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**ANIONIC SALTS IN THE PREPARTUM DIET AND ADDITION OF  
SODIUM BICARBONATE TO COLOSTRUM REPLACER AND THEIR  
EFFECTS ON IgG ABSORPTION IN THE NEONATE**

By

Kimberley M. Morrill

B.S. University of New Hampshire, 2006

THESIS

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Masters of Science

In

Animal Science

December, 2008

UMI Number: 1463231

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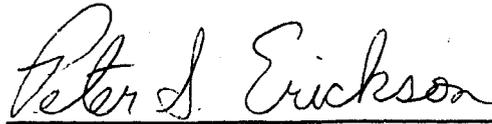
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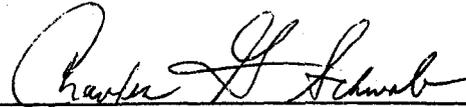
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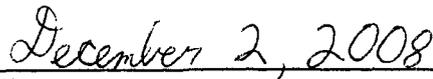
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## ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr. Peter Erickson, for his support throughout both my undergraduate and graduate education and for giving me the opportunity to further my education in dairy science.

I am also grateful to the members of my committee Dr. Charles Schwab and Dr. Mike Van Amburgh, who have provided guidance and support over the past two years.

Thank you to the former Department of Animal and Nutritional Sciences and the Department of Biological Sciences for providing me with financial support, teaching experience and the opportunity to work with an outstanding group of faculty members.

A special to thank you to Dr. Andrew Conroy, and the Thompson School of Applied Science for the teaching opportunity that was provided to me this past semester.

Thank you to Debbie Haines at the University of Saskatchewan for providing me with the colostrum replacer, and a sounding board when I wanted to discuss why I think I saw what I did!

To Nancy Whitehouse: Thank you for your guidance with my research and statistics. And thank you for all of your help when I came into your office every five minutes with a new question.

Jon Whitehouse: Thank you for your help sorting cows, delivering calves and not getting too mad at me when I mixed up your dry cows.

John Weeks, Roger Comeau and all the student employees at the Fairchild Teaching and Research Center: Thank you for your help moving cows, replacing transponders, taking colostrum samples and calling me anytime one of my cows was calving.

Aarron Palmer: Thank you for fixing the Calan doors in the pack barn.

I need to send a big thank you to my fellow graduate students Sue Marston, Erin Shea, Sarah Boucher, Scott Talbot, Kelly Greenbacker and Amanda Rivers.

Sue: Thank you for keeping me sane early on when my project kept changing, and during my project when I was too tired to function. Thank you for taking care of my cows & calves so that I could go to Holland. Don't get too frustrated you are on the home stretch!

Erin: Thank you for your help in writing my proposals, and figuring out my BioStats homework.

Sarah: Thank you for your guidance and support when I thought I would never be able to start my project.

Scott: Thank you for your help with treatments on weekends, and for always being able to make me laugh.

Kelly: Thank you for your help with treatments. Good luck with your calf project!

Amanda: Thank you for making me laugh on a regular basis!

Thank you to Acelyn who was a great help in the lab and in the barn.

Last but not least, thank you to my family for your love and encouragement.

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## LIST OF ABBREVIATIONS

A = anionic diet and no sodium bicarbonate in colostrum replacer

C = basal diet and no sodium bicarbonate in colostrum replacer

ANa = anionic diet and sodium bicarbonate in colostrum replacer

CNa = basal diet and sodium bicarbonate in colostrum replacer

MR = milk replacer

CR = colostrum replacer

AEA = apparent efficiency of absorption

AUC = area under the curve

IgG = immunoglobulin G

IgM = immunoglobulin M

IgA = immunoglobulin A

DMI = dry matter intake

ME = metabolizable energy

NE<sub>l</sub> = net energy for lactation

DCAD = dietary cation anion difference

CP = crude protein

AA = amino acid

TMR = total mixed ration

E. Coli = Escherichia Coli

## **ABSTRACT**

### ANIONIC SALTS IN THE PREPARTUM DIET AND ADDITION OF SODIUM BICARBONATE TO COLOSTRUM REPLACER AND THEIR EFFECTS ON IgG ABSORPTION IN THE NEONATE

by

Kimberley M. Morrill

University of New Hampshire, December 2008

Forty Holstein cows were assigned to a 2 X 2 factorial arrangement of treatments in a completely randomized block design to one of four treatments: basal diet and no sodium bicarbonate in the colostrum replacer; basal diet and supplemental sodium bicarbonate in the colostrum replacer; anionic salts and no sodium bicarbonate in the colostrum replacer; anionic salts and supplemental sodium bicarbonate in the colostrum replacer. Calves received two doses of colostrum replacer  $\pm$  sodium bicarbonate at 0 h, 1 dose of colostrum replacer  $\pm$  sodium bicarbonate at 6 hours and milk replacer at 12, 24, 36 and 48 hours.

Calves receiving sodium bicarbonate had higher serum IgG levels at 24 hours, and higher apparent efficiency of absorption values as compared to calves that did not receive supplemental sodium bicarbonate. Feeding anionic salts during the pre-fresh period did not affect IgG absorption in calves.

**(Key Words: Calves, colostrum replacer, sodium bicarbonate, IgG)**

# CHAPTER I

## REVIEW OF LITERATURE

### Introduction

A healthy calf begins not at birth, but with optimal nutrition and management of the dam throughout the gestation period (Davis and Drakley, 1998). Nutrient demands by the developing fetus become greatly important during the last trimester, with 60 % of the total fetal weight gain occurring during the last two months of gestation (Eley et al., 1978). Feeding prefresh transition cows is a balancing act to provide adequate nutrients for growth, and metabolic needs of the fetus as well as the nutrients needed to maintain homeorhesis, and prevent metabolic disorders in the cow after parturition.

Anionic salts fed during late gestation are beneficial in reducing metabolic problems in cows after parturition (Block, 1984; Block, 1994; Charbonneau, 2005; Goff and Horst, 1997). However, limited research has suggested that the anionic salts may negatively affect the calf's ability to absorb immunoglobulin - G (**IgG**) from colostrum and achieve passive

transfer: serum IgG > 10.0 mg/mL (Quigley and Drewry, 1998). Failure to absorb immunoglobulins from the colostrum results in failure of passive transfer: serum IgG <10.0 mg/mL. Calves that do not achieve passive transfer are associated with excess mortality risk, increased risk of diarrhea, increased rates of respiratory problems, and negative effects on future health, longevity and performance parameters (Davis and Drackley, 1998).

Cows and other ruminants have a synepithelialchorial type of placental barrier, so that IgG do not pass to the developing offspring during fetal development (Akers, 2002). Newborn animals of many species rely on maternal colostrum to provide them with the nutrients needed to sustain life. Due to the placental structure, bovine calves are born agammaglobulinemic, without any measurable IgG or immunoglobulin M (**IgM**) and require high quality colostrum to provide them with sufficient amounts of immunoglobulins to provide passive immunity for the first 30 to 90 days (Guy et al.,1994) until the calf is able to build its own immune system.

## **Part 1. The Dry Period:**

### **Nutritional Needs of the Cow and Conceptus**

Nutrient demands by the developing fetus become particularly important during the last trimester of gestation, which coincides with the dry period for the cow. Unfortunately, dry cows and springers have traditionally been one of the most neglected groups of cattle in dairy operations (Davis and Drackley, 1998). This neglect has most likely arisen because dry cows have relatively low nutrient requirements as compared to their lactating herdmates. Nutrition and management throughout the dry period not only prepares the cow for her upcoming lactation, but it is also vital for the developing fetus.

In mammals, nutrients are utilized by tissues involved in maintenance, growth and for establishing body reserves. During pregnancy, two additional tissues utilize a substantial portion of maternal nutrients, the developing fetus and the mammary gland. These additional tissues differ from other body tissues in that they confer no advantage to the animal. Instead they make tremendous demands such that the total metabolism of the pregnant animal must be altered to accommodate these needs (Bauman and Currie, 1980). Estimation of the nutrient requirements for pregnancy by the factorial method requires knowledge of the rates of nutrient accretion in conceptus tissues (fetus, placenta,

fetal fluids and uterus) and the efficiency with which dietary nutrients are utilized for conceptus growth (NRC, 2001).

The partitioning of nutrients to various body tissues involves two types of regulation, homeostasis and homeorhesis. Homeostatic control involves maintenance of physiological equilibrium or constant conditions in the internal environment. Homeorhesis is defined as the orchestrated or coordinated changes in metabolism of body tissues necessary to support a physiological state (Bauman and Currie, 1980). Nature has accorded a high priority to the functions of pregnancy and milk secretion, allowing nutrients to be partitioned for these functions at the expense of other metabolic processes even to the point that a disease state is created (Bauman and Currie, 1980).

### **Dry Cow Nutrient Requirements**

Nutrient intake is a function of dry matter intake (**DMI**) and nutrient density of the diet (NRC, 2001). Factors that influence DMI in prepartum cows include many factors. Body condition affects intakes with fat cows having lower intakes compared to normal cows (Hayirli et al., 2002). Ration composition and nutrient content may influence prepartum DMI, with diets high in energy or energy and protein increasing DMI (Minor et al., 1998 and VandeHaar et al., 1998). Another factor affecting DMI is the change in concentration of many hormones in the blood prior to

parturition (Grummer et al., 1990). A final factor that can affect DMI prior to parturition is something that can potentially be prevented by correct nutrition, the development of metabolic disorders; cows that are developing hypocalcemia have a lower prepartum DMI as compared to cows that are able to maintain calcium homeostasis (Goff and Horst, 1997). This could potentially be due to the loss of muscle tone, which adversely affects rumen function and rate of passage.

Hayirli et al. (2002) reviewed 16 different experiments done at eight universities to determine the effects of animal and dietary factors on DMI. They observed that DMI decreased 32.2 % during the final 3 wk of gestation, and 89 % of that decline occurred during the final week of gestation ( $P < 0.001$ ). This decrease in DMI over the last 3 wk of gestation can have detrimental effects on both the cow and the growing fetus.

### Energy

The energy requirement for maintenance and pregnancy of dairy cattle increases 23% during the last month prepartum, and the amount of metabolizable energy (**ME**) required by a pregnant animal at term is 75% above that of a non-pregnant animal of the same weight (Moe and Tyrrell, 1971). Inadequate energy intake during the prefresh period can lead to mobilization of body lipids, and an increase in the plasma

concentration of nonessential fatty acids, which then increases the risk for metabolic problems in the fresh period.

The energy required for gestation is assumed to be low when the day of gestation is less than 190 d and the maximum gestation length is set to 279 d, with longer gestation periods resulting in no change in energy requirements. The ME requirement for gestation is described as:

$$\text{ME (Mcal/d)} = [(0.00318 \times D - 0.0352) \times (\text{CBW}/45)]/0.14$$

Where D= day of gestation and CBW is calf birth weight in kilograms (NRC, 2001). To convert ME to net energy for lactation (**NE<sub>L</sub>**) an efficiency of 0.64 was used; therefore the NE<sub>L</sub> requirement for pregnancy is:

$$\text{NE}_L(\text{Mcal/d}) = [(0.00318 \times D - 0.0352) \times (\text{CBW}/45)]/0.218$$

Where D= day of gestation and CBW is calf birth weight in kilograms (NRC, 2001).

The NRC (2001) recommendation for energy density in diets fed to prefresh transition cows and heifers is 1.62 Mcal NE<sub>L</sub>/kg DM. The NRC (2001) states that this amount of energy may not provide sufficient energy

to meet requirements of heifers during a significant portion of the prefresh transition period and possibly of mature cows during the final few days prior to calving. However, feeding more than 1.62 Mcal NE<sub>L</sub>/kg DM may increase intake of rapidly fermentable carbohydrates and adversely affect rumen fermentation and DMI.

### Protein

Bell et al. (1995) suggests the factorial estimation of the total absorbed protein requirement during late pregnancy requires addition of the requirement for conceptus growth to those for maintenance and metabolic fecal N, plus any additional pregnancy related requirements, such as that for mammogenesis, and dry secretions during late pregnancy. This research suggests that during the last 2 months of pregnancy a mature, multiparous cow weighing 714 kg should need at most 780 g/d of absorbed protein.

According to the NRC (2001) there is insufficient evidence to support feeding diets with more than 12% crude protein (**CP**) to mature cows during the prefresh transition period. The current NRC (2001) recommendation is to feed 12% CP for mature cows and 15% CP for heifers during the prefresh transition period. The CP content of a pre-fresh heifer diet needs to be higher than a pre-fresh transition cow diet because the heifer is still growing. Protein and AA requirements for

mammary growth during the prefresh period have not yet been determined.

### Minerals

Minerals are an integral part of all biological functions including expression and regulation of genes, enzyme systems that regulate cellular function, osmotic balance, detoxification, acid-base balance and structural roles. Macrominerals are important structural components of bone and other tissues and serve as important constituents of body fluids. They account for about 9 to 10% of the DM in the fetus, and much of the increase in total mineral content during late lactation results from Ca and P accretion in the skeleton of the calf (House and Bell, 1993).

