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**ADDITION OF SODIUM BICARBONATE TO COLOSTRUM: EFFECTS ON IgG
ABSORPTION AND HEMATOCRIT IN NEONATAL CALVES**

BY

Colleen E. Chapman

B. S. University of Rhode Island, May 2009

THESIS

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Master of Science

in

Animal Science

September, 2011

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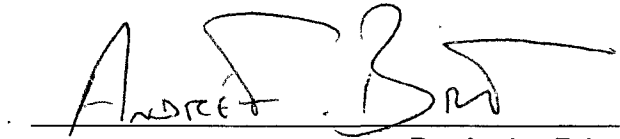


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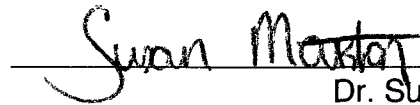
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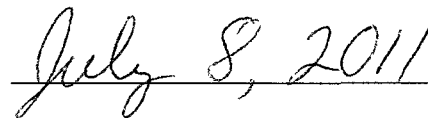
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ACKNOWLEDGEMENTS

This would not have been possible if it were not for the help and support from so many people.

First, I would like to thank the Department of Biological Sciences and the University of New Hampshire for giving me the opportunity to continue my education in animal science and providing me with financial support so that I could conduct research in the area of dairy science and gain valuable teaching experience.

I would also like to thank the George Walker Milk Fund for providing me with financial support as well. Without their help, this project would not have been possible.

Thank you to my committee members for serving on my committee and helping me through this journey.

Dr. Peter Erickson: Thank you for believing in me and taking me on as your graduate student. You have helped me so much through these past two years at UNH and I could not have done this without you. You were always there to listen to me and give me encouraging words when things got a little crazy. I learned so much from you and you really are an awesome professor and advisor! I will miss you!

Dr. Andre Brito: Thank you for being on my committee. You have helped me become a better scientific writer and I have learned a great deal from you in these past 2 years.

Dr. Susan Marston: Thank you for guiding and supporting me throughout this experience and giving me hope that it is all worth it in the end!

I also want to thank Nancy Whitehouse for taking time to teach me and answer all my questions on statistics as well as being there for me when I needed help taking blood samples. Also, thanks for being patient with me when I had no idea how to use and understand SAS.

I want to give a big thanks to Jon Whitehouse, John Weeks, Roger Comeau, and Sandy Boyle as well as the rest of the barn crew for calling me at all hours of the day and night when a bull calf was born and for helping me move the huge bull calves. I still need to work on my upper body strength...A special thanks to Jon for letting me take some of his colostrum and cheering me up when I was stressed or exhausted from little sleep.

I could not have gotten through this experience without the help of the other DNRC graduate students: Scott, Shara, Meagan, and Rosie. Thanks for always listening to me and being there for me when things got challenging. You were always there to calm my worries and cheer me up. And Rosie: you are one of my best friends and I couldn't have gotten through this without you. Thanks for helping me tube my first calf at midnight and driving me to school during a snowstorm because we both knew I wouldn't have made it there with grimace. 😊 And of course, thanks for being a shoulder to cry on and celebrating my achievements with me. I'm going to miss you!

Finally, I want to thank my family and friend's. You were always there to reassure and encourage me throughout this whole process. Although at times it was difficult, you never let me give up and I couldn't have done it without your love and support!

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LIST OF ABBREVIATIONS

CR = colostrum replacer

MR = milk replacer

AEA = apparent efficiency of absorption

AUC = area under the curve

Ig = immunoglobulin

IgG = immunoglobulin G

IgM = immunoglobulin M

IgA = immunoglobulin A

E. coli = Escherichia coli

ABSTRACT

ADDITION OF SODIUM BICARBONATE TO COLOSTRUM: EFFECTS ON IgG ABSORPTION AND HEMATOCRIT IN NEONATAL CALVES

by

Colleen E. Chapman

University of New Hampshire, September 2011

Twenty-six Holstein bull calves born from primiparous and multiparous cows without dystocia were assigned to a randomized complete block design to one of two treatments within each block: colostrum with no supplemental sodium bicarbonate or colostrum with 30 g of supplemental sodium bicarbonate. Calves were fed colostrum from nine different batches with an immunoglobulin G (IgG) concentration of 82.05 ± 8.45 g/L and the total amount of IgG fed of 329.89 ± 34.56 g at 0 h and 6 h postpartum. Calves were fed 2 L of milk replacer (MR) at 24, 36, and 48 h postpartum. The addition of sodium bicarbonate had no effect on IgG absorption. Serum IgG concentrations at 0, 6, 12, 24, and 48 h postpartum were not different between calves supplemented with or without 30 g of sodium bicarbonate to colostrum. Area under the curve (AUC) for IgG was also not affected by the sodium bicarbonate treatment and apparent efficiency of

absorption (AEA) was not improved by 30 g of supplemental sodium bicarbonate.

(Key words: Calves, colostrum, sodium bicarbonate, IgG)

CHAPTER I

REVIEW OF LITERATURE

Introduction

Successful calf and heifer management starts with the first feeding of colostrum. Due to the synepitheliochorial placenta of the cow, the calf is born agammaglobulinemic because there is negligible transfer of immunoglobulins (Ig) from the dam to the fetus prior to parturition. Therefore, the calf has an immature immune system with little protection against pathogens. High quality colostrum should be fed soon after birth as a source of nutrients and growth factors, to optimize Ig absorption, and decrease migration of pathogenic microorganisms across the intestinal epithelium to prevent mortality and morbidity.

Colostrum is the first mammary secretion that provides a highly concentrated source of Ig, especially IgG, which is the main contributor to passive immunity in the neonate (Butler, 1969). Colostrum also provides fat, lactose, vitamins, minerals, and water that are essential nutrients for calf health and development. After parturition, calves should receive 4 L of high quality colostrum within the first 24 h when the gut is open to the transport and absorption of large macromolecules such as Ig and other intact proteins. Maximum Ig absorption from colostrum occurs before 12 h after parturition with

the highest rates occurring before 6 h to provide passive immunity to the newborn calf (Shea et al., 2009). To achieve passive transfer, calves need a serum IgG concentration of ≥ 10 g/L at 24 h postpartum (NAHMS, 2007). Failure of passive transfer has been shown to result in reduced growth rates, increased risk of disease and death, increased risk of being culled, and decreased milk production in their first lactation (Smith and Foster, 2007). There are products available that can serve as colostrum replacements (CR) and colostrum supplements to boost the Ig content of poor quality colostrum when high quality colostrum is not available; however, they do not consistently provide successful passive transfer. Providing neonates with early and adequate intake of high quality colostrum is essential to their survival and future production.

Part I: Colostrum

Colostrum is the initial mammary secretion that provides the nutrients and Ig necessary to protect the neonate against pathogens and to enhance its growth and development. Providing early and adequate intake of high-quality colostrum is the most important factor that influences calf health and survival (Davis and Drackley, 1998). The composition of colostrum varies in comparison to milk and is the first concentrated source of nutrients such as protein, especially Ig, fat, lactose, water, vitamins, and minerals for the calf after parturition. The colostrum components provide a complete diet for the neonate, support growth of the intestinal mucosa, enhance intestinal absorptive capacity, and stimulate digestive functions of the small intestine as well as establishing passive immunity in neonatal calves (Hammon and Blum, 2002).

During mammary development, colostrogenesis succeeds growth and differentiation of mammary ductular and alveolar tissue and precedes the preparturient onset of copious milk secretion (Barrington et al., 1999). Colostrogenesis is defined as the prepartum transfer of Ig from maternal circulation into mammary secretions (Barrington et al., 2001). The transfer of Ig is initiated during the last weeks of gestation and quickly declines prior to parturition (Barrington et al., 1999). Several hundred grams to over 3 kg of Ig are transferred from blood circulation into the mammary gland during this time (Larson et al., 1980). It is a distinct, physiological, and functional stage of mammary gland development that is prominently different from the gland's primary role of milk

production (Barrington et al., 2001). The high concentration of Ig, specifically IgG₁, is the primary difference between colostrum and milk.

Relatively little is known about the mechanisms and regulations of colostrogenesis compared to other stages of mammary gland development. The relationship between lactogenesis and IgG₁ transport suggests that the hormones, which initiate lactation such as prolactin, the principal lactogenic hormone, may also suppress colostrogenesis (Barrington et al., 1999). Guidry et al. (1980) showed a variation in colostrum components and concentrations among individual quarters on the same udder, which show that local mechanisms can also affect colostrogenesis.

The origin of Ig found in mammary secretions are both humoral, arising from the blood stream and then transferred to lacteal secretions, and local, arising from production by plasmacytes, which are located adjacent to the secretory epithelium (Larson et al., 1980). The 3 types of Ig present in bovine colostrum are IgG, IgM, and IgA. These colostrum Ig are a concentrated source of serum antibodies that are essential for the neonate to gain sufficient immunity to be able to survive until their own immune system is fully developed (Larson et al., 1980).

The transfer of IgG from blood circulation across the mammary barrier into colostrum occurs via a highly specific transport mechanism within the secretory alveolar cells. The secretory alveolar cells have specific and different binding sites on their surface for both subclasses of IgG: IgG₁ and IgG₂ (Larson, 1979).

