relationship between the intake of selenium and vitamin E on the incidence of retained placentae in dairy cows. Vet. Rec. 93:641-643.

Tudor, G. D. 1972. The effect of pre- and post-natal nutrition on the growth of beef cattle I. The effect of nutrition and parity of the dam on calf birth weight. Austr. J. Agri. Res. 23:389–395.

Vickers, M. H., B. H. Breier, W. S. Cutfield, P. L. Hofman, and P. D. Gluckman. 2000. Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. Am. J. Physiol-Endocrinol Metab. 279:E83-E87.

Vieira-Neto, A., K. N. Galvão, W. W. Thatcher, and J. E. P. Santos. 2017. Association among gestation length and health, production, and reproduction in Holstein cows and implications for their offspring. J. Dairy Sci. 1–16.

Vonnahme, K. A., M. J. Zhu, P. P. Borowicz, T. W. Geary, B. W. Hess, L. P. Reynolds, S. P. Ford. 2007. Effect of early gestational undernutrition on angiogenic factor expression and vascularity in the bovine placentome. J. Anim. Sci. 85:2464–2472.

Wang, S., E. H. Mcdonnell, F. A. Sedor, and J. G. Toffaletti. 2002. pH Effects on Measurements of Ionized Calcium and Ionized Magnesium in blood. Arch. Path. and Lab. Med. 126:947–950.

Warnes, K. E., M. J. Morris, M. E. Symonds, I. D. Phillips, J. J. Clarke, J. A. Owens, and I. C. McMillen. 1998. Effects of increasing gestation, cortisol and maternal undernutrition on hypothalamic neuropeptide Y expression in the sheep fetus. J. Neuroendocrinol. 10:51-57

Weich, W., E. Block, and N. B. Litherland. 2013. Extended negative dietary cation-anion difference feeding does not negatively affect postpartum performance of multiparous dairy cows. J. Dairy Sci. 96:5780–5792.

Welbourne, T. C. 1976. Acidosis activation of the pituitary-adrenal-renal glutaminase I axis. Endocrinol. 99:1071–1079.

Wittum, T.E. and L. J. Perino. 1995. Passive immune status at postpartum hour 24 and long-term health and performance of calves. Am. J. Vet. Res. 56:1149-1154.

Zhu, M. J., S. P. Ford, W. J. Means, B. W. Hess, P. W. Nathanielsz, and M. Du. 2006. Maternal nutrient restriction affects properties of skeletal muscle in offspring. J. Physiol. 575:241–250.

BIOGRAPHICAL SKETCH

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