Trace minerals often serve as components of metalloenzymes and enzyme cofactors or as components of hormones. A fetus is completely dependent on its dam via the placenta for its supply of essential trace elements. Copper, Mn, Zn and Se are often the most limiting trace elements for the fetus and neonate for normal development, and deficiencies of these elements can impair fetal growth and can cause death (Abdelrahmand and Kincaid, 1993).

The mineral status of newborn calves depends not only on the mineral intake from colostrum but also from the mineral transfer via the

placenta that occurs during gestation (Kume and Tanabe, 1993). Mineral balance during the prefresh period is vital to maintain metabolic homeostasis, and prevent metabolic disorders post partum.

Calcium:

Calcium is essential for formation of skeletal tissue, transmission of nervous tissue impulse, excitation of skeletal and cardiac muscle contraction, blood clotting and as a component of milk. Calcium is also involved in the activity of numerous enzymes and serves as a second messenger.

The developing fetus requires a negligible amount of Ca until the last trimester of pregnancy, when the fetal skeleton begins to become calcified. House and Bell (1993) estimated that Ca accretion rate in the conceptus increased from 2.3 g/d at 190 d of gestation to 10.3 g/d at 280 d of pregnancy. The absorbed calcium requirement to meet the demands of the uterus and conceptus is described by the exponential equation of House and Bell (1993) for any given day of gestation beyond 190 as:

$$\text{Ca (g/day)} = 0.0245 e^{(0.05581 - 0.00007 t)t} - 0.02456 e^{(0.05581 - 0.00007(t-1))(t-1)}$$

Where t = day of gestation.

When the loss of Ca exceeds intake and what body reserves can replenish, hypocalcemia can occur and result in the loss of nerve and muscle function, and lead to parturient paresis. Insufficient Ca in the prefresh diet can lead to a decrease in bone calcification, and decreased growth in the growing fetus.

Phosphorus:

Phosphorus is intimately involved in the acid-base buffer systems of blood and other bodily fluids, in cell differentiation, and as a component of cell walls and cell contents. Estimated P accretion rates in the conceptus increased from 1.9 g/d at 190 d of gestation to 5.4 g/d at 280 d of gestation (House and Bell, 1993).

The requirement for absorbed P to meet demands of the conceptus for any day beyond 190 days of gestation is described by the exponential equation:

$$\text{Absorbed phosphorus (g/d)} = 0.02743e^{(0.05527 - 0.000075 t)t} - 0.02743e^{(0.05527 - 0.000075 (t-1))(t-1)}$$

Where t = days of gestation (house and Bell, 1993).

Insufficient P in the diet may result in unthriftiness, inappetence, and poor growth (NRC, 2001). Acute hypophosphatemia (less than 2 mg P/dl

of plasma) may occur if cows are fed low dietary P and challenged by extra demands for P in late pregnancy due to accelerated fetal growth, and colostrogenesis (NRC, 2001).

### Sodium:

Sodium, Cl and K in proper concentrations and balance, are indispensable for a number of physiologic functions including heart function and nerve impulse conduction and transmission. Sodium also plays a role in the sodium-potassium adenosine triphosphate enzyme (Na-K ATPase) responsible for creating electric gradients for nutrient transport.

Cattle evolved without abundant dietary Na to meet nutritional needs. Therefore, the body developed a tenacious ability to conserve Na, via the kidney and efficient absorption from the lower small intestine and large intestine (NRC, 2001). Daily maintenance requirement for absorbed Na in non lactating pregnant cattle is 1.5 g/100 kg body weight (NRC, 2001). The Na requirement for the conceptus is 1.39 g/d from 190 to 279 days of gestation.

### Chlorine:

Chloride is the major anion in the body involved in regulation of osmotic pressure, making up more than 60% of the total anion equivalents in the extracellular fluid.

Typically chlorine is provided in the diet in a salt form which is solubilized, releasing the negatively charged chloride ion for absorption. The maintenance requirement for absorbed chlorine is 2.25 /100 kg of body weight (NRC, 2001). The pregnancy requirement for chlorine is 1.0 g/d from 190 days of gestation to parturition (NRC, 2001).

Potassium:

Potassium is involved in osmotic pressure, acid-base regulation, water balance, nerve impulse transmission, muscle contraction, oxygen and carbon dioxide transport: in phosphorylation of creatine, pyruvate kinase activity, as an activator or co-factor in many enzymatic reactions, in cellular uptake of amino acids and synthesis of protein, carbohydrate metabolism and in maintenance of normal cardiac and renal tissue (NRC, 2001).

Potassium must be supplied daily in the diet because there is little storage in the body and the animal's requirement for potassium is highest of all the mineral element cations. The NRC (2001) recommends that the daily maintenance requirement of absorbed K in non-lactating cattle is 0.038 g/kg of body weight plus 2.6 g/k of dietary DMI. The requirement of absorbed K of the conceptus is 1.027g/d from 190 to 270 d of gestation.

## **Dry Period Feeding Strategies**

A primary goal of the prefresh transition cow diet is to provide adequate nutrition prepartum to prevent metabolic disorders that may occur prior to or immediately after parturition. This can be done by balancing a diet to have a negative dietary cation-anion difference **(DCAD)**.

Dietary cation–anion difference is defined as milliequivalents of  $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{=})$  per kilogram of DM and has a direct impact on blood acid-base metabolism, which in turn works to regulate bone cell function and  $\text{Ca}^+$  homeostasis (Arnett, 2003). To calculate the DCAD in meq/100g DM, the following equation is used:

$$[(\% \text{Na} / 0.023) + (\% \text{K} / 0.039)] - [(\% \text{Cl} / 0.0355) + \% \text{S} / 0.016]$$

The particular minerals ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{S}^{=}$ ) have been chosen to calculate DCAD because their importance in ruminant metabolism lies in their indirect participation in osmotic balance, acid-base balance and integrity and pumping mechanisms of cell membranes (Block, 1994).

The anions  $\text{Cl}^-$  and  $\text{S}^{=}$  should be balanced in a ration against the cations  $\text{Na}^+$  and  $\text{K}^+$  to optimize the physiological functions of the animal, however, the bioavailability of some elements may affect the equation.

The DCAD concept for prefresh transition cows involves the use of compounds added to feed to induce a mild, metabolic acidosis in the cow. The DCAD of the diet does not determine the acidogenic or alkalogenic properties of feeds but can affect metabolic processes in the animal by the absorption and metabolism of these ions (Block, 1994).

Manipulation of acid-base difference makes it possible to maintain the cows in a metabolic acidosis during the critical period that precedes calving, resulting in an increase in calcium mobilization and a decrease in hypocalcemia (Riond, 2001). Bone acts as a "fail – safe" mechanism to buffer  $H^+$  if the kidneys and lungs are unable to maintain acid-base balance within physiological limits (Arnett, 2003). Feeding acidifying diets causes a decrease in the activity of osteoblasts and an increase in resorption pit formation on osteoclasts which releases cations (including Ca) into the blood in order to help correct the pH (Arnett, 2003; Riond, 2001).

Arnett (2003) suggests that osteoclasts are particularly sensitive to pH changes at approximately 7.1, such that pH reductions of only a few hundredths of a unit causes a doubling of resorption pit formation; below a pH of about 7.0 the stimulatory effects begin to plateau. Low cation-anion difference prepartum can also mitigate hypocalcemia peripartum

via increased urinary Ca, blood ionized Ca, and responsiveness to Ca homeostatic hormones (Block, 1994).

Reduced cation-anion differences prepartum have been related to reduced severity of udder edema, which is likely related to an increase in renal loss of water and unchanged water intake (Block, 1994). Dry cow diets that are high in K, Na or both alkalinize the cow's blood and increase the susceptibility for milk fever (NRC, 2001).

The DCAD of a diet can be manipulated by forages and the amounts of forage fed. Forages that are high in  $\text{Na}^+$  and / or  $\text{K}^+$  should be fed in small amounts or not fed at all during the dry period, while forages that are low  $\text{Na}^+$  and  $\text{K}^+$  should be utilized at higher rates. Unfortunately, the nutrient content of available forages does not always allow for the DCAD to reach the recommended levels. To help lower the DCAD of the pre-fresh transition cow ration, anionic salts can be added to the diet.

### **Anionic Salts**

Anionic salts are compounds that contain high amounts of the negatively charged fixed anions  $\text{Cl}^-$  and  $\text{S}^-$  relative to the positively charged cations  $\text{Na}^+$  and  $\text{K}^+$ . Anionic salts cause rations to be more acidic, increasing the absorption of dietary Ca, and stimulating mobilization of bone Ca due to improvement in parathyroid hormone receptor sites (NRC, 2001). This is beneficial in the prefresh period to

prevent metabolic problems after calving. Monitoring the urine pH of cows during the week prior to parturition has proven to be an effective means of assessing effectiveness of anionic salts. For optimal control of subclinical hypocalcemia, Holsteins on a negative DCAD diet should have a urine pH between 6.2 to 6.8, while Jerseys should have a urine pH between 5.0 to 5.5 (Riond, 2001).

### **Parturient Paresis**

Parturient paresis, occurs when cows are unable to compensate for the dramatic increase in  $\text{Ca}^+$  needed for colostrogenesis at calving, and the onset of lactogenesis. Parturient paresis affects 4.9% of the dairy cattle in the United States each year (USDA, 2007).

Normally  $\text{Ca}^+$  homeostatic mechanisms maintain blood  $\text{Ca}^+$  concentration between 7 and 10 mg/dl. The  $\text{Ca}^+$  homeostasis of multiparous dairy cattle is often disturbed by the sudden increase in demand for  $\text{Ca}^+$  at the initiation of lactation. During the dry period, only  $\text{Ca}^+$  lost to the fetus and endogenous fecal drain needs to be replaced. As colostrogenesis and lactogenesis begin an additional 25 g of  $\text{Ca}^+$ /d is needed in the  $\text{Ca}^+$  pool (Riond, 2001). The  $\text{Ca}^+$  that is absorbed from feed is momentarily insufficient to make up for this loss, blood serum  $\text{Ca}^+$  concentration drops below 5 mg/dl (NRC, 2001) and hypocalcemia

develops. Low prepartum  $\text{Ca}^+$  can also lead to difficult calving, prolapsed uterus, retained placenta and stasis of the digestive tract. Cows that suffer from clinical parturient paresis have an increased risk for retained fetal membranes, displaced abomasum, mastitis (Curtis et al., 1985 and Gröhn et al., 1989) and decreased milk yield (Block, 1984) as compared with cows who did not suffer clinical parturient paresis.

The degree of hypocalcemia experienced will depend on the amount of  $\text{Ca}^+$  leaving the extracellular  $\text{Ca}^+$  pool and the rate at which the  $\text{Ca}^+$  homeostasis system can replace that  $\text{Ca}^+$  loss. An important determinant in the risk for parturient paresis is the acid – base balance of the cow at the time of parturition.

In a study done by Block (1984) cows were fed one of two diets, an anionic diet or a cationic diet. The cows on the cationic diet had a 47.4% incidence of milk fever, compared to the cows on the anionic diet that had a zero incidence of parturient paresis. In the same study it was seen that cows receiving the anionic diet had higher plasma ionized  $\text{Ca}^+$  concentration as compared to cows fed the cationic diet. This was the result of an increase in bone mobilization, as indicated by increased levels of hydroxyproline.

Block (1994) suggests that negative DCAD in rations for prepartum cows prevents a decline in blood  $\text{Ca}^+$  at the initiation of lactation by one

or more of the following mechanisms: increasing the rate of bone mobilization of  $\text{Ca}^+$  directly, increasing the rate of bone mobilization of  $\text{Ca}^+$  indirectly via increased excretion (reduced retention) of  $\text{Ca}^+$ , or increase in intestinal absorption of  $\text{Ca}^+$ .

Lowering the DCAD in the prefresh period may also decrease the risk for other parturient diseases such as retained fetal membranes and displaced abomasum, because the incidence of parturient paresis, even sub-clinical increases the risk factor of these diseases.

### **Impacts of a Negative DCAD**

The benefits of anionic salts on preventing milk fever must be weighed against the potential negative effects. Some observed effects of feeding anionic salts prepartum have been a reduced DMI (Charbonneau, 2006; Moore et al., 2002), metabolic acidosis that is not always corrected and the potential to reduce the neonates ability to absorb IgG from colostrum (Guy et al., 1996).

### **Reduced DMI**

Some anionic salts are unpalatable and a high inclusion rate can decrease feed intake (Charbonneau, 2006; Moore et al., 2000). A second reason why DMI is reduced is from the discomfort caused by the mild acidosis that is created by the addition of the anionic salts to the diet (Vagnoni and Oetzel, 1998).

Moore et al. (2002) reported that feeding anionic salts to prepartum heifers significantly reduced the energy balance and increased liver triglyceride content. However, cows being fed the same anionic salts did not experience reduced energy balance or increased liver triglyceride content. This suggests that anionic salts should not be fed to prepartum heifers.

Decreasing feed intake prior to parturition can lead to a negative energy balance, and increase the incidence of metabolic disorders after parturition. If a decrease in DMI is observed, the ration needs to be rebalanced to adjust the energy content to reflect that actual intake.