The colostral IgG are first bound to the surface receptors that are concentrated to certain areas on the basal membrane of the cell surface, which induces a pinocytotic engulfment of the area into the cell-forming vesicles lined with the cell's basal membrane (Larson, 1979). These vesicles then move through the cell to discharge their contents at the apical membrane into the luminal secretions (Larson, 1979).

Even though IgG makes up the largest portion of Ig present in bovine colostrum, IgM and IgA are also present in smaller quantities. These other 2 types of Ig are derived primarily from local synthesis by plasmacytes; however, in some instances, small amounts may be derived from the blood (Larson, 1979).

Composition of Colostrum

Colostrum not only contains numerous Ig, which protect the neonate against disease, it is also a rich and concentrated source of nutrients, having nearly twice as much total solids as whole milk which is vital to the calf's survival (Table 1). The composition of colostrum is important in satisfying the nutritional requirements of neonatal dairy calves, particularly for nutrients that only minimally cross the placenta, such as the fat-soluble vitamins (Kehoe et al., 2007). Carbohydrates and fat are essential in providing energy and fuel for the newborn, while vitamins and minerals are important cofactors for enzymes and body maintenance (NRC, 2001).

Colostrum also contains physiologically active components such as leukocytes, cytokines, insulin-like growth factors, and hormones, which are

Table 1: Composition of Holstein colostrum and milk

Variable	Colostrum	Milk
Fat, %	6.7	3.6
Total Protein, %	14.0	3.2
Casein, %	4.8	2.5
Immunoglobulins, g/L	60.0	0.9
IgG, g/L	50.50	0.80
IgM, g/L	4.2	0.05
IgA, g/L	3.9	0.2
Lactose, %	2.7	4.9
Calcium, %	0.26	0.13
Zinc, mg/100mL	1.22	0.30
Iron, mg/100mL	0.2	0.05
Vitamin A, μ g/100mL	295	34
Vitamin E, μ g/fat	84	15
Vitamin B12, μ g/100mL	4.9	0.6
Folic Acid, μ g/100mL	0.8	0.2
Choline, mg/mL	0.7	0.13
Riboflavin, μ g/mL	4.83	1.47

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

important in support of growth, development, and immunocompetence of the intestine (Davis and Drackley, 1998). Due to stimulating gut development, colostrum seems to improve postnatal nutrient uptake and stimulates plasma concentrations of insulin and leptin to promote anabolic processes in neonatal calves (Hammon and Blum, 2002).

Protein

Colostrum is made up of 14% total protein compared to 3.2% in milk. The protein content of colostrum and milk varies widely depending on genetics, health conditions, and environmental factors such as nutrition, stage of lactation, age, breed, season, and geographical location (Marnila and Korhonen, 2002a).

Proteins are divided into 2 main classes: the casein, which include 4 different types, and the whey portion, which include β -lactoglobulin, α -lactalbumin, Ig, lactoferrin, and serum albumin as well as other minor whey proteins. The whey protein fractions remain soluble at a pH of 4.6 where the caseins become precipitated.

Protein is required for growth and muscle development as well as the enhancement of the neonate's immune system. Large amounts of amino acids are needed for the development of the digestive tract during the immediate post-natal period and most are provided by colostrum (Davis and Drackley, 1998).

The whey protein, α -lactalbumin, is an important early source of amino acids because of its low retention time in the abomasum and its high rate of intestinal digestion (Yvon et al., 1993). The whey protein, β -lactoglobulin, also has a short

retention time in the abomasum and a rapid degradation in the small intestine making it a major source of amino acids as well during the first 7 h after consumption of colostrum (Yvon et al., 1993). The whey protein, β -lactoglobulin, is found in colostrum in concentrations of an average of 14 g/L (Marnila and Korhonen, 2002b). Casein, however, is a delayed source of amino acids because of its coagulation in the abomasum but releases significant amounts of calcium and phosphorus when digested to become available to the neonate (Ng-Kwai-Hang, 2002).

Another whey protein found in colostrum, known as lactoferrin, acts as a selective antibiotic because it binds to iron and renders it unavailable to the bacterial population in the digestive tract. In bovine colostrum, the concentration of lactoferrin varies from 1 to 2 g/L. Lastly, the whey protein, serum albumin, is involved in the transport, metabolism, and distribution of ligands, maintains osmotic pressure of blood, and also protects the body against pathogens.

Immunoglobulins are part of the whey protein class in colostrum. They function in providing protection against microbial pathogens. The mechanisms provided by colostral Ig are: augmenting phagocytosis and cell-mediated cytotoxicity reactions by leucocytes, agglutination of bacteria, neutralization of microbes and activation of the complement system (Marnila and Korhonen, 2002b). They are able to be absorbed into the intestine intact due to the limited acid secretion and decreased proteolytic activity in the neonatal digestive tract, along with the low retention time of Ig in the abomasal coagulum (Davis and

Drackley, 1998). However, the Ig are susceptible to trypsin, but colostrum contains a trypsin inhibitor. Trypsin inhibitor is found in very high concentrations in the first milking colostrum, but then decreases quickly in subsequent milkings, which is parallel with changes in colostral Ig concentrations (Piñeiro et al., 1978). It is important that Ig are absorbed intact to provide the neonate with their initial immunity until their own immune system fully develops.

Immunoglobulins

Immunoglobulin is a term referring to a family of high molecular weight proteins that share common physio-chemical characteristics and antigenic determinants (Butler, 1969).

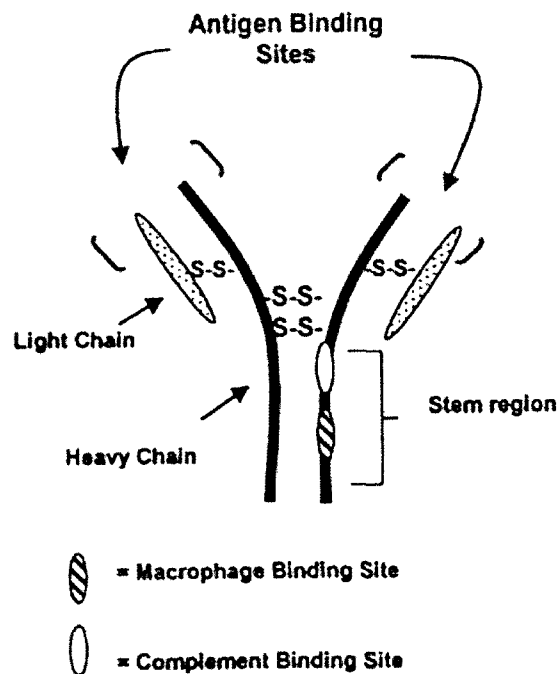


Figure 1. Structure of an immunoglobulin. (Adapted from Akers, 2002).

The 3 classes of Ig found in the bovine are IgG, IgM, and IgA and the basic structure of all are similar.

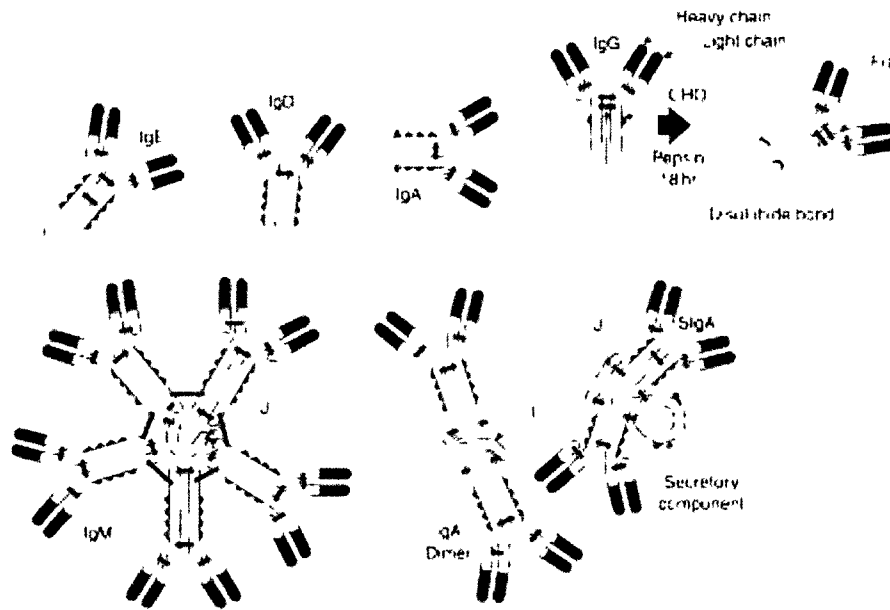


Figure 2. Structure of the five classes of immunoglobulins. (Marnila and Korhonen, 2002b)

Each Ig are monomers or polymers of 4-chain molecules consisting of two identical light polypeptide chains with a molecular mass of about 23 kDa and two identical heavy polypeptide chains, each with a molecular mass of 53 kDa (Marnila and Korhonen, 2002b). The complete Ig molecule has a molecular mass of about 160 kDa. The light chains are attached to the heavy chains by a disulfide bond and the two heavy chains are held together by disulfide bonds near a hinge region which gives the molecule the flexibility needed in antibody-antigen interactions (Marnila and Korhonen, 2002b). Immunoglobulins include all molecules with antibody activity along with chemically related normal or pathological proteins (Butler, 1969). The classification of Ig is primarily based on

their physio-chemical and antigenic properties as well as antibody specificity. The Ig are most effective in providing immune protection when all 3 are present in colostrum (Logan et al., 1974).

Immunoglobulin G

The principle Ig in the bovine is IgG and comprises 85-90% of the serum and whey Ig (Klaus et al., 1969). It is the main contributor to systemic immunity (Davis and Drackley, 1998). Immunoglobulin is further divided into IgG₁ and IgG₂, with IgG₁ accounting for 80-90% of the total IgG (Larson et al., 1980). The 2 subclasses of IgG differ antigenically and in amino acid composition (Butler, 1969). The IgG₁ class is selectively transported into lacteal secretions of the mammary gland from blood circulation and is the principle Ig for passive immunization of the calf (Butler, 1969). The IgG₂ class is found in high amounts in serum, but occur in lower concentrations in milk, colostrum, and saliva (Butler, 1969). There are no significant differences between the serum concentration of IgG₁ and IgG₂, but IgG₁ is the primary Ig of the lacteal and salivary secretions (Butler, 1969). Although IgG₁ is concentrated in the mammary gland, there is no selectivity between the IgG types in the calf's gut (Butler, 1969).

Immunoglobulin M

Immunoglobulin M is antigenically distinct and makes up less than 10% of the serum and colostrum Ig (Klaus et al., 1969). This Ig is essential for the primary immune response and complement fixation by enhancing the inflammatory response and stimulating direct destruction of bacteria, as well as being an

effective agglutinating antibody (Butler, 1969). Immunoglobulin M is an important contributor to early immunity as well as assisting in intestinal immunity against enteric pathogens (Logan et al., 1974).

Immunoglobulin A

Immunoglobulin A is found in the lowest concentrations of serum and colostral Ig (about 5%) and is locally synthesized in the mammary lymphoid and epithelial tissue (Butler, 1969; Klaus et al., 1969). However, in human milk and colostrum, IgA comprises about 90% (Marnila and Korhonen, 2002b). Because production of secretory IgA is less in bovine than in many other species, many of the functions of IgA in other species are assumed by IgM and IgG in the calf (Butler, 1969).

Fat

Newborn calves are born with relatively small energy reserves, with only 3% body fat compared to 16% body fat for human infants (Faulkner, 1983). However, most of the lipid content is structural as opposed to reserve fat, and is not able to contribute to the energy needs of the neonate. If adequate colostrum is not fed to the neonate immediately after birth, the 3% body lipid content will be depleted within 18 h after parturition (Okamoto et al., 1986).

Holstein colostrum contains 6.7% fat compared to only 3.6% fat in milk (Table 1). The intake energy content of colostrum is calculated by using kcal/g values for lactose, non-Ig protein, and milk fat. The calculated value of energy content for colostrum is 1.16 kcal/g, which is greater than the energy content of

0.69 kcal/g for milk (Davis and Drackley, 1998). However, the energy content of colostrum is not consistent and can vary greatly with the fat content. This increase in fat and energy content from colostrum is essential for thermogenesis and maintenance of body temperature (Davis and Drackley, 1998).

Carbohydrates: Lactose

Lactose also provides energy to the calf for thermogenesis and maintenance of body temperature (Davis and Drackley, 1998). However, colostrum only contains 2.7% of lactose compared to 4.9% in milk (Table 1). According to the osmoregulatory theory, the production of milk depends on the presence of lactose (Marnila and Korhonen, 2002a). The whey protein, α -lactalbumin, plays an important role in the biosynthesis of lactose by being the modifier in the lactose synthesis complex. The carbohydrate source is limited to lactose or simple sugars because of the inability of the calf to digest other disaccharides or starch at such an early age (Siddons et al., 1969).

Vitamins and Minerals

Colostrum, when compared to whole milk, is a concentrated source of both vitamins and minerals (Table 1) and is the major source of these nutrients for newborn calves. Fat-soluble vitamins, specifically vitamins A, D, and E, are important components in colostrum because they do not cross the placental barrier in significant amounts (Quigley and Drewry, 1998). Since colostrum is the primary source of these nutrients for the calf, supplementing the dam with these

fat-soluble vitamins will ensure that adequate amounts will be transferred into the lacteal secretions.

Other vitamins, such as folic acid, choline, and riboflavin, and the minerals Zn and Fe, are found to be higher in concentration in colostrum than whole milk (Akers, 2002). While most minerals are found in sufficient amounts in colostrum, concentrations depend on diet and parity of the dam, and can result in deficiencies in the calves if supplementation is not provided (Kehoe et al., 2007). Ensuring adequate amounts of vitamins and minerals are essential for growth and development of the calf's digestive system and initiation of its own metabolism (Davis and Drackley, 1998).

Water

Water is the most important nutrient and is essential for optimal growth, consumption of dry feed, and calf survival (NRC, 2001). It plays an important role in all chemical reactions of the body as well as being a solvent for nutrients, a natural lubricant, helps eliminate waste products, and is a thermoregulator to maintain homeostasis.

Diarrhea is the most common cause of calf death in the United States, and 10 - 12% of body weight can be lost as water along with losses of important electrolytes, such as sodium, chloride, and potassium, which provide ideal functioning of organs and cells (NRC, 2011). Dehydration is the cause of death, and not directly from the infectious agents. Free choice water should be provided to the calf along with colostrum and milk to prevent dehydration and calf death.

Factors Affecting Colostrum Quality

The concentration of Ig in maternal colostrum, more specifically the IgG content, is the factor that determines colostrum quality. High quality colostrum should contain at least 50 g/L of IgG (Godden, 2008). Finding the Ig content in colostrum is important in determining the amount of IgG the calf will consume and can potentially absorb to ensure passive transfer of immunity. Passive transfer is dependent on the IgG concentration of maternal colostrum (Nocek et al., 1984).

There is a great deal of variation in Ig content among cows. Pritchett et al. (1991) collected colostrum samples from 919 Holsteins and determined the mean IgG₁ content was 48.2 g/L, with a standard deviation of 21.9 g/L. Several factors can influence the nutrient composition and Ig concentration of maternal colostrum such as quantity, breed, parity, the environment, and management practices.

Quantity

The quantity of colostrum produced at the first milking will affect the IgG concentration and colostrum quality. The increased milk weight at first milking is negatively correlated with IgG₁ concentration (Pritchett et al., 1991). The increase in milk volume could most likely be diluting the IgG₁ accumulation in the mammary gland. Pritchett et al. (1991) observed a first milk volume of less than 8.5 kg for Holstein cows would be expected to increase the percentage of IgG₁ concentration in colostrum from 64 to 77% for second-lactation cows and 74 to 81% for third-lactation cows and can be used as a criterion to select better-quality

colostrum. Immunoglobulin G₁ is the Ig highest in concentration in bovine colostrum and the predominant Ig absorbed from the calf's intestine to systemic circulation during the first 24 h postpartum (Butler, 1969).

Breed

Another factor influencing colostrum quality is breed. Guy et al. (1994) used 15 beef (Charolais X Hereford) and 13 dairy (Holstein) cows to study the physiological basis of breed differences in IgG₁ concentration in colostrum. Results showed that beef cows have greater colostrum IgG₁ concentrations of 113.4 g/L compared to 42.7 g/L in dairy cows. Serum IgG₁ concentrations averaged 8.4 g/L in beef cows and 5.7 g/L in dairy cows between 28 d and 24 d prepartum and declined to 6.7 g/L in beef cows and 1.4 g/L in dairy cows between 4 d prepartum and calving. The greater decrease in serum IgG₁ concentration from dairy cows suggest that more IgG₁ was transferred into the mammary gland of dairy cows; however, the dairy cows had a lower colostrum IgG₁ concentration, which can be due to a dilution effect associated with greater lactogenic activity of dairy cows (Guy et al., 1994).

Colostrum Ig concentration also varies between individual dairy breeds. Muller and Ellinger (1981) compared total Ig content of colostrum from 5 breeds of dairy cattle and found total Ig concentration of colostrum was the lowest in Holstein cows (55.9 g/L). Results showed colostrum Ig concentration for Holstein cows was numerically lower than Guernsey (63.1 g/L) and Brown Swiss cows (65.7 g/L). The colostrum Ig concentration for Holstein cows was also lower than

Ayrshire (80.8 g/L) and Jersey cows (90.4 g/L) ($P < 0.05$). The differences of Ig concentration between breeds could be attributed to genetic and/or dilution effects.

Parity

Parity has been shown to affect colostrum quality in several studies (Devery-Pocius and Larson, 1983; Gulliksen et al., 2007; Kruse, 1970).

Colostrum from primiparturient heifers generally has a lower content of IgG than colostrum from older cows and colostral IgG content may be lower for second lactation cows compared with those with at least 4 parities (Devery-Pocius and Larson, 1983; Gulliksen et al., 2007; Kruse, 1970). Researchers observed that total IgG₁ reached peak concentrations in the third and fourth lactations, nearly doubling the total IgG₁ content in primiparturient heifers (Devery-Pocius and Larson, 1983). The reason for this could be due to the highly specialized transport system of IgG into the blood and may not be fully functional until later lactations.

Corbett (1991) observed the Ig concentration in colostrum from primiparturient heifers to be 28 g/L and the level increased to 59 g/L in second lactation, 82 g/L in third lactation, and 73 g/L in fourth lactation cows. The increasing IgG content with increasing parity could be due to the fact that primiparturient heifers have not been exposed to as many herd-specific pathogens, and therefore, the antibodies against those antigens are not present in the blood to be transferred into the colostrum.