#### Metabolic Acidosis

The blood's acid - base balance is tightly regulated, because even a minor change from the normal pH range can severely affect many organs. Metabolic acidosis, when the blood pH is below 7.35, results an over abundance of acid in the blood or a loss of bicarbonate from the blood. Metabolic acidosis caused by a negative DCAD diet develops because the amount of acid within the body is increased through ingestion of a substance that is metabolized to an acid. Mild metabolic acidosis is beneficial in how it helps with Ca mobilization from the bone in the transition cow; however there are potential negative effects. Metabolic acidosis is a complicating factor in a number of diseases that

affect cattle, including ketosis, lactic acidosis, enterotoxigenic diarrhea in calves and some enteric diseases of adult cattle (Bigner, et al., 1997). If the metabolic acidosis is not corrected immediately after calving more problems can persist, and the preventative measures put forth during the prefresh transition period will be lost.

Bigner et al. (1997) tested three different Na<sup>+</sup> compounds to determine which was best to treat metabolic acidosis. Metabolic acidosis was induced by feeding a diet that was high in anions for 7 d. Treatments were orally administered in equivalent amounts of Na<sup>+</sup> in the form of NaCl, NaHCO<sub>3</sub> or Na<sup>+</sup> propionate. Both NaHCO<sub>3</sub> and Na<sup>+</sup> propionate were equally effective in correcting the acid-base balance of blood.

#### Effects on the Neonate

Calves born from cows fed anionic diets might be affected by respiratory or metabolic acidosis (Guy et al. 1996), which might, in turn affect apparent efficiency of absorption (**AEA**), and the acquisition of passive transfer (Quigley and Drewry, 1998). Boyd (1989) reported that calves suffering from respiratory acidosis had reduced colostrum IgG absorption. The prevalence of respiratory acidosis immediately postpartum could also inhibit the ability of the neonate to adapt to the extrauterine environment (Besser et al., 1990 and Quigley and Drewry, 1998). Respiratory acidosis develops when the lungs do not expel carbon

dioxide adequately (Arnett, 2003) and blood pH < 7.2 (Bleul et al., 2007). Respiratory acidosis may persist for 24 h after birth.

Guy et al. (1996) reported that calves that were born from cows fed a cationic diet (+445 meq/kg of DM) had higher serum IgG<sub>1</sub> concentration at 24 h than did calves from cows fed more acidotic diets (+75 meq/kg of DM). Conversely, Tucker et al. (1992) reported that the DCAD of the diet (-30 or +90 meq/kg of DM) fed to 120 dry cows and heifers did not affect the acid-base status or the plasma mineral content of the resulting calves. This is an area that deserves more attention to determine if there is an effect of an anionic diet on the newborn.

### **Part II: Nutritional Needs of the Newborn Calf: Colostrum**

Achieving early and adequate intake of high quality colostrum is the single most important management practice influencing calf health and survival. Colostrum components exert effects on the gastrointestinal tract, produce transient systemic metabolism and endocrine changes and have long lasting effects on immunoprotection as well as nutritional status of the newborn calf (Bösze, 2008).

Colostrum arises during a distinct physiological and functional stage of mammary gland development that is markedly different from the gland's primary role of milk production (Barrington et al., 2001). In

domestic ruminants, the primary difference between colostrum and milk is the high concentration of colostral immunoglobulins, specifically immunoglobulin G<sub>1</sub> (**IgG<sub>1</sub>**). Colostrogenesis, or the prepartum transfer of immunoglobulins from maternal circulation into mammary secretions, is a discrete and finite stage (Barrington et al., 2001). Transfer of immunoglobulins begins several weeks prior to parturition and ceases abruptly immediately prior to parturition (Brandon et al., 1971). During this period, up to 500 g/week of IgG are transferred into mammary secretions (Goff and Horst, 1997).

The mechanism and regulation of colostrogenesis is not as well known as that of the other stages of mammary gland development. There is clear evidence that colostrogenesis is regulated in part by lactogenic hormones (Barrington, 2001), and is affected by local mechanisms within the mammary gland. A simple example of this is the variation in IgG content and composition of secretions between individual quarters of the same udder (Guidry et al., 1980).

Immunoglobulin G are transferred from the bloodstream, across the mammary barrier by a specific transport mechanism. Receptors on the mammary alveolar epithelial cells capture IgG from the extracellular fluid and the molecule undergoes endocytosis, transport and finally release into the luminal secretions (Larson, 1980).

Selective transport of IgG<sub>1</sub> into colostrum requires two separate functions. Specific receptors for IgG<sub>1</sub> must be present on the basal plasma membrane of the secretory cells, positioned for capture of the ligand from the extracellular fluid. In addition, mammary epithelial cells must be able to internalize and transcytose IgG<sub>1</sub> in order to deliver it into the luminal secretions (Barrington et al., 2001).

Smaller amounts of Immunoglobulin A (**IgA**) and IgM are largely derived from local synthesis by plasma-cytes in the mammary gland (Godden, 2008) and a smaller amount transported from the blood (McGuirk and Collins, 2004).

Colostrogenesis ceases prior to, or at the onset of lactation, suggesting that the hormones necessary for lactogenesis are likely candidates for regulating the cessation of IgG transfer (Barrington, 2000). Winger et al. (1995) concluded that pharmacological doses of dexamethasone given to cows actively concentrating IgG<sub>1</sub> in mammary secretions resulted in a sharp decline in IgG<sub>1</sub> concentrations. This result is consistent with the accepted role of glucocorticoids as part of the lactogenic complex that triggers the onset of milk secretion. However, glucocorticoids can cause induction of premature parturition and it could be the termination of pregnancy, rather than specific hormonal effects

that may account for the decrease in IgG transfer to the mammary gland (Barrington et al., 2001).

### **Colostrum Composition**

Other than immunoglobulins which provide disease protection, colostrum also contains many other nutrients vital to the calf's survival (Table 1). The carbohydrates, fat, and protein in colostrum are essential as fuels to the newborn (NRC, 2001). The vitamins and minerals are essential as co-factors for enzymes and general maintenance parameters. Increasing evidence in calves and other species indicate that colostrum also provides maternal leukocytes, growth factors, hormones, cytokines, and nonspecific antimicrobial factors all of which are necessary to stimulate growth and development of the digestive tract and other organ systems (Davis & Drackley, 1998; Hammon and Blum, 1998; NRC, 2001).

### **Proteins**

Bovine colostrum is 14% protein, this is much greater than the protein concentration in milk which is 3.2% protein. The major proteins present in colostrum include the family of caseins,  $\beta$ -lactoglobulin,  $\alpha$ -lactoglobulin, immunoglobulins, lactoferrin and various minor whey proteins such as transferrin and serum albumin. Proteins within colostrum provide not only nutrition for the neonate but also enhancement of the

immune system, act as a defense against pathogenic bacteria, viruses and yeast, and development of the gut and its functions (Bösze, 2008).

The biological properties of proteins in colostrum facilitate nutrient assimilation and peptides with regulatory activity likely influence the growth and differentiation of various neonatal tissues (Talukder et al., 2001). The effect of these proteins depends on the absorption and transport of colostrum macromolecules from the gut of the lumen to specific tissues. Large quantities of amino acids (**AA**) are also needed for the rapid protein accretion that occurs independent of IgG accumulation in the digestive tract (Davis & Drackley, 1998). The majority of the protein's composition in colostrum is immunoglobulins.

### Immunoglobulins

The limited acid secretion and low proteolytic activity in the digestive tract of newborn calves, coupled with the retention of the IgG in the abomasal coagulum, favor passage of intact IgG into the intestine (Davis & Drackley, 1998).

Immunoglobulins refer to a family of high molecular weight proteins that share common physico-chemical characteristics and antigenic determinants. These proteins occur in the serum and other body fluids of animals and possess  $\gamma$ - or slow  $\beta$ -electrophoretic mobility (Butler, 1969). All immunoglobulins are composed of polypeptide chains referred to as

heavy (long) chains and light (short) chains that vary in composition of both component amino acids and prosthetic groups (Larson, 1980). The classification of immunoglobulins is based on the antigenic and physico-chemical characteristics of these proteins.

There are three identified classes of immunoglobulin in cattle, IgG, IgA, and IgM. These immunoglobulins account for 85 to 90 %, 5 % and 7 %, respectively of the total Ig in colostrum (Godden, 2008). These different classes may share antigenic similarities, but have different physico-chemical features. In addition to class differences among immunoglobulins, smaller antigenic and physico-chemical differences in the heavy polypeptide chains within a class give rise to subclasses (Butler, 1969 ). In cattle, this is seen with two subclasses of IgG: IgG<sub>1</sub> and immunoglobulin G<sub>2</sub> (**IgG<sub>2</sub>**). These subclasses differ slightly in their heavy chains and are in nearly equal concentrations in the blood (Larson, 1980).

### Immunoglobulin G

Bovine IgG is subdivided into two classes, IgG<sub>1</sub> and IgG<sub>2</sub>. It is the most abundant and most extensively studied immunoglobulin in the cow. Approximately 85-90% of the serum and whey immunoglobulins are in the IgG class (Klaus, 1969). The two subclasses of IgG differ antigenically and in AA composition. Immunoglobulin G<sub>2</sub> is found in high amounts in serums, but occur in lower concentrations in milk, colostrum and saliva (Butler,

1969 ). ImmunoglobulinG<sub>1</sub> is the principal immunoglobulin for passive immunization of the calf (Butler, 1969).

### Immunoglobulin M

Bovine IgM is an antigenically distinct macroglobulin, comprising less than 10% of serum and colostrum immunoglobulins (Klaus, 1969).

Immunoglobulin M is a more effective antibody than IgG in agglutination, phage neutralization, complement fixation and hemolysis (Butler, 1969).

Much of the efficiency of IgM can be explained on the basis of size and number of antibody combining sites.

### Immunoglobulin A

Immunoglobulin A has been shown to be antigenically distinct from IgG and IgM by immunodiffusion. Bovine secretory IgA is composed of four alpha chains, four light chains and one molecule of glycoprotein-a (Butler, 1969). The alpha chains are antigenically and physiochemically distinct from the heavy chains of IgM and IgG, while the light chains are identical to those occurring on other bovine immunoglobulins (Butler, 1969). Immunoglobulin A is capable of neutralizing and preventing the entry of potentially harmful antigens into the host (Bösze, 2008).

### Fat

The newborn calf is born with relatively small energy reserves, with only 3% of the body weight made of lipids. Much of this lipid content is

structural and is unable to contribute to energy needs of the calf. This small amount of fat in the calf would be mobilized within 18 h of life in the absence of feed intake. Colostrum from Holstein cattle contains 6.7 % fat as compared to milk which contains 3.6 % fat (Table 1; Foley and Otterby, 1978). The gross energy content of colostrum can be calculated by using the kilo calorie values for lactose, non-immunoglobulin protein and fat. Davis and Drackley (1998) calculated the average energy content of colostrum to be 1.16 kcal/g. This is considerably greater than the energy content of milk which is 0.69 kcal/g. The energy content of colostrum can vary greatly depending on the fat content. The energy provided by fat and lactose in colostrum is essential for thermogenesis and maintenance of body temperature.

#### Vitamins & Minerals

Colostrum is the primary source of vitamins and minerals for newborn calves and many of the minerals and vitamins are more concentrated in colostrum as compared to milk (Table 1). This may be an evolutionary strategy to ensure supply of adequate amounts of these minerals and vitamins for the newborn calf to initiate its own metabolism successfully and for development of the digestive system (Davis and Drackley, 1998).

Fat soluble vitamins A, D, and E do not cross the placental barrier resulting in colostrum being the primary source of these nutrients for the calf (Quigley and Drewry, 1998). For vitamins and minerals colostrum is more concentrated than milk in Zn, Fe, folic acid, choline and riboflavin (Akers, 2002).

### **What Affects Colostrum Quality?**

The concentration of Ig in the maternal colostrum is important in determining the amount of Ig the calf consumes and can potentially absorb. Colostral IgG concentration is the primary factor that can affect passive transfer (Nocek et al., 1984), and is the hallmark for evaluation of colostrum quality. High quality colostrum has an IgG concentration greater than 50 g/L (Godden, 2008). Various factors can affect the concentration of Ig, and nutrient composition of colostrum, these factors include, but are not limited to parity, quantity of colostrum produced, breed, environmental and management strategies.

#### **Parity**

Research has shown that colostrum from second and later lactation cows has a higher concentration of Ig than that of first parity cows (Devery-Pocius and Larson, 1983 and Shearer et al., 1985). Devery-Pocius and Larson (1983) observed that total IgG<sub>1</sub> reached a maximum concentration in the third and fourth lactation and nearly doubled as

compared to the first lactation. Total IgG<sub>2</sub> was lowest in first lactation cows and increased with lactation number, only lactation number 5 to 8 had significantly greater values compared to lactation 1 animals. Serum IgM and IgA did not show any trend with age.

Immunoglobulin G is transported from the blood to the colostrum by a highly specialized transport system, this data suggests that the transport system may not mature until later lactations, and is coincident with maximum mammary gland development. This result is also supported by the thought that first parity cows have been exposed to fewer antigens than older cows and therefore produce lower quantities of colostrum antibodies.

### Quantity

A second factor that can affect the IgG concentration within colostrum is the quantity produced at first milking. Weight of first milk colostrum was found to be the variable most highly correlated (negatively) with colostrum IgG<sub>1</sub> concentration (Pritchett et al, 1991).

### Breed

Guy et al. (1994) investigated the physiological basis of breed differences in IgG<sub>1</sub> concentration in colostrum. Utilizing 15 beef cows and 13 dairy cows, it was observed that overall IgG<sub>1</sub> concentration was greater in colostrum secretions from the beef cows (113.4 mg/ml) than from

dairy cows (42.7 mg/ml). The conclusion from this study was that dairy cows transfer more IgG into secretion than did beef cows, but colostral IgG concentrations were lower in dairy cows due to the dilution effect of the greater volume of colostrum produced by the dairy cows as compared to the beef cows.

Muller and Ellinger (1981) compared total IgG content of colostrum among various breeds of dairy cattle, Holstein cows produced colostrum with total Ig content (5.6 %) that was numerically lower than for Guernsey (6.3 %) and Brown Swiss cows (6.6 %), and was statistically lower than Ayrshire (8.1%) and Jersey cows (9.0 %). These differences could be attributed to genetic differences and/or dilution effects.

#### Climate/Environment

One factor that effects colostrum composition is often times out of our control. While adequate ventilation, and cooling systems help reduce the effects of climate, it still remains a viable factor is effecting colostrum quality.

Nardone et al. (1997) reported that when primiparous cows were exposed to high air temperatures (temperature – humidity index = 82 from 0900 to 2000 h and temperature humidity index = 76 from 2100 to 0800 h) it markedly affected colostrum composition, as compared to cows exposed to thermal comfort (temperature – humidity index = 65). For the first four

milking, colostrum from cows under high air temperatures had lower mean percentages of total protein (7.5%) as compared to their herdmates who were exposed to thermal comfort (9.0%). Further analysis of colostrum protein fractions showed that heat stress reduced the concentration of casein, lactalbumin, and IgG, and IgA, but did not reduce the percentage of lactoglobulin or the concentration of IgM.

### Management Factors

Aside from parameters that can not be changed (age, breed...) management factors can also play a role in altering colostrum composition and IgG concentration.

New mastitis infections often occur during the dry period, and this is an area that affects colostrum quality. Gulliksen et al. (2008) reported that a somatic cell count of >50,000 cells/mL was the only test-day result found to be significant for the production of colostrum with very low IgG values. Mastitis infections can be prevented during the dry period by proper management of dry cow and close up pens and with the use of antibiotics at dry off and teat sealants to prevent bacteria and other microorganisms from entering the udder.

Milking pre-partum heifers and cows has become a common management practice on some dairies as a way to reduce udder edema, and prevent mastitis in older cows. While this practice can be

beneficial to the cow, it can reduce the quality of the colostrum. This is because IgG transfer into milk is largely completed prior to parturition. Pre-milking cows, or cows that have excessive leakage prior to calving, will result in a loss of IgG from the mammary gland, and will result in colostrum with lower IgG concentrations (Kruse, 1970).

Additional management factors that can potentially influence colostrum composition and quality include the prefresh diet, body condition score prior to calving (Odde, 1988) and the on farm vaccination program.

### **How to Test Colostrum**

Determining the quality of maternal colostrum is a beneficial management practice that is quick, easy and cost effective to the producer.

Fleenor and Stott (1980) created a practical field method for measuring immunoglobulin concentration in bovine colostrum. Utilizing data collected from 14 colostrum samples it was determined there was a linear relationship between colostrum specific gravity and immunoglobulin concentration. A colostrometer was developed by incorporating means and percent coefficients of variation for colostrum constituents into a conventional hydrometer. The colostrometer can be used for qualitative assessment of colostrum or to estimate the quantity of Ig.

Currently only 13% of all dairy operations evaluate colostrum quality prior to feeding (USDA, 2007). Farms that have a herd size of 500 head or more are more likely to evaluate colostrum quality (45.2%) as compared to farms with less than 100 head (7.6%). The most common methods of colostrum measurement on farms that measure colostrum quality are the use of a colostrometer (43.7% of operations) and visual appearance (41.6% of operations) (USDA, 2007).

A colostrometer can not detect or predict incipient failure in passive transfer of immunity. However, it is a valuable tool that can be utilized to make a quick assessment on colostrum quality.

### **How to Improve Colostrum Quality**

Colostrum is vital to the survival of the newborn calf, however high quality colostrum is not always available. To be classified as colostrum of satisfactory quality, international recommendations set a minimum concentration of 50 g of IgG/L (Besser et al., 1985 and 1990). In a study done utilizing Norwegian Red cattle, 1,250 colostrum samples had IgG concentrations ranging from 4 to 235 g/L with 57.8% of the samples having less than adequate IgG concentration (Gulliksen, et al., 2008). Whether the cow is down, it is the middle of the night, or the cow is leukosis positive, the newborn calf needs colostrum. Research has been done on ways to improve colostrum, to create colostrum supplements that can be added

to maternal colostrum and colostrum replacers, which have been created as a total replacement for maternal colostrum.

### Bovine Serum Products

Bovine serum products are one of the least expensive sources for colostrum supplements, because bovine blood is plentiful and considered a waste product from slaughter houses, it also contains high levels of IgG.

Arthington et al. (2000) compared the absorptive efficiency of IgG from a commercial bovine serum product, cow colostrum (positive control), and two commercial milk derived colostrum supplements. Plasma IgG concentrations at 24 hours were 12.1, 6.8, 2.2, and 3.5 g of IgG/L for colostrum, bovine serum, supplement 1 and supplement 2. While calves that received cow colostrum had a higher plasma IgG concentration, apparent efficiency of absorption was greatest for the calves receiving the bovine serum product. This is a direct relation to the initial amount of IgG fed to the calves. The results of this study indicate that when maternal colostrum is not available, serum derived IgG may be used to supplement the calves with a concentrated source of IgG.

Although bovine serum derived colostrum supplements have proven to more effective than other supplements, they no longer can be used in countries where the use of animal proteins has been banned. This is due to the concern of diseases such as Bovine Spongiform

Encephalopathy being transferred from animals to humans during consumption.

### Colostrum Supplement

Colostrum supplements were developed because of the high number of calves that had inadequate absorption of IgG and the lack of high quality maternal colostrum. Colostrum supplement products are designed to provide extra IgG (typically 25 – 45 g/dose) to neonatal animals during the period of macromolecule transport (Davenport et al., 2000). Current colostrum supplements provide exogenous IgG from bovine lacteal secretions, eggs and bovine serum. Colostrum supplements refer to products that are intended to provide <100 g of IgG/dose and are not formulated to completely replace colostrum (Quigley et al., 2001).

Hopkins and Quigley (1997) performed a study to determine if the addition of a colostrum supplement to maternal colostrum affected serum IgG concentration and efficiency of absorption. Fifty two Holstein heifer and bull calves were blocked by sex and randomly assigned to receive 3.8 L of maternal colostrum in one feeding, 1.9 L in two feedings or 1.9 L in two feedings plus 272 g of colostrum supplement at the first feeding. Blood was collected via the jugular vein at 0, 24 and 48 h and analyzed for IgG. Serum IgG levels were lower ( $P < 0.01$ ) for calves that received 1.9 L in two feedings plus 272 g of colostrum supplement at the first feeding as

compared to calves that received 1.9 L in two feedings. However, serum IgG concentrations at 48 h did not differ among treatments. The researchers suggested that if high quality colostrum is available to be fed addition of colostrum supplement appears unnecessary.

### Colostrum Replacer

While colostrum provides the calf with the antibodies needed to obtain passive transfer, it can also be the first exposure of pathogens to the calf. Escherichia coli, leukosis and Johne's disease can all be transmitted from cow to calf via colostrum. Early exposure to these pathogens represents significant health and economical problems. Colostrum replacers were originally created as a way to provide calves with the needed antibodies when high quality maternal colostrum is not available, or when it is known that a cow carries a specific disease that can be transmitted via the colostrum. In a recent study it was observed that 94.7 % of dairies feed colostrum to their calves, but the remaining 5.3 % feed a colostrum replacer due to Johne's disease (Fulwider et al., 2008).

Colostrum replacers should provide >100g of IgG/dose, the minimum dose required for the calf to achieve a predicted final serum IgG concentration of >10.0 mg/mL (Quigley et al., 2001, 2002). A colostrum replacer must also provide the nutrients required by the calf to

thermoregulate and establish homeostasis; this includes adequate protein, energy, vitamins and minerals.

Swan et al. (2007) studied passive transfer of IgG and preweaning health in Holstein calves fed maternal colostrum and colostrum replacer. Serum IgG concentrations between days 1- 8 were higher for calves that received maternal colostrum ( $14.8 \pm 7.0$  mg/mL) as compared to calves that received the colostrum replacer ( $5.8 \pm 3.2$  mg/mL). Various studies have been done to compare maternal colostrum to colostrum replacer and similar results were observed (Smith and Foster, 2007)

Shea (2007) performed a study that compared the passive transfer of calves being fed colostrum replacer with or without supplemental lactoferrin. Calves that were fed 1 dose of colostrum replacer without supplemental lactoferrin within 90 min of birth, had a 24 h average serum IgG of 10.7 g/L. Calves that received 2 doses of colostrum replacer without supplemental lactoferrin (1 dose within 90 min of birth and a second dose at 12 h) had a 24 h average serum IgG of 14.4 g/L. This research suggests that colostrum replacer is adequate to be fed as a complete replacement for maternal colostrum and will allow the calf to achieve passive transfer.

## **Passive Transfer**

Cattle have a six layer placenta which inhibits the transfer of serum proteins from the cow to the calf, unless there is a damaged placentation (Redman, 1979). Due to this placental structure newborn calves are born agammaglobulinemic, and passive immunity protection is achieved by the transport of macromolecules found in colostrum through the intestinal epithelium into the bloodstream of the neonate. Immunoglobulin G that is absorbed within the first 24 h after birth remains an effective protective mechanism until the immune system of the calf matures. Attainment of passive immunity can be affected by many external factors: colostrum IgG concentration, colostrum volume, age at which colostrum is consumed and calf stress.

### **How IgG is Transferred to the Calf**

The cow, like other ruminants has a syndesmochorial placenta, immunoglobulins can not cross the placenta, and calves rely on colostrum for passive immunity. Immunoglobulin G is transferred to the calf via the colostrum. Neonatal calves are pseudomonogastics, whose gastrointestinal tract is characterized by a marked growth rate. Intestinal absorption of large molecular proteins and peptides by pinocytosis decreases through day seven postnatally, whereby protein digestion by lysosomes within enterocytes is replaced by digestion in the GIT lumen

and on enterocytes borders (Blum, 2006). It has been suggested that absorption of IgG in the small intestine within the first 24 -36 hours occurs through non-selective endocytosis of macromolecules (Stott, 1979).

There are two phases of absorption of macromolecules from the intestinal lumen to the blood: A) uptake or internalization within intestinal epithelium and B) transport or subsequent expulsion of the macromolecules into systemic circulation (Leccee and Morgan, 1962). Stott et al. (1979) suggested that colostrum feeding stimulates pinocytosis in the endocytes, followed shortly by cessation of internalization of macromolecules. Other researchers suggest that intestinal selectivity does exist among the IgGs and absorption does not appear to occur entirely by non-specific endocytosis of macromolecules (Sangild, 2003).

In a study done by Sangild (2003) using pig and lamb fetuses, it was observed that intestinal macromolecule uptake is present in the fetus during the last weeks of gestation, but is markedly less in the fetus than the neonate. This observation suggests that the ability to take up and transfer intact protein from the epithelium into the circulation is a very time-specific process that develops close to term, and is not merely a result of intestinal immaturity.

The degree of humoral immunological protection depends on the amount and the time of colostrum uptake, the colostrum immunoglobulin

quality and concentration and the intestinal capacity to absorb immunoglobulins (Sangild, 2003).

Closure of the intestinal permeability to colostral immunoglobulins in the calf was originally thought to occur spontaneously with a progressively increasing rate after 12h postpartum, with mean closure near 24 h (Stott, 1979). More recent research has shown that gut closure appears to be an energy dependent process, because both hypoxia at birth (Tyler and Ramsey, 1991) and insulin-mediated hypoglycemia (Tyler and Ramsey, 1993) delayed gut closure. With increasing age there are progressively fewer intestinal epithelial cells capable of pinocytotic activity, colostral uptake and transmission of colostral constituents into circulation (Stott, 1979 III).

Shortening the absorption period may be beneficial to reduce the intake of bacteria into the intestines and blood stream. Absorbed IgG is then transferred through the lymphatic system into peripheral circulation; this remains an effective protective mechanism until the specific immune system of the calf matures (Redman, 1979).

#### Rate of Absorption

The rate and pattern of colostral immunoglobulin absorption has been determined from the determination of the interaction of three factors: starting age of colostrum feeding, amount of colostrum fed and

time after feeding. The rate of absorption and period of absorption determine concentration of serum Ig in neonatal calves and their passive immunity potential (Stott et al. 1979).