Climate/Environment

Another factor that can have effects on colostrum composition is the environment and climate. When cows are exposed to temperatures outside their thermoneutral zone (10 - 20°C), many of their normal body functions are compromised. Respiration rate, intake, fluid consumption, and basic metabolic rate are all affected, which can have an impact on colostrogenesis and the efficacy of Ig transfer from circulation into the mammary gland. Heat stress can decrease dry matter intake and intake of needed nutrients is reduced because of increased metabolic demands (Akers, 2002). Providing the cows with sufficient ventilation and cooling systems, can help reduce the effects of the climate.

Nardone et al. (1997) observed that colostrum from primiparous heifers exposed to high ambient temperatures (31.5°C) had lower mean percentages of total protein (7.5%) compared to 9.0% total protein in primiparous heifers who were exposed to thermal comfort (26°C) ($P < 0.05$). Analysis of the colostrum protein fractions showed that heat stress reduced the concentration of colostrum casein, α -lactalbumin, IgG, and IgA; however, IgM and β -lactoglobulin were unaffected (Nardone et al., 1997). The decrease of colostrum IgG could be the affect of reduced blood flow to the mammary gland because of the increase in air temperature (Nardone et al., 1997). The researchers also suggest the concentration of colostrum IgA decreased because heat stress impaired the immune reactivity of the mammary gland plasmacytes that produce IgA.

Management Factors

Management factors can also affect colostrum quality and relate to the care and condition of the dam prepartum. A huge health concern is mastitis during the dry period, which can result in lower IgG concentrations. Mastitic infections can be prevented during the dry period by maintaining clean and dry pens to reduce the growth of pathogens. Proper management of the dry cow by the use of antibiotics at dry off and teat sealants will prevent bacteria and other pathogenic microorganisms from entering the mammary gland. These practices will decrease the occurrences of environmental mastitis and other diseases that could have a negative impact on colostral IgG content.

Prepartum milking of heifers and cows is becoming a common management practice for some dairies as a method to reduce udder edema. Although this technique will alleviate edema and be beneficial to the dam, it will reduce colostrum quality. The transfer of IgG into the mammary gland is almost complete in the few weeks leading up to parturition; therefore, the prepartum milking of cows or extensive leakage of colostrum from the mammary gland results in the loss of IgG content after parturition and decreasing the quality of colostrum (Kruse, 1970).

Other management factors that potentially affect colostrum composition and quality include diet, body condition score prior to calving, vaccination programs, and length of dry period. Prefresh cows that are fed restricted amounts of energy and crude protein have been shown to negatively affect colostrum

quality and may affect the transfer of passive immunity to calves (Quigley and Drewry, 1998). Establishing an appropriate vaccination program is also beneficially because it will ensure exposure of cows to antigens and result in production of specific IgG that is transferred into maternal colostrum from circulation. Dry periods that are too short (less than 3 weeks) can have negative affects on colostrum IgG because it does not allow for sufficient time for IgG to accumulate in the mammary gland (Dixon et al., 1961).

Recently, researchers have been studying the effects of pasteurization on colostrum quality. Elizondo-Salazar and Heinrichs (2009) evaluated the effects of feeding heat-treated colostrum or unheated colostrum of different bacterial counts on passive transfer of immunity in neonatal dairy calves. The researchers observed heat treatment of colostrum at 60°C for 30 min reduced colostrum bacteria concentration yet maintained colostrum IgG concentration and viscosity at similar levels to the control treatment. Calves fed heat-treated colostrum had significantly greater IgG concentrations at 24 h (26.7 g/L) and greater apparent efficiency of absorption (AEA) of IgG (43.9%) compared with calves fed unheated-low bacteria colostrum (IgG = 20.1 g/L; AEA = 32.4%).

The mechanism for this is unknown; however, Johnson et al. (2007) hypothesized that bacteria in colostrum may bind free IgG in the gut lumen or directly block uptake and transport of IgG molecules across intestinal epithelial cells, therefore, interfering with passive absorption of colostrum Ig.

Testing Colostrum Quality

Accurate determinations of Ig concentrations in colostrum require radial immunodiffusion assays; however, the use of colostrometers and refractometers are a fast and easy way to ensure quality maternal colostrum is being fed to the neonate. The colostrometer was developed by Fleenor and Scott (1980) and is a practical technique that allows for quick on-farm assessment of the Ig concentration in bovine colostrum. Using colostrum samples taken from 14 Holstein cows within 24 h postpartum, it was determined that there was a linear relationship between colostral specific gravity and Ig concentration, and no correlations between specific gravity and fat, non-protein nitrogen, or casein (Fleenor and Scott, 1980). The colostrometer evaluates colostrum quality as good (50 - 140 g/L), moderate (20 - 50 g/L), or poor (< 20 g/L). The colostrometer reading, however, is highly dependent on temperature of colostrum and should be tested under room temperatures around 20-25°C because measurements at temperatures lower than 20°C will overestimate colostral Ig content and readings taken at temperatures greater than 20°C will underestimate colostral Ig content (Davis and Drackley, 1998).

Brix refractometers are also being used to test colostrum quality. These measure the refractive index and the total solids in colostrum, which are highly correlated to the Ig content. It is easy to perform on the farm because only 2 drops of colostrum are needed. A brix reading of < 19.9% is considered poor, 20.0% - 21.9% is fair, and 22% or more is considered good quality colostrum.

Both are inexpensive (around \$40-80) and are valuable instruments that can be used to provide a convenient way to determine colostrum quality, thereby ensuring sufficient immune protection for the neonate.

Improving Colostrum Quality

Colostrum is crucial to the health and survival of the neonate; however, high quality colostrum is not always available. High quality colostrum is classified as having a minimum concentration of 50 g/L of IgG (Besser et al., 1985; Godden, 2008), but not all colostrum produced meets this criteria. In a study testing the quality of colostrum from 1,250 cows, researchers found 57.8% of the samples collected had less than 50 g/L of IgG with an IgG concentration range of 4 to 235 g/L (Gulliksen, et al., 2007).

There has been a lot of interest in products that can serve as CR and colostrum supplements to boost the Ig content of poor quality colostrum when fresh or frozen high quality colostrum is not available or if producers are trying to prevent the spread of diseases such as Johne's or leukosis. However, because colostrum from the dam of a calf provides antibodies that are specific for certain antigens found on that farm, it is difficult to develop a colostrum substitute that would provide protection against all pathogens on all farms. If only poor quality colostrum is available, a colostrum supplement may be added to colostrum to attempt to enhance colostrum quality and disease resistance.

Colostrum Supplements

Inadequate absorption of IgG from colostrum that leads to failure of passive transfer and/or the absence of high quality colostrum sufficient to meet minimum IgG intake of neonates has prompted the development of colostrum supplements. Colostrum supplements are aimed to provide additional IgG to the neonate during the time of macromolecular transport (Davenport et al., 2000). Currently, colostrum supplements are providing exogenous IgG from bovine lacteal secretions as well as bovine serum and eggs. These products are intended to provide < 100g of IgG/dose as well as other nutrients required by the calf and are not formulated to completely replace colostrum (Quigley et al., 2002). Colostrum supplements do not provide the necessary energy and vitamins or contain the amino acid profile of maternal colostrum; therefore, they are used in conjunction with maternal colostrum as a means of increasing the amount of IgG fed to the newborn calf (Quigley et al., 2002).

Santoro et al. (2004) used 48 Holstein bull calves and fed 2 L of high quality pooled colostrum (> 50 g/L of IgG) with or without trypsin inhibitor or fed one packet (454 g) of colostrum supplement (IgG = 10%) that was suspended in 2 L of warm water with or without trypsin inhibitor. The researchers found that calves fed colostrum supplement had lower serum IgG concentrations with a mean of 4.55 g/L at 24 h after birth than with calves fed maternal colostrum (14.6 g/L at 24 h after birth); however, the addition of trypsin inhibitor was not beneficial to the colostrum supplement or maternal colostrum. Apparent efficiency of

absorption was similar among treatments, which suggests that feeding more colostrum supplement could result in adequate serum IgG (Santoro et al., 2004).

A study conducted by Hopkins and Quigley (1997) used 52 Holstein bull and heifer calves to determine whether 3.8 L of colostrum in one feeding or divided into two equal feedings would influence serum IgG concentrations at 24 h of age and if the addition of 272 g (25g of IgG) of colostrum supplement (First Milk Formula; Land O' Lakes, Ft. Dodge, IA) to maternal colostrum affected serum IgG concentration and efficiency of IgG absorption. At 24 h postpartum, serum IgG concentrations were lowest for calves fed two colostrum feedings with supplement compared to two feedings without supplement; however, at 8 h postpartum, serum IgG concentrations did not differ among treatments. The researchers suggest that the lack of increase in serum IgG with colostrum supplement could have been due to the small amount of IgG provided by the supplement and the low efficiency of absorption that has been observed with colostrum supplements (Hopkins and Quigley, 1997). When high quality colostrum is fed, the addition of a colostrum supplement is unnecessary and the poor apparent efficiency of absorption from colostrum supplements may be due to the presence of excessive amounts of non-IgG protein that compete with macromolecular binding sites in the intestine (Davenport et al., 2000).