Stott et al. (1979) determined that the highest rates of absorption, based on serum IgG concentration occurred during the first 4h after feeding and that calves fed 0.5 and 1.0 L of colostrum showed a much slower rate of absorption during the second 4 h. In the same study Stott et al. (1979) also showed that age at first feeding had little influence on rate of absorption up to 12 h postpartum, and from that point on, absorption rates decreased at a progressive rate with increasing age. Calves born in the U.S. that are removed from their dam immediately after birth were hand fed colostrum an average of 3.3 hours after birth (USDA, 2007).

#### Amount of Absorption

Aside from the period of absorption and the rate of absorption, the amount of IgG absorption also affects passive transfer. Stott et al., (1979) showed that both the age of the calf when colostrum was fed and the amount fed are two major factors determining the IgG concentration in serum of the post-colostral calf.

To achieve successful passive transfer a 43 kg (90 lb) Holstein calf needs to be fed a minimum of 100 g of IgG in the first colostrum feeding

(Davis and Drakley, 1998). This means that the volume of colostrum that the individual calf needs to receive is dependant upon the IgG concentration of the colostrum. Due to the fact that many producers do not test colostrum, it is suggested to feed colostrum at 10 to 12% of the calf's bodyweight (Godden, 2008).

Immunoglobulin G concentration in blood serum is a direct indicator of calfhood mortality risk. Hancock (1985) utilized 10 commercial dairy herds to determine mortality risk at various IgG concentrations. Herds were identified as being a low mortality herd, 0 to 2.8% mortality rate or a high mortality herd, 5.8% to 13% mortality rate. The week one mean IgG concentration of calves from the low mortality herd was 9.8 mg/mL whereas calves in high mortality herds only achieved a concentration of 7.3 mg/mL. This suggests a difference between optimum IgG absorption and adequate IgG absorption. Calves in the low mortality herds had lower serum IgG concentrations and were still at a lower mortality risk, as compared to their counterparts. This suggests that calves from healthier herds may not require as much as colostral IgG, as compared to calves from poorer herds. During week three and four, IgG levels in calves from the higher mortality herds were greater compared to calves in lower mortality herds.

However, calves from the herds with a low mortality rate may not require the same amount of IgG as do calves in herds with higher mortality rates (Hancock, 1985).

### **Testing for Passive Transfer**

One method that is used to determine passive transfer is measuring the serum IgG concentration. Calves with a serum IgG level of  $>10\text{mg/mL}$  is considered to have achieved passive transfer. Testing serum IgG levels 24 to 48 h after birth can be used for prospective risk assessment of individual calves, and retrospective evaluation of sick calves (Hancock, 1985). Serum IgG measurement has also been recommended as a means of evaluating efficiencies of management procedures when there is an excess of morbidity among calves within a herd.

Currently only 2.1 percent of U.S. dairy operations routinely monitor serum protein levels in heifer calves (USDA, 2007). Large dairies ( $>500$  head) are leading the industry with 14.5% of these dairy operations routinely monitoring serum protein levels, as compared to small herds ( $<100$  head) with only 1.1 percent of operations routinely monitoring serum protein levels (Table 2).

### **Factors that Affect Passive Transfer**

There are various factors that affect passive transfer in the newborn calf. The primary factors that influence passive transfer are the amount of

quality colostrum fed and the time postpartum of feeding (Nocek, 1984). Other aspects that influence passive transfer include environment and parturient reproductive abnormalities, this includes parity of dam, calving ease, month of birth, maximum environmental temperature on day of birth (Stott et al, 1975) sex of calf, placental retention and dystocia (Donovan, 1986).

### Colostrum Ig Concentration

Besser et al. (1985) observed that there was a significant negative correlation between efficiency of absorption and mass of immunoglobulin fed for both IgG and IgM. In this study calves that were fed colostrum with less IgM absorbed a higher proportion of the IgM than calves fed colostrum with more IgM. IgG showed similar results with a higher efficiency for absorption when less was fed, however, calves that received colostrum with a higher IgG level had higher serum IgG levels as compared to calves that received the lower amount of IgG. This information suggests that the transport system for IgM and IgG can become saturated if overloaded, and it may be more beneficial to the calf to feed it smaller amounts of colostrum more often.

Jaster (2005) evaluated quality, quantity and timing of colostrum feeding on IgG<sub>1</sub> absorption in Jersey calves and observed that calves fed colostrum with higher concentrations of total ingested IgG<sub>1</sub> had

significantly higher serum IgG<sub>1</sub> concentrations than calves fed low IgG<sub>1</sub> colostrum. It was also observed that calves receiving 2 L of high IgG<sub>1</sub> colostrum at birth and 12 h has higher mean apparent efficiency of IgG<sub>1</sub> absorption as compared to calves receiving 4 L of high IgG<sub>1</sub> colostrum at birth, calves receiving 4 L of low IgG<sub>1</sub> at birth and calves receiving 2L of low IgG<sub>1</sub> at birth and 12 h. This research suggests that to maximize IgG absorption calves should receive 2 separate feedings of high quality colostrum to maximize IgG<sub>1</sub> absorption.

#### Seasonal Effects

Seasonal variations in passive transfer in calves have been demonstrated in several temperate regions. Mean monthly serum IgG<sub>1</sub> concentration were lowest in winter and increased during spring and early summer and peaked in September, when the greatest environmental stress was cold (Boyd, 1972 & Gay, 1965 & Gay 1983). In a similar study (Donovan et al. 1986) the opposite was seen, however this study was done in a subtropical climate where intense heat was the greatest environmental stress. Stott et al, (1975) observed that calves exposed to hotter, less desirable environments responded by having higher mortality, higher serum corticosteroid concentration and lower serum IgG<sub>1</sub> concentrations at 2 and 10 d after birth.

This variation in pattern suggests that management practices may play an important role in seasonal variation. People may not be as motivated to spend the extra time to tend to newborn calves when the weather is at one extreme or the other (Donovan et al., 1986).

### Colostrum pH

Maternal colostrum has an average pH of 6.17 (Tsioulpas et al., 2007). The chemical compositions of colostrum replacers and colostrum supplements may affect the efficiency that IgG are absorbed by the calf (Quigley, 2000). Differences in AEA among colostrum supplements may be due to differences in ingredient composition, chemical characteristics, including pH and IgG concentration.

Quigley et al, (2000) studied the effect of varying pH on AEA of a colostrum supplement derived from edible-grade bovine serum. Lifeline Calf Nutritional Colostrum Supplement (American Protein Corporation, Ames, IA) was utilized in the study and had a pH of 7.5 when reconstituted in 2 L of water. Using sodium citrate the pH of the colostrum supplement was altered from 7.5 to 7.0, 6.0 and 5.0. Blood samples were obtained at 0 and 24 h and analyzed for IgG. Plasma IgG levels and AEA of calves did not differ significantly among treatments. This suggests that a change in pH of colostrum supplement in the range of 5.0 to 7.5 does not alter IgG absorption.

## Dystocia

Dystocia is a major cause of weakness, morbidity and mortality in dairy calves. A prolonged and difficult calving can cause acidosis and hypoxia in the calf, both of which have major detrimental effects (Davis & Drakley, 1998).

Donovan et al. (1986) reported that dystocia was associated with lower neonatal serum protein concentration. Odde (1988) observed similar results in a study conducted to determine the effect of body condition at calving and calving difficulty on calf vigor and calf serum immunoglobulin concentration. A calving difficulty score of 1 through 3 was assigned to each calf. Calves with a calving score of 1 (unassisted delivery) had a significantly higher serum IgG<sub>1</sub> and serum IgM concentration as compared to calves with a calving score of 2 or 3.

The reduced IgG serum concentration in dystocia calves could be related to the increase in endogenous corticosteroid release, and their effect on closure of the intestinal wall. Another explanation for this is that calves that experience dystocia often lack vigor, and may lack the ability to nurse off the dam, or from a bottle. To help reduce failure of passive transfer among dystocia calves, managers should tube feed any calves that can not suckle.

## Sex of the Calf

Odde (1988) observed that heifer calves had higher serum IgG<sub>1</sub> level at 36 h as compared to bull calves (2355.4 mg/dl and 2037.4 mg/dl, respectively). Odde (1988) hypothesized that this result was due to the higher degree of calving difficulty for bull calves.

Failure to achieve passive transfer increases the risk of neonatal septicemia and mortality. It has been estimated that a certain frequency of hypogammaglobulinemia cannot be avoided under practical farm conditions due to variations in birth weight, immunoglobulin concentration in colostrum, dose of colostrum, age at first feeding and possibly to genetically determined ability to absorb immune globulins (Bush, 1980). If failure of passive transfer occurs, calves can be treated with the administration of plasma at a dosage of 20 mL/kg I.V. (Weaver, 2000). If plasma treatment is not an option, calves that suffer failure of passive transfer can still survive if they are placed in a clean environment with low exposure to infectious pathogens (Weaver, 2000). It is also important to remember that neonates that do achieve passive transfer can easily suffer disease if placed in a dirty environment or exposed to bacterial pathogens, or other disease causing organisms.

### **PART III. Sodium Bicarbonate**

Sodium Bicarbonate has successfully been used to correct acidosis (pH < 7.2) in calves (Bleul et al., 2005, Koch & Kaske, 2008, Michna et al., 1996) when injected intravenously.

Ayers and Besser (1992) evaluated the effects of alkalizing agents, administered prior to feeding colostrum, on absorption of IgG, blood-gas and acid-base values. Significant least square means effects were detected for sodium bicarbonate treatment on blood pH (+0.04 units), PCO<sub>2</sub> (+ 4.1 mm of Hg), and HCO<sub>3</sub> concentrations (+ 4.4 mEq/L). Absorption of colostral IgG was not affected by sodium bicarbonate treatment or the altered blood-gas and/or acid-base status.

Sodium bicarbonate has also been used as a method to buffer acidified and fermented colostrum. Foley et al. (1978) observed that neonatal calves receiving buffered colostrum had higher serum IgG concentrations compared to calves that received fermented colostrum. Addition of sodium bicarbonate to fermented colostrum (Jenny et al., 1983) and to acidified colostrum (Eppard et al., 1981) improved feed intake when compared to acidified or fermented colostrum that did not contain additional sodium bicarbonate. Kellaway et al. (1977) and Emerick (1976) reported that bovine colostrum with supplemental sodium

bicarbonate may improve performance of calves fed high concentrate diets pre- and post weaning.

Aside from feed intake parameters, it has been reported that Sodium bicarbonate in human milk and bovine colostrum can inhibit the growth of *Escherichia coli* 0111 (**E. coli**) (Bullen et al., 1972 and Griffiths and Humphreys, 1977). Bullen et al. (1972) reported that human milk and bovine colostrum that contained supplemental sodium bicarbonate to bring the pH up to 7.2 and 7.5 had a powerful bacteriostatic effect; however when sodium bicarbonate was added in a smaller amount to bring the pH of milk and colostrum to only 6.8 and 6.95 the bacteriostatic effect was not seen. Griffiths and Humphreys (1977) determined that the sodium bicarbonate and not the pH change was the cause of the bacteriostatic effect by altering the pH of colostrum with sodium bicarbonate and NaOH. Both colostrum samples were altered to reach a pH of 7.4, however only the colostrum containing the sodium bicarbonate had a bacteriostatic effect. This research suggested that the bacteriostatic action of milk and colostrum is due to the combined action of antibody and lactoferrin and is dependent on sodium bicarbonate to counteract the iron-mobilizing effect of citrate which is normally present in milk and colostrum. These results suggest that adding supplemental

sodium bicarbonate to colostrum or colostrum replacer in an amount that increases the pH to 7.2 could reduce the risk of *E. coli* in newborn calves.

### **Conclusion**

Large volumes of research have been done on management practices to improve calf survival and management of transition cows. However, limited research has been done on how treatment of the pre-fresh transition cow affects the neonate's ability to thrive after parturition. Anionic salts, when used correctly, are a useful tool to dairymen to prevent metabolic problems after calving, however their impact on the neonate need to be researched. Sodium bicarbonate has been successfully used to correct acid-base balances in calves suffering from acidosis caused by diarrhea. If calves are born with respiratory acidosis it is possible that addition of sodium bicarbonate to the colostrum or colostrum replacer may help in correcting the acidosis and increasing the chances of survival, and other health parameters in the newborn calf.