Lactoferrin has also been added to CR in a study conducted by Shea et al. (2009). Lactoferrin is an iron binding glycoprotein that is found in concentrations of 1 to 2 g/L in colostrum. It has been shown to be important for intestinal

development and the development of the immune system and inhibits bacterial growth in the small intestine. However, the researchers found that supplementing lactoferrin at 0, 0.5, or 1 g/d to calves fed CR had a negative effect on apparent efficiency of IgG absorption (Shea et al., 2009). The cause of this is unknown but researchers suggest that lactoferrin would have to be fed in conjunction with another component of maternal colostrum such as lysozyme or with another substance such as sodium bicarbonate. Results from these studies propose that absorption of IgG could be a more complex process than passive transfer and more research needs to be performed to have a better understanding.

Bovine serum products are used as inexpensive colostrum supplements aimed to increase IgG absorption in the newborn calf. The products are derived from bovine blood from slaughterhouses, which is a waste product that contains high levels of IgG.

Arthington et al. (2000) compared the absorptive efficiency of IgG of a commercial bovine serum product, bovine colostrum, and two commercial milk-derived IgG supplements. Plasma IgG concentrations at 24 h were 12.1 g/L for colostrum, 2.2 g/L and 3.5 g/L for the two milk-derived supplements, and 6.8 g/L for the bovine serum product. Although, plasma IgG concentrations were highest in calves fed colostrum, calves receiving bovine serum-derived IgG had greater efficiency of IgG absorption. This is in direct relation to the initial amount of IgG fed to the calves (90 g/L for bovine serum products vs. 200 g/L for colostrum). The results of this study indicate that in the absence of high quality colostrum,

serum-derived IgG may be used to supply calves with a concentrated source of IgG, which can be readily absorbed (Arthington et al., 2000). However, none of the supplements provided calves with adequate 24 h blood IgG concentrations of ≥ 10 g/L. This could be due to the profile of antibody specificity in a colostrum product is different from the antigenic reservoir on a dairy farm and indicates that these products need to be studied further to examine the influence of supplementing varying qualities of maternal colostrum with serum-derived IgG before being used as a commercial product to feed to newborn calves.

Although this study suggests that serum-derived IgG supplements are more effective than lacteal or egg-based supplements, they are currently banned in some countries where the use of animal proteins is prohibited. This is due to the concern of spreading and transferring diseases such as bovine spongiform encephalopathy not only from animal to animals, but animal to humans as well.

Colostrum Replacers

Colostrum provides newborn calves with antibodies to protect against disease before its own immune system can develop, but it can also be the source of exposure to different pathogens. The transfer of many diseases from dam to fetus can be passed through colostrum that include *Escherichia coli* (*E. coli*), leukosis, and Johne's disease. Early exposure of these disease-causing agents can be detrimental to the calves' health as well as cause financial problems for producers. For this reason, CR have been developed to provide the neonate with Ig for disease protection when high quality colostrum is unavailable or when

dams are known carriers of certain diseases that can transfer the pathogens through colostrum to the calf. Fulwider et al. (2008) observed 94.7% of the 90,162 dairy cows on 113 dairies in 5 states he surveyed, fed colostrum while the remaining 5.3% fed a CR due to incidence of Johne's disease.

Colostrum replacers should contain > 100g of IgG/dose, which is the minimum amount required to achieve a serum IgG concentration of ≥ 10 g/L at 24 h after birth and (Quigley et al., 2002). A CR also must provide nutrients required by the calf, which includes energy from carbohydrates and lipids that are needed for the calf to thermoregulate and establish homeostasis, protein as a source of amino acids for gluconeogenesis and protein synthesis, as well as vitamins and minerals (Quigley et al., 2002).

Studies have shown that CR has been found to be an acceptable alternative to maternal colostrum; however, absorption of IgG is lower in serum concentrations of calves fed the replacer compared to maternal colostrum (Smith and Foster, 2007). Swan et al. (2007) studied the effects of IgG absorption and preweaning health in Holstein calves fed maternal colostrum or a plasma-derived CR. Maternal colostrum had an IgG concentration of 76.7 ± 30.0 g/L and the CR contained 125 g of IgG. The mean serum IgG concentrations between days 1 and 8 were higher for calves that received maternal colostrum (14.8 ± 7.0 g/L) compared to calves that received CR (5.8 ± 3.2 g/L). One reason for this is the mass of IgG administered to the calf at first feeding. Calves fed maternal colostrum received an average dose of 291.5 g of IgG in 3.8 L at the first feeding,

however, the CR calves were fed 125 g of IgG in one dose or 170 g of IgG over 2 feedings.

Researchers have been studying the addition of sodium bicarbonate to colostrum replacer and its effect on absorption of IgG, but there have been dissimilar results (Cabral et al., 2011; Morrill et al., 2010; Table 2). Morrill et al. (2010) fed CR in 2 feedings with 132 g of IgG \pm 19.5g of sodium bicarbonate at the first feeding and 66g of IgG \pm 9.75g of sodium bicarbonate at the second feeding. The researchers observed higher serum IgG levels and higher AEA for calves receiving supplemental sodium bicarbonate in CR compared to those calves that did not (IgG 16.3 g/L vs. 13.2 g/L and AEA 31.2% and 26.1 respectively; $P < 0.05$; Figure 3). However, when Cabral et al. (2011) added varying amounts of sodium bicarbonate to CR (0 g, 15 g, 30 g, and 45 g), results showed that 45 g of sodium bicarbonate had a negatively linear trend ($P < 0.08$) on intestinal absorption of IgG, while 30 g of sodium bicarbonate had numerically higher serum IgG concentrations at 24 h after birth (Figure 4). Apparent efficiency of absorption of IgG at 24 h showed a negative linear effect ($P < 0.05$) when dietary levels of sodium bicarbonate were increased from 0 to 45 g (Cabral et al., 2011). More research in the work of sodium bicarbonate with CR as well as maternal colostrum needs to be conducted to determine how sodium bicarbonate works in the newborn calf.

Table 2. Comparing the results of Morrill et al. (2010) and Cabral et al. (2011)

	Morrill et al. (2010)				Cabral et al. (2011)			
	Treatments ¹							
Item	C	CNa	A	ANa	0	15	30	45
IgG fed	198 g				211.6 g or 216.8 g			
Serum IgG g/L 24 h	13	15	13	17	16.85	16.04	17.16	14.52
AUC ² , IgG g/L*h	562	575	534	688	679.92	622.15	681.99	561.72
AEA ³ , %	26.8	29.6	25.5	32.9	32.09	32.53	31.58	30.19

¹ Treatments: C = no anionic salts in prepartum diet, no supplemental sodium bicarbonate in CR; CNa = treatment C, supplemental sodium bicarbonate in CR; A = anionic salts in the prepartum diet, no supplemental sodium bicarbonate in CR; ANa = treatment A, supplemental sodium bicarbonate in CR; 0 = 0 g of sodium bicarbonate; 15 = 15 g of sodium bicarbonate; 30 = 30 g of sodium bicarbonate; 45 = 45 g of sodium bicarbonate.

² AUC = Area under the curve; $0.5 \times \text{difference in time} \times \text{difference in IgG concentration}$ (Phillips and Taylor, 1973).

³ AEA = Apparent efficiency of absorption; $(\text{plasma IgG [g/L]} \times \text{body weight [kg]} \times 0.092/\text{IgG intake}) \times 100\%$ (Quigley et al., 1998).

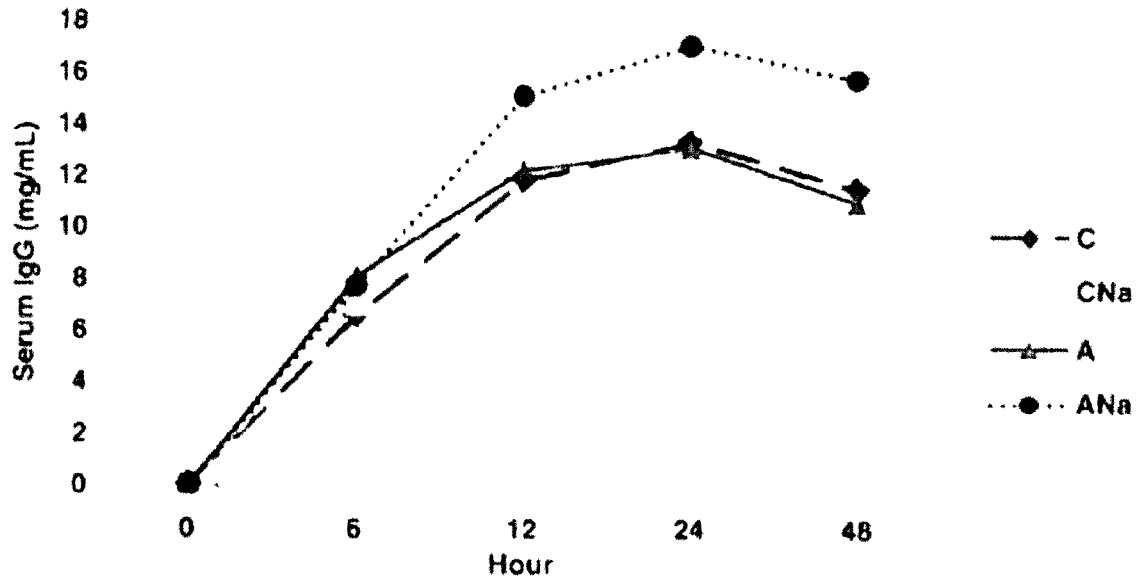


Figure 3. Serum IgG concentrations of calves born from cows fed either the control diet (no anionic salts) or the anionic diet with or without sodium bicarbonate added to colostrum replacer. C = no anionic salts in prepartum diet, no supplemental sodium bicarbonate in CR; CNa = treatment C, supplemental sodium bicarbonate in CR; A = anionic salts in the prepartum diet, no supplemental sodium bicarbonate in CR; ANa = treatment A, supplemental sodium bicarbonate in CR.

(From Morrill et al., 2010)

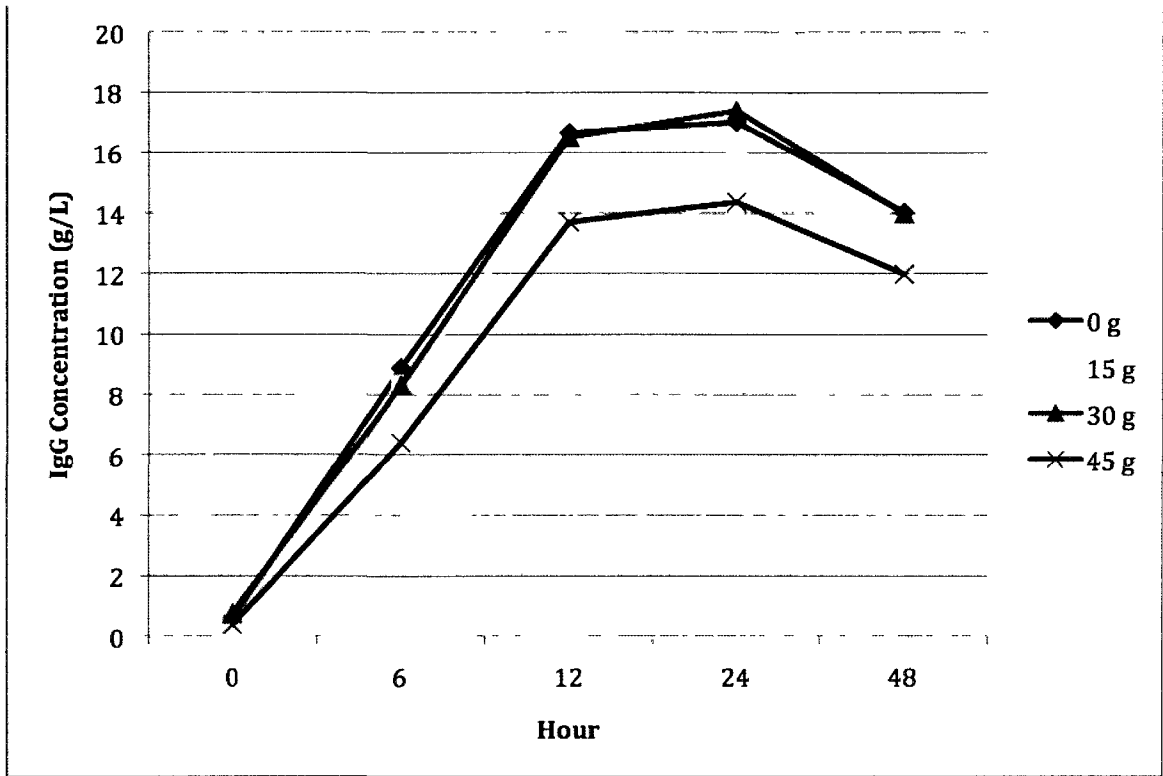


Figure 4. Serum IgG concentration for calves receiving 0, 15, 30, or 45g of sodium bicarbonate added to the colostrum replacer.

(From Cabral et al., 2011)

Part II: Maximizing Passive Transfer to the Neonate

Calves are born agammaglobulinemic because there is negligible transfer of Ig from the dam to the calf prior to birth. Neonatal calves have an immature immune system with little protection against pathogens. This is due to the synepitheliochorial placenta of the dam that prevents the passage of maternal antibodies into the fetus. The gut of the newborn calf is nonselective and open to the transport and absorption of large macromolecules such as Ig and other intact proteins for only about the first 24 h of life. The loss of absorptive capacity begins soon after and progresses continuously until gut closure is completed around 24 h (Stott et al., 1979). Neonatal calves acquire immunity through passive transfer of maternal antibodies from colostrum. Delaying colostrum feeding can result in decreased ability to absorb colostral Ig and decreased serum Ig concentrations. Research has shown that calves with inadequate Ig concentrations have reduced growth rates, increased risk of disease and death, increased risk of being culled, and decreased milk production in their first lactation (Smith and Foster, 2007). Ingestion and absorption of colostral immunoglobulins soon after birth are essential in the survival and future production of the neonate.

The USDA National Animal Health Monitoring System (NAHMS) states the percentage of heifer calves with serum IgG levels below 10 g/L or had failure of passive transfer had decreased about one-half from 1991 to 2007. The Dairy 2007 study found that 14.1% of heifer calves had adequate passive transfer and 19.2% had failure of passage transfer compared to over 40% of heifer calves with

failure of passive transfer in 1991 (USDA, 2007). The percentage of unweaned calves that died also decreased from 10.5% in 2002 to 7.8% in 2007; however, in the 2007 study, only healthy heifer calves that received colostrum were sampled; therefore, the percentage of calves failing passive transfer could be much larger. These results show how the advances in calf research is improving calf management on dairy farms, but their needs to be even more awareness of the risks involved with failure of passive transfer.

Passive Transfer

The bovine placenta is morphologically classified as a cotyledonary placenta, due to the cotyledons, which attach the placenta to the uterine wall and is structurally classified as a synepitheliochorial placenta because it consists of 6 layers (Schatten and Constantinescu, 2007). These characteristics determine what substances are allowed to pass through the placenta from the dam to the fetus.

Due to the synepitheliochorial placenta, the transfer of serum Ig are inhibited from dam to fetus. Neonates are born without a developed immune system unless there was some damaged placentation (Redman, 1979). Passive immunity protection and Ig absorption is achieved by the transport of macromolecules found in maternal colostrum through the intestinal epithelium and into the bloodstream of the neonate postnatally. The majority of the absorption occurs within the first 24 h of life and declines rapidly. The Ig absorbed from the maternal colostrum into the circulation of the newborn calf in

the first 24 h provides immunological protection until its own immune system develops.

Transfer of IgG to the Calf

Neonatal calves are considered preruminants because their rumen is immature and nonfunctional and their abomasum constitutes about 50% of the total tissue weight of the stomach. This makes a newborn calf's digestive system similar to nonruminants such as swine and humans. The newborn calf's gastrointestinal tract is growing at a fast rate. Intestinal absorption of large molecular proteins and peptides via pinocytosis decreases through the seventh day after birth (Blum, 2006). Also, protein digestion by lysosomes within enterocytes is replaced by digestion in the lumen of the gastrointestinal tract and on the cell membrane of the enterocytes (Blum, 2006). A study conducted by Stott et al. (1979) showed IgG absorption in the small intestine occurring within the first 24 to 36 h is through non-selective endocytosis of macromolecules.

In newborn calves, absorption of macromolecules from the intestinal lumen to the blood occurs in two phases: 1) uptake or internalization within the intestinal epithelium via pinocytosis and 2) the transport and subsequent expulsion of the macromolecules into systemic circulation (Stott et al., 1979). There is some disagreement as to the specificity of macromolecule uptake. Some researchers suggest that colostrum feeding stimulates pinocytosis in the endocytes, which is quickly followed by cessation of macromolecule internalization (Stott et al., 1979), whereas others researchers infer that intestinal

selectivity does exist between IgG and absorption does appear to have specificity for certain macromolecules during endocytosis (Sanglid, 2003).

The study conducted by Sanglid in 2003 used pig and lamb fetuses to evaluate the uptake of macromolecules by the intestine during the last weeks of gestation in utero. Sanglid (2003) observed that macromolecule uptake in the intestine is present in the fetus but is markedly increased in the neonate. This observation suggests that the ability to uptake and transfer intact protein from the epithelium into circulation is a specific process that develops near the time of parturition and is not only a result of immaturity of the intestine. Sanglid (2003) also found that the degree of humoral immunological protection depends on the amount and timing of colostrum feeding as well as the colostral Ig concentration, quality, and intestinal capacity of the neonatal calf to absorb Ig.

The closure of intestinal permeability to colostral Ig was thought to occur spontaneously with age at a progressively increased rate after 12 h postpartum, with a mean closure time occurring near 24 h postpartum (Stott et al., 1979). However, recent research has shown that the process of gut closure appears to be an energy-dependent process considering that both hypoxia at birth (Tyler and Ramsey, 1991) and insulin-mediated hypoglycemia (Tyler and Ramsey, 1993) delay gut closure. As the neonate is increasing in age, the epithelial cells progressively lose their capacity and ability to perform pinocytotic activities, thereby reducing colostral Ig uptake and transmission of colostral components into circulation (Stott et al., 1979). When the Ig is absorbed, it is transferred into

the lymphatic system and onto the peripheral circulation, which allows for IgG to remain effective in immune protection until the immune system of the calf matures (Redman, 1979).

Extending the absorption period may be beneficial in increasing the amount of Ig absorbed through the intestines; however, it could be harmful to the health of the calf by increasing the ability for bacteria and other pathogens to be absorbed directly into the blood stream. Therefore, shortening the absorption period may protect the newborn calf from prolonged exposure to the unselective absorption of pathogens (Stott et al., 1979).