**Table 1.** Physical characteristics and composition of colostrum and whole milk

Item	Colostrum (no. of postpartum milking)					Milk
	1	2	3	4	5	
pH	6.32	6.32	6.33	6.34	6.33	6.5
Total solids (%)	23.9	17.9	14.1	13.9	13.6	13.9
Total fat (%)	6.7	5.4	3.9	4.4	4.3	4.0
solids not fat (%)	16.7	12.2	9.8	9.4	9.5	8.8
Total Protein (%)	14	8.4	5.1	4.2	4.1	3.1
casein (%)	4.8	4.3	3.8	3.2	2.9	2.5
Immunoglobulins(%)	6.0	4.2	2.4	...	...	0.09
IgG (g/100ml)	3.2	2.5	1.5	...	...	0.06
IgA (%)	3.9	...	...	...	...	0.2
IgM (%)	4.2	...	...	...	...	0.05
Lactose (%)	2.7	3.9	4.4	4.6	4.7	5.0
Ash (%)	1.11	0.95	0.87	0.82	0.81	0.74
Ca (%)	0.26	0.15	0.15	0.15	0.15	0.13
Mg (%)	0.04	0.01	0.01	0.01	0.01	0.01
K (%)	0.14	0.13	0.14	0.15	0.14	0.15
Na (%)	0.07	0.05	0.05	0.05	0.05	0.04
Cl (%)	0.12	0.1	0.1	0.1	0.1	0.07
Zn (mg/100 ml)	1.22	...	0.62	...	0.41	0.3
Mn (mg/100 ml)	0.02	...	0.01	...	0.01	0.004
Fe (mg/100 ml)	0.2	...	...	...	...	0.05
Cu (mg/100ml)	0.06	...	...	...	...	0.01
Co (mg/100ml)	0.5	...	...	...	...	0.1
Vitamin A (µg/100 ml)	295	190	113	76	74	34
Vitamin E (µg/g fat)	84	76	56	44	31	15
Riboflavin	4.83	2.71	1.85	1.8	1.76	1.47
Choline	0.7	0.34	0.23	0.19	0.16	0.13

Adapted from Akers, 2002; Foley and Otterby, 1978)

Table 2. Colostrum management parameters, broken down by herd size

	Small (Fewer than 100)	Medium (100 - 499)	Large (500 or more)	All Operations
Age at first feeding (h)	3.4	3.3	2.8	3.3
Estimate IgG levels (%)	7.6	19.8	45.2	13
Pasteurize colostrum (%)	0.2	0.9	6.4	0.8
Monitor serum IgG (%)	1.1	2.4	14.5	2.1

## CHAPTER II

# ANIONIC SALTS IN THE PREPARTUM DIET AND ADDITION OF SODIUM BICARBONATE TO COLOSTRUM REPLACER AND THEIR EFFECTS ON IgG ABSORPTION IN THE NEONATE

### Introduction

A healthy calf begins not at birth, but with optimal nutrition and management of the dam throughout the gestation period (Davis and Drakley, 1998). Nutrient demands by the developing fetus become greater during the last trimester, with 60 % of the total fetal weight gain occurring during the last 2 months of gestation (Eley et al., 1978). Feeding prefresh transition cows is a balancing act to provide adequate nutrients for growth and metabolic needs of the fetus as well as the nutrients needed to maintain homeorhesis, and prevent metabolic disorders in the cow after parturition.

Anionic salts are often fed during late gestation, and are beneficial in reducing metabolic problems in cows after parturition (Block, 1984;

Block, 1994; Goff and Horst, 1997; Charbonneau, 2005;). However, limited research has suggested that feeding anionic salts to prepartum cows may negatively affect the neonate's ability to absorb IgG from colostrum and achieve passive transfer: serum IgG  $\geq$  10.0 mg/mL (Quigley and Drewry, 1998).

Cows and other ruminants have a synepithelialchorial type of placental barrier, so that IgG does not pass to the developing offspring during fetal development (Akers, 2002). Newborn animals of many species rely on maternal colostrum to provide them with the nutrients needed to sustain life. Due to the placental structure, calves are born agammaglobulinemic, without any measurable IgG or IgM and require high quality colostrum to provide them with sufficient amounts of Ig to provide passive immunity for the first 30 to 90 d of life (Guy et al., 1994) until the calf is able to build its own immune system. Calves that do not achieve passive transfer are associated with excess mortality risk, increased risk of diarrhea, increased rates of respiratory problems, and negative effects on future health, longevity and performance parameters (Davis and Drackley, 1998).

Sodium bicarbonate has been used as a method to buffer acidified and fermented colostrum. Foley et al. (1978) observed that neonatal calves receiving buffered colostrum had higher serum IgG concentrations

compared to calves that received fermented colostrum. Addition of sodium bicarbonate to fermented colostrum (Jenny et al., 1983) and to acidified colostrum (Eppard et al., 1981) improved feed intake when compared to acidified or fermented colostrum that did not contain additional sodium bicarbonate. Bullen et al. (1972) reported that bovine colostrum that contained supplemental sodium bicarbonate had increased bacteriostatic activity. Kellaway et al. (1977) and Emerick (1976) reported that bovine colostrum containing supplemental sodium bicarbonate may improve performance of calves fed high concentrate diets pre- and post weaning.

The objectives of this experiment were 1) to determine if feeding anionic salts pre-partum affected the neonate's ability to absorb colostrum IgG and obtain passive transfer and 2) to determine if supplementing CR with sodium bicarbonate would improve the neonate's ability to absorb IgG and obtain passive transfer.

## **Materials and Methods**

### **Experimental Design and Treatment Diets**

This experiment was reviewed and approved by the University of New Hampshire Institutional Animal Care and Use Committee (Approval # 071007).

Forty multiparous Holstein close up dry cows and their resulting calves were used in this study. Calves were assigned to one of ten blocks. Blocking was done according to expected date of birth. Using a table of random numbers calves within each block were assigned one of four treatments: 1) dam's fed a prepartum ration with +77 DCAD, calves were fed CR without additional sodium bicarbonate **(C)**; 2) C and calves were fed CR with supplemental 19.5 g of supplemental sodium bicarbonate / dose of CR **(CNa)**; 3) Dams were fed a prepartum ration with -100 DCAD and calves were fed CR without supplemental sodium bicarbonate **(A)**; 4) A and calves were fed 19.5 g of supplemental sodium bicarbonate /dose of CR **(ANa)**. Cows started on the treatment diets 21 d prior to their expected calving date. Actual days on treatment averaged 18 d for prepartum cows.

Cows were housed in a pack barn bedded with sawdust and fed individually via Calan doors (American Calan, Northwood, NH). Cows were fed once daily at 12:00. The ingredient composition of the basal diet is presented in Table 3. The proportions of all feedstuffs were kept constant throughout the study. Dietary treatment levels of anionic salts were adjusted based on individual cow intakes to achieve the correct DCAD. SoyChlor™ (West Central Soy, Ralston, IA) and calcium sulfate were weighed out in the laboratory, top-dressed to the diet and manually

Ranger; American Calan, Inc. Northwood, NH). The basal diet was mixed using fresh feed before each feeding. Feed offered was adjusted daily to achieve 5 to 10 % orts. Within the first 15 min of feeding, the anionic salt treatment was top-dressed and hand mixed into the freshly delivered TMR. Orts were collected and weighed daily.

Corn silage and hay crop silage samples were taken daily and analyzed for DM. Composites were made weekly and frozen. Alfalfa hay was sampled via core-sampling upon delivery.

Frozen samples of forages, TMR and orts were dried in a forced-air convection oven at 60°C (VWR Scientific, NJ). The dried silages and hay samples were ground to pass a 1-mm screen using a Wiley Mill (Thomas Scientific, Swedesboro, NJ). Composites were made of each dried feed. Composites were sent to Analab (Fulton, IL) for wet chemistry. Samples were analyzed for DM, CP, NDF, ADF, Ca, P, Mg, K, Cl, and Na according to the AOAC methods (1999).

The DM of the CR and MR was determined by drying samples in a forced-air convection oven (VWR Scientific Inc., West Chester, PA) at 60°C for 24 h. Samples from CR and MR were collected from each bag and stored at -20°C. After the completion of the experiment, samples were composited and sent to Agri-King (Fulton, IL) for nutrient analysis. Samples

were analyzed for CP, fat, lactose, Ca, P, Mg, K, Na and Fe according to the AOAC methods (1999).

### **Metabolic Disorders**

All cows were monitored for sign of parturient paresis and retained placenta for 15 d postpartum.

### **Blood Collection for Immunoglobulin G**

Blood samples were collected via jugular venipuncture before the first feeding of CR (within 90 min of birth, referred to as 0 h) and at 6, 12, 24, and 48 h after birth. Samples were collected in 5-mL tubes. Samples were allowed to clot at room temperature for at least 3 h and then centrifuged (CentraMP4R; International Equipment Company; Needham HTS, MA) at 3300 x g at 25°C for 20 min. Serum samples were stored at -20°C until analyzed for IgG by radial immunoassay (Cardiotech Services, INC; Louisville, KY).

Apparent efficiency of IgG absorption (**AEA**) at 24 h of age was estimated using the equation:  $(\text{plasma IgG [g/L]} \times \text{body weight [kg]} \times 0.09 / \text{IgG intake}) \times 100\%$  (Quigley et al., 1998). The IgG concentration at 6, 12, 24 and 48 h and area under the curve (**AUC**) were analyzed.

### **Urine Collection for pH**

Urine samples of all prepartum cows were collected Monday, Wednesday and Friday at 11:00 from mid stream urine. Samples were

analyzed within 60 min using an Orion pH meter (Orion Research; Boston, MA).

### **Statistical Analysis**

Daily DMI data was analyzed as a randomized block design using the repeated measures determined in the MIXED procedure of SAS (SAS® Institute, 2001) according to the following model:

$$Y = \mu + B_i + A_k + D_m + L_n + AD_{km} + AL_{kn} + ADL_{kmn} + e_{ijk}$$

Where:

Y = the dependent variable,

$\mu$  = the overall mean,

$B_i$  = the random effect of block  $i$  ( $i = 1, \dots, 10$ ),

$A_k$  = the fixed effect of the  $k^{\text{th}}$  anionic salt level ( $k = +77, -100$ ),

$D_m$  = the fixed effect of the  $m^{\text{th}}$  day ( $m = 0, \dots, -21$ ),

$L_n$  = the fixed effect of  $n^{\text{th}}$  lactation ( $n = 2, 3$ ),

$AD_{km}$  = the fixed interaction between the  $k^{\text{th}}$  anionic salt level, and the  $m^{\text{th}}$  day,

$AL_{kn}$  = the fixed effect interaction between the  $k^{\text{th}}$  anionic salt level and the  $n^{\text{th}}$  lactation,

$ADL_{kmn}$  = the fixed interaction between the  $k^{\text{th}}$  anionic salt level, and the  $m^{\text{th}}$  day and the  $n^{\text{th}}$  lactation,

$e_{ikmn}$  = the residual error.

In this model, the random effect of cow within block subclass is used as the error term for the effect of treatments. The residual errors are errors within cow across time and represent errors from repeated measurements in the experimental units (cows) was modeled using a first-order autoregressive covariance structure. First order autoregressive resulted in

the smallest Bayesian information criteria of the three covariate structures tested: compound symmetry, unstructured and first autoregressive. (Littell et al., 1996). Degrees of freedom were calculated using the Satherwaite option of MIXED procedure (SAS, 2001). Least square means were determined for treatment. Significant treatment effects were noted at  $P \leq 0.05$ . The PDIFF option in SAS® was used to test treatment differences among least square means.

Urine pH were transformed to H<sup>+</sup> ion concentration and reduced to weekly averages prior to statistical analysis. Weekly averages of H<sup>+</sup> ion concentration was analyzed as a randomized block design. using the repeated measures determined in the MIXED procedure of SAS (SAS® Institute, 2001) according to the following model:

$$Y_{ijk} = \mu + b_i + A_k + W_l + AW_{kl} + e_{ikl}$$

Where:

Y = the dependent variable,

$\mu$  = the overall mean,

$b_i$  = the random effect of block i (i = 1,...10),

$A_k$  = the fixed effect of the k<sup>th</sup> anionic salt level (j = +100, -100),

$W_l$  = the fixed effect of the l<sup>th</sup> week (k = 0,... -3),

$AW_{kl}$  = the fixed effect of the interaction between the k<sup>th</sup> anionic salt level and the l<sup>th</sup> week,

$e_{ikl}$  = the residual error.

In this model, the random effect of cow within block subclass is used as the error term for the effect of treatments. The residual errors are errors

within cow across time and represent errors from repeated measurements in the experimental units (cows) was modeled using a first-order autoregressive covariance structure. First order autoregressive resulted in the smallest Bayesian information criteria of the three covariate structures tested: compound symmetry, unstructured and first autoregressive. (Littell et al., 1996). Degrees of freedom were calculated using the Satherwaite option of MIXED procedure (SAS, 2001). Least square means were determined for treatment. Significant treatment effects were noted at  $P \leq 0.05$ . The PDIFF option in SAS® was used to test treatment differences among least square means.

The FREQ procedure with the CHISQ option of SAS 9.1® (SAS Institute, 2001) was used to determine the relationship between anionic salts prepartum and the incidence of milk fever and retained placenta.

Calf birth weight was analyzed as a randomized block design using the MIXED procedure of SAS® (SAS Institute, 2001) according to the following model:

$$Y = \mu + B_i + A_k + G_o + e_{iko}$$

Where:

Y = the dependent variable,

$\mu$  = the overall mean,

$B_i$  = the random effect of block I ( $I = 1, \dots, 10$ ),

$A_k$  = the fixed effect of the  $k^{\text{th}}$  anionic salt level ( $k = +77, -100$ )

$G_o$  = the fixed effect of the  $o^{\text{th}}$  gender ( $o = \text{male, female}$ ),

$e_{iko}$  = the residual error.

Degrees of freedom were calculated using the Satterwaite option of the MIXED procedure of SAS® (SAS Institute, 2001). Least square means were determined for treatment. Significant treatment effects were noted at  $P \leq 0.05$ .

Calf IgG, AEA and AUC data was analyzed as a 2 x 2 factorial design using the MIXED procedure of SAS® (SAS Institute, 2001) according to the following model:

$$Y_{ijk} = \mu + b_i + S_j + A_k + SA_{jk} + e_{ijk}$$

Where:

Y = the dependent variable,

$\mu$  = the overall mean,

$b_i$  = the random effect of block i (i = 1,...10),

$S_j$  = the fixed effect of the j<sup>th</sup> sodium bicarbonate level (j = 0, 9.75),

$A_k$  = the fixed effect of the k<sup>th</sup> anionic salt level (k = +77, -100),

$SA_{jk}$  = the fixed effect of the interaction between the j<sup>th</sup> sodium bicarbonate level and the k<sup>th</sup> anionic salt level,

$e_{ijk}$  = the residual error.

Degrees of freedom were calculated using the Satterwaite option of the MIXED procedure of SAS (SAS® Institute, 2001). Least square means were determined for treatment. Significant treatment effects were noted at  $P \leq 0.05$ . The PDIFF option in SAS® was used to test treatment differences among least square means.

## **Results**

Chemical analysis of feedstuffs fed to transition cows is presented in Table 4. Chemical analysis of CR and MR are presented in Table 6.

Dry matter intake for pre-partum cows was lower ( $P \leq 0.05$ ) on days 8, 5, 4, and 1 prior to parturition for cows receiving anionic salts as compared to cows receiving only the basal diet (Figure 1). Mean dry matter intake for pre-partum cows was decreased ( $P < 0.01$ ) by the addition of anionic salts. Dry matter intake averaged 12.3 kg for cows not receiving anionic salts and 11.4 kg for cows receiving anionic salts.

Monitoring urine pH has proven an effective means of assessing the effectiveness of anionic salts (Riond, 2001). Urine pH prior to the start of the study averaged 8.27 for cows that received the basal diet and 8.10 for cows that received the anionic salt treatment. As expected, urine pH was reduced ( $P \leq 0.05$ ) for cows receiving anionic salt 1 wk prior to parturition to a pH of 6.19 as compared to 7.72 for cows not receiving anionic salts (Figure 2).

Incidences of milk fever and retained placenta were not different between cows C and A (Table 5). Calving ease was not different Between C and A cows.

Body weight was not different among calves born from C and A cows (42.9 kg and 42.4 kg respectively). There was a trend ( $P \leq 0.10$ ) for

bull calves to have a higher body weight at birth as compared to heifer calves (44.4 kg and 40.9 kg, respectively).

At birth serum IgG values for all calves were 0 g/mL. There was no effect of feeding anionic salts to the pre-partum cows on serum IgG concentrations of the calves (Figure 3). Calves that received sodium bicarbonate in CR had higher serum IgG values at 24 h ( $P < 0.05$ ) as compared to calves that did not receive supplemental sodium bicarbonate in CR (Table 7). All but 3 of the 40 calves on study attained blood serum IgG concentration  $\geq 10$  mg/mL by 24 h, resulting in successful passive transfer. Area under the curve for IgG was not affected by the use of anionic salts in the prepartum diet. However, AUC was greater for the calves fed supplemental sodium bicarbonate in CR as compared to calves that did not receive supplemental sodium bicarbonate in CR ( $P < 0.05$ ).

There was no effect on AEA of IgG when anionic salts were fed prepartum. There was an increase ( $P < 0.05$ ) in AEA of IgG when supplemental sodium bicarbonate was added to CR compared to no supplemental sodium bicarbonate.

### **Discussion**

Dry matter intake decreased 2.1 % between 3 wk pre-partum to 2 wk pre-partum in cows receiving anionic salts and decreased an

additional 10 % the week prior to parturition. This reduction in DMI may represent a response to the metabolic acidosis that is induced by anionic salts (Vagnoni and Oetzel, 1998), or by the decreased palatability of the diet caused by the addition of anionic salts (Moore et al., 2002; Charbonneau, 2006). Cows that did not receive anionic salts had a 5.4 % increase in DMI between 3 wk pre-partum to 2 wk pre-partum, and a 0.4 % decrease in DMI the week prior to parturition. The increase in overall DMI of 5 % from the start of the pre-partum diet to parturition disagrees with previous research indicating a decrease in DMI prior to parturition (Hayirli et al., 2002).

The observed urine pH value for cows fed the anionic salts falls into the target pH range of 6.2 to 6.8 that is suggested to increase mobilization of bone Ca (Riond, 2001). The results obtained in this study support the use of measuring urine pH to monitor preparturient cows that are fed reduced DCAD diets (Goff et al, 2004).

Metabolic problems that were observed in this study do not follow the trend that is often observed with prepartum feeding of anionic salts. Seven of the 40 cows on the study were treated for parturient paresis after parturition. Two of the cows received no anionic salts, while 5 did receive anionic salts. This differs from previous research in which prepartum cows fed anionic salts had no incidences of parturient paresis (Block, 1984).

However, of the cows receiving anionic salts that developed milk fever all but one was in her 4<sup>th</sup> or greater lactation.

Five of the forty cows on the study had retained placentas. Four of these cows' received anionic salts prepartum and one did not receive anionic salts prepartum. Two of the four cows that received anionic salts prepartum that had retained placentas were also treated for milk fever. Three of the four cows that had retained placenta and received anionic salts had a calving ease score of three. The one cow that was not receiving anionic salts and had a retained placenta had a calving ease score of one.

Body weight of calves was not different from cows receiving anionic salts prepartum as compared to cows receiving no anionic salts. When sex of the calf was statistically analyzed a trend ( $P \leq 0.10$ ) was observed, with bull calves weighing 8.25% more than the heifer calves.

Serum IgG concentrations at 24 h from calves receiving sodium bicarbonate were higher ( $P < 0.05$ ) for calves receiving supplemental sodium bicarbonate, as compared to their counterparts who did not receive supplementation. These results differ from Ayers and Besser (1992) who reported that absorption of colostrum IgG was not affected by sodium bicarbonate treatment (3 mEq/kg of BW) when administered intravenously. Earlier research showed that addition of sodium

bicarbonate to human milk and bovine colostrum inhibited the growth of *E. coli* 0111 (Griffiths and Humphreys, 1977). James et al. (1985) reported that uptake of globulin proteins across the gut was reduced when bacteria were present. Corley et al. (1977) suggested that nonspecific pinocytosis of bacteria could block absorption of Ig molecules. Therefore, it is possible that IgG uptake was increased with the addition of sodium bicarbonate in the CR because *E.Coli* was reduced, and IgG absorption was not reduced.

Serum IgG concentrations for calves on treatment A reached peak IgG levels (17.61 mg/mL) at 24 h, whereas calves from treatments C, CNa and ANa reached peak levels (19.3, 20.0 and 21.7 mg/mL) at 12 h. This suggests that addition of sodium bicarbonate to CR may inhibit on maturation of the gut wall, and delay gut closure.

The calves that did not obtain blood serum IgG concentration  $\geq 10$  mg/mL by 24 h were all bull calves, and were on treatments C, CNa, and A. All calves on treatment ANa obtained passive transfer. The three calves that did not obtain passive transfer all had a calving ease score of one, whereas all dystocia calves (n = 11) achieved passive transfer. This differs from previous research that indicates that dystocia calves are less likely to obtain passive transfer (Davis and Drakley, 1998; Donovan et al., 1986; Odde, 1988). When calves with a calving ease score of 1 and 2 were

removed from data analysis, sodium bicarbonate increased IgG absorption ( $P \leq 0.03$ ). This suggests that dystocia calves may benefit from sodium bicarbonate supplementation; however, these data were collected from only 6 of the 40 calves.

Apparent efficiency of IgG absorption at 24 h was greater ( $P \leq 0.05$ ) for calves receiving supplemental sodium bicarbonate (40.5 %) as compared to calves not receiving sodium bicarbonate (37.8 %), indicating that sodium bicarbonate in CR is beneficial in the absorption of colostral IgG. Area under the curve for IgG was greater for the calves fed supplemental sodium bicarbonate in CR as compared to calves that did not receive supplemental sodium bicarbonate ( $P < 0.05$ ). This further supports the idea that supplemental sodium bicarbonate in CR increases the ability of the calf to absorb IgG.

### **Conclusion**

The feeding of anionic salts pre-partum did not have an effect on IgG absorption in the neonate. The addition of sodium bicarbonate to the colostrum replacer increased IgG absorption and AEA at 24 h. The addition of sodium bicarbonate to the CR also allowed all dystocia calves to obtain passive transfer. Future research in this area should follow calves through the weaning process to determine if the addition of sodium

bicarbonate to CR has had any effects on health and growth parameters.

**Table 3:** Ingredient and chemical composition of prepartum diets.

Item	Control	Anionic
Dietary ingredients, % of dietary DM		
Corn silage	59.3	55.4
Grass haylage	4.0	3.7
Alfalfa hay	17.5	16.0
Straw	2.1	1.8
Calcium carbonate	0.3	0.2
Molasses	0.4	0.3
Soy 48	2.2	1.9
Soy hulls	6.0	5.5
Corn meal	1.3	1.2
Steam flaked corn	1.0	0.9
Ground beet pulp	4.6	3.8
Mineral mix	1.3	1.2
SoyChlor	---	7.6
Calcium sulfate	---	0.5
Calculated nutrient content, % dietary DM		
DM, %		
CP, %	11.1	12.0
ADF, %	32.1	29.7
NDF, %	48.1	45.5
Ca, %	1.2	1.2
P, %	0.2	0.3
Mg, %	0.4	0.3
K, %	1.4	1.3
Cl, %	0.5	0.9
Na, %	0.3	0.1
DCAD	77.0	-100.0

**Table 4:** Chemical composition of feedstuffs in prepartum diet.

Item	%DM	CP	ADF	NDF	Ca	P	Mg	K	Cl	Na
Corn silage	24.8	7.2	28.8	46.3	0.3	0.2	0.2	1.0	0.3	0.0
Haylage	22.3	15.6	41.4	54.3	0.9	0.3	0.2	2.4	0.3	0.0
Alfalfa hay	88.5	21.6	28.8	35.6	1.4	0.3	0.3	2.4	0.3	0.0
Soy 48	89.1	52.0	4.8	7.2	0.3	0.7	0.3	2.1	0.0	0.0
Steam flaked corn	85.3	8.7	3.3	7.8	0.0	0.2	0.1	0.3	0.1	0.0
Soy hulls	90.8	10.7	51.0	73.2	0.6	0.1	0.0	1.2	0.1	0.1
Straw	93.1	4.7	57.6	83.1	0.4	0.1	0.1	0.9	0.2	0.1
Molasses	74.3	5.8	0.2	0.2	0.2	0.0	0.3	6.1	0.0	1.5
Corn meal	88.1	9.1	3.4	9.5	0.0	0.3	0.1	0.4	0.1	0.0
Calcium carbonate	100.0	---	---	---	8.0	0.0	2.1	0.1	0.0	0.1
Ground beet pulp	88.3	10.0	23.1	45.8	0.9	0.1	0.2	1.0	0.2	0.3
Soychlor™ <sup>1</sup>	85.0	26.4	13.5	19.9	4.7	0.4	2.8	0.5	9.8	0.1

<sup>1</sup> Manufactured by West Central Soy, Ralston, IA.

**Table 5.** Incidence of metabolic disorders in post partum cows.

	Treatment <sup>1</sup>				CHISQ
	A		C		
	Incidence <sup>2</sup>				
	Y	N	Y	N	
Milk Fever (%)	12.5	37.5	5	45	0.212
Retained Placenta (%)	10	40	2.5	47.5	0.151

<sup>1</sup> A = anionic salts in prepartum diet, C = no anionic salts in the prepartum diet.

<sup>2</sup>Y= metabolic disorder, N = no metabolic disorder

**Table 6:** Nutrient analysis on a DM basis of colostrum replacer and milk replacer.

Item	CR <sup>1</sup>	MR <sup>2</sup>
DM, %		
CP, %	57.33	20.48
Fat, %	20.60	19.93
Lactose, %	9.59	38.14
Ca, %	0.81	1.02
P, %	0.74	0.62
K, %	0.61	1.63
Mg, %	0.13	0.13
Na, %	0.25	0.61
Fe, ppm	6.00	111.00
Zn, pp,	69.00	0.00
IgG (g/dose)	66.00	---

<sup>1</sup> Alta Gold Colostrum Replacer. Saskatoon Colostrum. Saskatoon, Canada.

<sup>2</sup> Milk Formula, Non-medicated. Blue Seal Feeds. Londonderry, NH.