Rate of Absorption

Rate of absorption of Ig in the neonate can be affected by the age at first feeding of colostrum and the amount of colostrum fed. These factors determine the concentration of serum IgG in the neonate and both exhibit a linear response such that as age at colostrum feeding increases, Ig concentration decreases and as the amount of colostrum fed increases, up to 2 L, serum Ig concentration increases (Stott et al., 1979). Shea et al. (2009) observed that most of the IgG absorption occurs before 12 h after birth with the highest rates occurring before 6 h (Figure 5). The researchers also found that 2 feedings of colostrum at 0 h and 12 h after birth increased IgG uptake. The 2007 dairy study conducted by the USDA's NAHMS, found that on average, calves that were removed from their dam immediately after parturition received hand-fed colostrum 3.3 h after birth

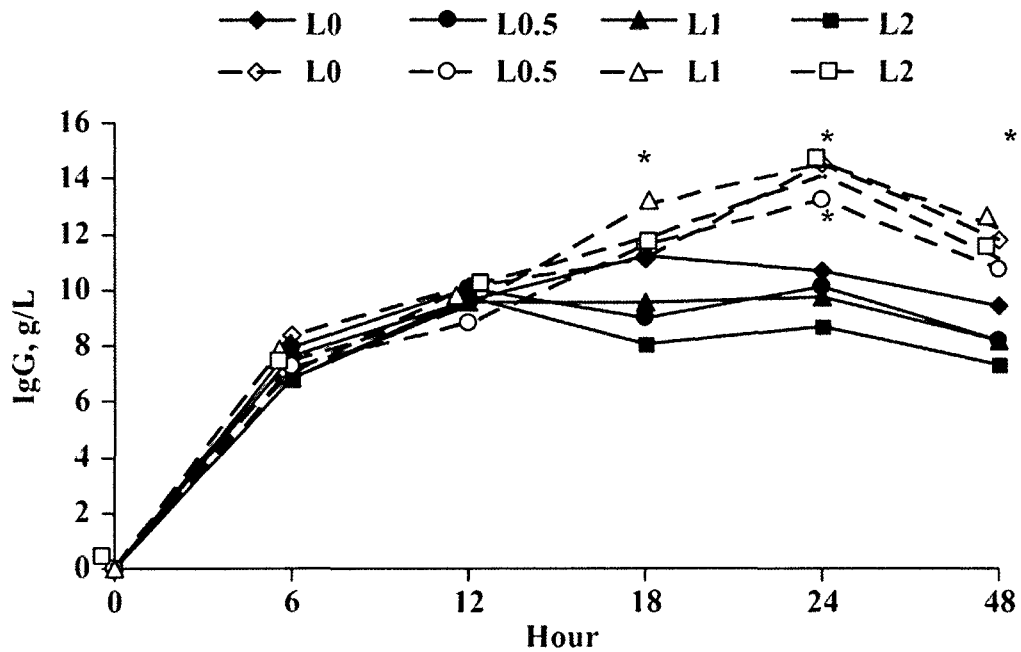


Figure 5. Serum IgG concentration (g/L) of calves fed 1 (closed symbol) or 2 (open symbol) doses of colostrum replacer with varying amounts of lactoferrin over the first 48 h of life. L0 = 0 g of lactoferrin, L0.5 = 0.5 g of lactoferrin, L1 = 1 g of lactoferrin, L2 = 2 g of lactoferrin. The largest SEM was 0.87 and occurred for all time points in calves fed 1 dose of colostrum replacer with L2. *Calves fed 2 doses of colostrum replacer had greater ($P < 0.05$) plasma IgG than those fed 1 dose of colostrum replacer.

(From Shea et al., 2009)

and 40.1% of calves received 4 quarts or more of colostrum, while 16.8% of calves received 2 quarts or less during the first 24 h.

Amount of Absorption

To achieve successive passive transfer with a serum IgG concentration of greater than 10 g/L at 24 h postpartum, it is recommended that a 43 kg Holstein calf be fed a minimum of 100 g of IgG and ideally close to 150 g in the first feeding of colostrum (Davis and Drackley, 1998). To account for variability in colostrum quality, a standard recommendation has been to feed 2 quarts of colostrum within the first few hours of life, followed by an additional 2 quarts within 12 h (Davis and Drackley, 1998). However, because many producers do not test the quality of colostrum before feeding, researchers have now suggested they feed colostrum at 10 to 12% of the calf's body weight (Godden, 2008). This will ideally ensure that adequate colostral IgG's are fed to the neonate to achieve passive transfer.

Testing for Passive Transfer

The transfer of IgG from dam to neonatal calf through colostrum is known as passive transfer, which can be determined by 2 methods: measuring serum IgG concentrations or serum protein levels. A calf's passive transfer status is excellent if its serum IgG level is 15 g/L or more and adequate if its serum IgG level is 10 to 14.99 g/L. A calf has failure of passive transfer if its serum IgG level is below 10 g/L. Serum IgG concentrations can be measure using radial immunodiffusion assays and is considered to be the most accurate method of

determining the calf's status of passive transfer. Testing serum IgG levels in calves between 24 and 48 h postpartum can be a relatively simple method for evaluating colostrum management programs and individual calf health status (Hancock, 1985).

Measuring serum protein levels is another way to determine passive transfer. This can be measured by a refractometer, which is less accurate but gives the producer a general idea of the calf's serum IgG concentrations in the blood. A serum total protein concentration of at least 5.5 g/dL is correlated with successful passive transfer of immunity in healthy calves (Tyler et al., 1996). According to USDA (2007), only 2.1% of U.S. dairy herds routinely measured passive transfer via serum proteins in their heifer calves. Only 14.5% of larger dairies (> 500 head) routinely monitored serum protein levels compared to smaller operations (< 100 head) at only 1.1%.

Factors that Affect Passive Transfer

Achievement of successful passive transfer is primarily affected by colostrum quality and quantity as well as age at first feeding of colostrum (Nocek et al., 1984). Other factors that influence passive transfer in the neonate include prepartum feeding of the dam (Guy et al., 1996), seasonal effects (Donovan et al., 1986), colostrum pH (Quigley et al, 2000), dystocia (Davis and Drackley, 1998), breed (Roy, 1980), and sex of the calf (Odde, 1988).

Colostrum Ig Concentration

The concentration of IgG in colostrum affects passive transfer. Besser et al.

(1985) observed a significant negative correlation between efficiency of absorption and amount of Ig fed for both IgG ($P < 0.001$) and IgM ($P < 0.01$). Researchers observed calves that were fed colostrum with lower amount of IgG and IgM absorbed a higher proportion of IgG and IgM than those fed a higher mass of IgG and IgM. These results suggest that the transport systems for IgM and IgG become saturated if overloaded. This could mean that there is a specific receptor for the IgG and IgM mechanisms of absorption because passive transfer should not reach a saturation point. Additionally, it indicates that the theory of absorption being nonselective maybe false.

Researchers have found that when a higher mass of IgG is fed, the apparent efficiency of absorption (AEA) may decrease, but the serum IgG concentrations increased (Besser et al., 1985). Jaster (2005) evaluated the *quality, quantity, and timing of the colostrum feeding on IgG₁ absorption in Jersey calves*. Researchers observed that calves fed higher concentrations of total IgG₁ resulted in higher serum IgG₁ concentrations compared to calves fed lower concentrations. It was also observed that calves receiving 2 feedings (0 and 12 h after birth) of 2 L of high IgG₁ concentration (84 g/L) had higher AEA compared to calves receiving either 4 L of high IgG₁ colostrum in 1 feeding or calves receiving 2 feedings of 2 L of low IgG₁ (31.2 g/L) colostrum. Mean serum IgG₁ concentration was 45.7 g/L for calves receiving 2 feedings of 2 L of high IgG₁ concentration and was 38.7 g/L for calves receiving either 4 L of high IgG₁ colostrum in 1 feeding, which suggests that calves should receive 2 separate

feedings of high quality colostrum to maximize IgG₁ absorption.

Prepartum Feeding of Anionic Salts

Prepartum feeding of anionic salts to cows is a method to reduce and prevent metabolic disorders during early prepartum by increasing the absorption of dietary calcium and stimulating mobilization of bone calcium due to the increase in parathyroid hormone receptor sites (Block, 1994; NRC, 2001). While this may be beneficial to the cow, researchers have found that calves born from dams that were fed anionic salts prepartum might be affected by respiratory or metabolic acidosis (Guy et al., 1996). Respiratory acidosis is caused by a decrease in respiration so that the lungs cannot expel carbon dioxide sufficiently and therefore causes a decrease in blood pH to below 7.2 (Bleul et al., 2007). Metabolic acidosis is a condition that occurs when the body produces too much acid or when the kidneys are not removing enough acid from the body. Although metabolic acidosis usually resolves within 2 h of birth, respiratory acidosis may persist for more than 24 h postpartum and may affect AEA and serum IgG concentrations. Guy et al. (1996) observed that calves born from cows fed cationic diets (+445 mEq/kg of DM) had higher serum IgG₁ concentrations at 24 h postpartum than calves from cows fed more acidotic diets (+75 mEq/kg).

Researchers have shown that respiratory and metabolic acidosis can decrease AEA and the ability of the calf to achieve passive transfer (Quigley and Drewry, 1998) because of a reduction in IgG absorption (Boyd, 1989).