**Table 7:** Overall means of IgG, area under the curve for IgG, and apparent efficiency of absorption.

	Treatments <sup>1</sup>				Contrasts			
	C	CNa	A	ANa	SE <sup>2</sup>	SB <sup>3</sup>	Anionic	Anionic*SB
Initial BW	43.5	42.29	42.54	42.31	1.34	---	0.69	---
Calving ease <sup>4</sup>	1.87	1.64	1.61	1.83	0.3	---	0.90	---
Serum, IgG g/L	15.7	20.0	17.6	21.6	2.0	0.04	0.36	0.93
24-h								
Range g/L	6.1 - 29.0	5.5 - 30.6	8.0 - 29.8	17.2 - 29.4				
AUC IgG (g/L*h)	697.6	745.3	625.8	879.78	73.9	0.04	0.67	0.17
AEA <sup>5</sup> , %	31.1	39.2	33.9	41.8	3.6	0.03	0.45	0.98

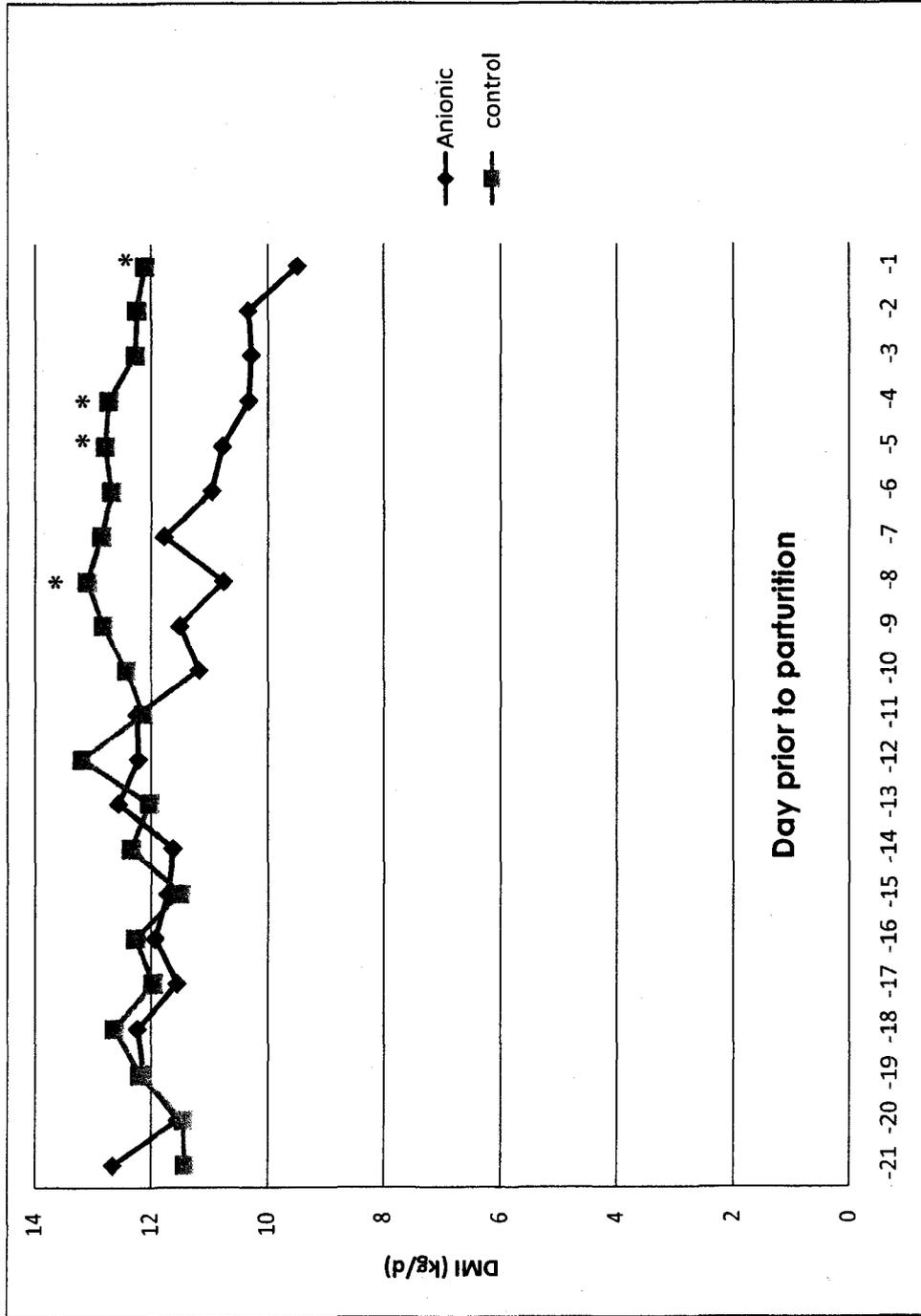
<sup>1</sup>Calves were born from cows that received a diet either with Anionic Salts or without Anionic Salts prior to parturition.

<sup>2</sup>SE = standard error.

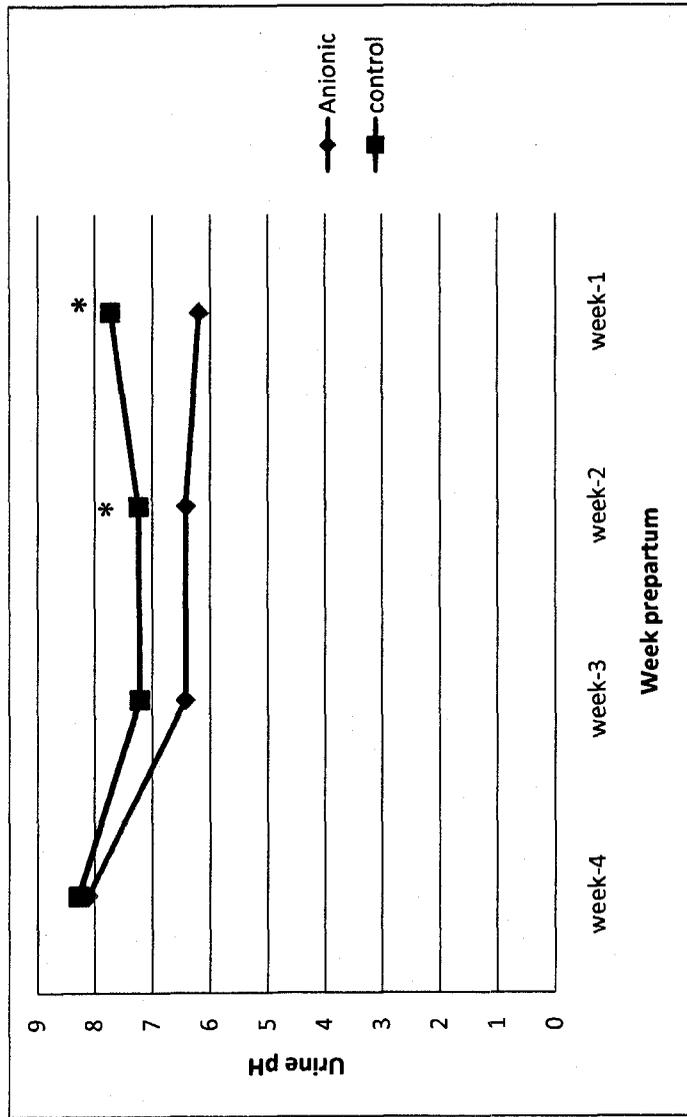
<sup>3</sup>SB = Sodium Bicarbonate

<sup>4</sup>Calving ease score 1 = unassisted delivery, 2 = assisted easy calving, 3 = assisted difficult calving.

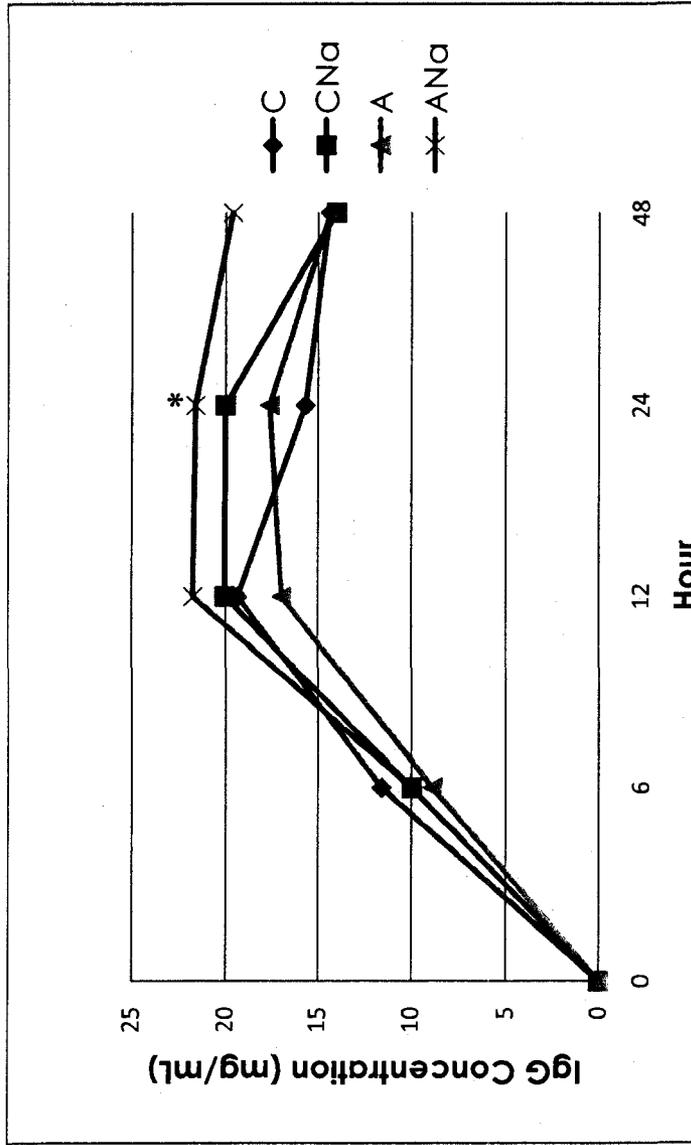
<sup>5</sup> AEA was calculated using the equation: (plasma IgG[g/L] x body weight [kg] x 0.09 / IgG intake x 100%)



**Figure 1 :** Dry matter intake (kg/day) of cows being fed either the basal diet or the basal diet + anionic salts. \*( $P < 0.05$ ). Cows fed the basal diet without anionic salts had greater DMI on 8, 5, 4 and 1 day pre-partum.



**Figure 2:** Urine pH of cows either receiving the no anionic salts in the prefresh diet or anionic salts in the prefresh diet. The largest SEM was 0.18 and occurred in all cows on both treatments for all time points. \* ( $P < 0.05$ ). Cows receiving basal diet + anionic salts had lower urine pH values 1 and 2 weeks prior to parturition.



**Figure 3:** Serum IgG concentrations of calves born from cows either receiving the basal diet or the basal diet + anionic salts with or without sodium bicarbonate added to the colostrum replacer. C = No anionic salts in pre-fresh diet, no supplemental sodium bicarbonate in CR, CNa = Treatment C and supplemental sodium bicarbonate in CR, A = anionic salts in pre-fresh diet and no supplemental sodium bicarbonate in CR, ANa = Treatment A and supplemental sodium bicarbonate in CR. The largest SEM was 2.5 and occurred in calves on treatment A at 12 h. \* ( $P < 0.05$ ) Calves receiving supplemental sodium bicarbonate had greater plasma IgG concentrations at 24 h.

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## **APPENDICES**

## APPENDIX A

# University of New Hampshire

Research Conduct and Compliance Services, Office of Sponsored Research  
Service Building, 51 College Road, Durham, NH 03824-3585  
Fax: 603-862-3564

29-Oct-2007

Erickson, Peter  
Animal & Nutritional Sciences  
Dairy Nutrition Research Center  
30 O'Kane Road  
Durham, NH 03824

**IACUC #:** 071007

**Project:** Anionic Salts and Their Effect on IgG Absorption in the Neonate

**Category:** B

**Approval Date:** 19-Oct-2007

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category B on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *the study involves either no pain or potentially involves momentary, slight pain, discomfort or stress.*

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

**Please Note:**

1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Roger Wells at 862-2726 or Julie Simpson at 862-2003.

For the IACUC,

  
Jessica A. Bolker, Ph.D.  
Chair

cc: File  
Whitehouse, Nancy

incorporated into the total mixed ration (**TMR**) at each feeding. Cows were moved to an individual maternity pen 1 day prior to the expected due date, or upon showing signs of parturition.

Calves were removed from their dam immediately after birth, weighed and placed in a naturally ventilated, enclosed calf room, in individual pens. Pens were bedded with kiln-dried sawdust. The calves remained in their pens for the duration of the study (48 h). Calves were assigned a dystocia score of 1 to 3 based on difficulty of calving; 1= unassisted delivery, 2 = assisted easy calving, 3 = assisted difficult calving. All calves received one dose (132 g of IgG) of Calf's Choice Total™ Gold (Saskatoon Colostrum Company; Saskatoon, SK Canada) CR at 0 h. Sodium bicarbonate was added (19.5 g) to the CR for the CNa and ANa calves to increase the pH from 6.0 to 7.0. A half dose (66 g IgG) of Calf's Choice Total™ Gold (Saskatoon Colostrum Company; Saskatoon, SK Canada) CR was fed at 6 h with or without sodium bicarbonate to alter the pH from 6.0 to 7.0. Two hundred and fifty four grams of milk replacer (**MR**) was reconstituted with 2 L of water and fed to the calves at 12, 24, 36 and 48 h.

### **Feed intake and Analysis**

The prepartum diet was fed to the cows as a TMR. It was prepared by weighing each ingredient and mixing in a mobile drum mixer (Data