Conversely, several other studies did not find any effect on IgG absorption or

AEA in calves born from dams fed anionic salts compared to calves born from dams not fed anionic salts (Drewry and Quigley, 1998; Morrill et al., 2010). The dissimilar results could have been due to the difference in magnitude of the dietary cation/anion balance since Morrill et al. (2010) fed control cows a +77 mEq/kg diet and treated cows a -100 mEq/kg diet while Guy et al. (1996) fed diets of +445 mEq/kg and +75 mEq/kg.

Vitamin C, Vitamin E, and Selenium

Selenium (Se) influences the immune response in several species of animals via the activation of phagocytosis by neutrophils, increased antibody production, and enhanced lymphocyte proliferation (Kamada et al., 2007). Calves are born with a Se deficiency; therefore, feeding Se after birth is an important technique for promoting the development of their own immune system and growth (Kamada et al., 2007). Kamada et al. (2007) observed that Se supplementation of 1.0 mg/kg in 4 feedings of colostrum, significantly increased IgG amount in the blood plasma of calves 24 h after birth by 20%. The researchers also found that the addition of 3.0 mg/kg in 1 feeding was more effective on IgG absorption with an increase of 42%. The increased IgG concentration in the blood continued for about 2 weeks. Kamada et al. (2007) suggests that the addition of Se to colostrum may directly activate the physiological pinocytosis of intestinal epithelial.

Abdelrahman and Kincaid (1995) observed increases in Se reserves in the liver, blood, and plasma Se concentrations when Se was supplemented in the

dam prepartum. The researchers also found colostrum concentrations of Se were increased by supplementing 3 mg/d of Se for 60 d prepartum. Although Abdelrahman and Kincaid (1995) did not measure IgG concentration in the calves born from the dams on the study, the calf's immunity could have been improved due to the known benefits of Se.

Vitamin C and vitamin E are antioxidants that have shown to influence the immune response of several animal species but supplementation has not been beneficial in calves (Hidiroglou et al., 1995). There were no differences in IgG₁ or IgG₂ uptake by vitamin C or vitamin E supplement but IgM tended to be higher in supplemented calves with both vitamins (Hidiroglou et al., 1995).

Seasonal Effects

The environment in which the calf is born may affect the absorption of Ig. In temperate climates, researchers have shown the mean monthly IgG₁ concentrations were lowest in winter and increased during spring and early summer peaking in September (Boyd, 1972; Gay et al., 1965; Gay et al., 1983). Conversely, Donovan et al. (1986) observed seasonal effects on Ig absorption were detected with the highest serum total protein occurring in February and March and lower total protein concentrations were associated with elevated environmental temperatures in the summer months. This could have been due to the fact that Donovan et al. (1986) study was conducted in a subtropical climate. The effects of ambient temperature outside the thermoneutral range for calves might involve direct effects on intestinal absorption and transport as well as the

ability of the calf to stand and nurse (Olsen, 1980; Quigley and Drewry, 1998). Heat stress might have been the cause for these results; however, the exact means by which the environment alters IgG absorption and serum protein concentrations are unknown (Donovan et al., 1986).

Colostrum pH

The average pH of maternal colostrum is around 6.17 (Tsioulpas et al., 2007). The chemical compositions of CR and supplements may affect the efficiency of IgG absorption in the calf (Quigley et al., 2000). Differences in the apparent efficiency of absorption (AEA) of IgG between CR and supplements maybe due to the variation in ingredient composition and chemical characteristics such as the IgG concentration and pH.

Quigley et al. (2000) studied the effects of varying pH levels on AEA of a *colostrum supplement derived from edible-grade bovine serum*. In this study, Lifeline Calf Nutritional Colostrum Supplement (American Protein Corporation, Ames, IA) was reconstituted with water and fed to calves to provide 45 g of IgG. The normal pH of the reconstituted colostrum supplement was 7.5. Sodium citrate (0, 0.4, 2.3, and 7.0 g/dose) was added to produce final pH in reconstituted product of 7.5, 7.0, 6.0, and 5.0, respectively. The 24 h mean plasma IgG levels for the 4 pH treatments of 7.5, 7.0, 6.0, and 5.0 were 6.57, 6.49, 5.76, and 7.19 g/L respectively. Apparent efficiency of absorption was 19, 20, 17, and 24% of the 4 respective pH treatments. These results indicate that a change in pH of colostrum supplements from 5.0 to 7.5 do not appear to affect IgG

absorption and were not statistically significant.

Cabral et al. (2011) added varying levels of sodium bicarbonate to CR and found a negative linear effect on IgG absorption. The treatments of sodium bicarbonate were 0 g, 15 g, 30 g, and 45 g which raised the pH of the CR to 6.10, 6.60, 6.90, and 7.10 respectively. However, work conducted by Morrill et al. (2010) found contradictory results. When the pH of CR was raised to 7.0 using sodium bicarbonate, researchers observed a 25% increase in IgG absorption (Morrill et al., 2010). The differences between the 3 studies could have been caused by the origin of the CR and colostrum supplements because Quigley et al. (2000) fed a serum-based colostrum supplement while Morrill et al. (2010) and Cabral et al. (2011) fed a lacteal-based CR. Another difference was the substance used to alter the pH of the colostrum. Quigley et al. (2000) used sodium citrate to lower the pH while Morrill et al. (2010) and Cabral et al. (2011) used sodium bicarbonate to raise the pH of the CR. The sodium bicarbonate could have had a bacteriostatic effect compared with the sodium citrate. This will be discussed further in Part III. Also, Quigley et al. (2000) fed a smaller amount of sodium than did Morrill et al. (2010) and Cabral et al. (2011), which may indicate the amount of sodium is causing the effect on IgG absorption.

There were also differences in feeding protocols. Morrill et al (2010) fed CR in 2 feedings compared to one large feeding in Cabral et al. (2011) study. The single feeding may have exceeded the intestinal capacity to absorb IgG by saturating the system, which did not allow for any further absorption of IgG.

Future research in the area of sodium bicarbonate and sodium alone, and the effect of colostrum pH on passive transfer needs to be conducted.

Dystocia

Dystocia is a major cause of weakness, morbidity, and mortality in dairy calves as well as increases the incidences of other postpartum disorders in cows. A prolonged and difficult calving may cause acidosis and hypoxia in the calf, which could negatively affect IgG absorption (Davis and Drackley, 1998).

Researchers have observed low neonatal serum protein concentrations in dystocia calves (Donovan et al., 1986; Odde, 1988). Odde (1988) evaluated the effects of body condition at calving and difficulty of calving on calf vigor and serum Ig concentrations. Each calf was assigned a calving difficulty score of 1 through 3. Calves with a calving score of 1 (unassisted delivery) had higher IgG₁ and IgM serum concentrations compared with calves with calving scores of 2 or 3. The lower serum IgG concentrations in dystocia calves could be the result of an increase in endogenous corticosteroid release and its subsequent effect on the closure of the intestinal wall. It could be also related to the fact that dystocia calves often lack vigor and therefore, compromise their ability to nurse off the dam. In order to help prevent failure of passive transfer in dystocia calves, producers should use an esophageal feeder to tube feed any calves that do not or cannot suckle.

Breed

Roy (1980) summarized several studies and found that breed differences

exist in the efficiency of Ig absorption. Jersey calves had higher 24 h IgG concentrations than Holsteins (16.47 and 11.12 g/L), and absorbed IgG with 21.9 ± 0.9% efficiency compared with 17.0% for Holsteins (Jones et al., 2004).

Researchers have also found that Holstein calves had a greater AEA than did Ayrshire calves or Friesian x Ayrshire calves, which could have been from differences in body weight, gender, blood volume, and metabolic state of the calf (Quigley and Drewry, 1998).

Sex of the Calf

The sex of the calf may also influence AEA and IgG absorption.

Researchers have shown that heifer calves generally have higher serum IgG concentrations than do bull calves (Odde, 1988; Roy, 1980). Odde (1988) observed that heifer calves had serum IgG₁ levels of 23.6 g/L compared with only 20.4 g/L for bull calves. It is not clear whether the sex of the calf may be related more to blood volume than to AEA or that the larger size of bull calves may influence the metabolic state of the calves due to the higher degree of calving difficulty for bulls, which would affect passive transfer (Quigley and Drewry, 1998).

Failure of Passive Transfer

Failure of passive transfer is indicated by a serum IgG concentration of less than 10 g/L at 24 h postpartum and increases the risk of septicemia and mortality in the neonatal calf. It has been determined that a certain frequency of hypogammaglobulinemia cannot be avoided under practical conditions due to variations in birth weight, Ig concentration in colostrum, volume of colostrum, age

at first feeding, and maybe due to a genetic disposition for Ig absorption ability (Bush, 1980). Calves that do not achieve passive transfer can be treated with plasma IV at a dosage of 20 mL/kg (Weaver et al., 2000). Although, calves are at a greater risk for developing disease, they can survive if they are placed in a clean environment with low exposure to infectious pathogens (Weaver et al., 2000). It is also still important to keep in mind that calves, whom have attained passive transfer, can easily suffer many diseases if placed in a dirty environment and exposed to several pathogens.

Part III: Sodium Bicarbonate

Research has shown that intravenously injecting 8.4% sodium bicarbonate (NaHCO_3) solution has been effective in improving the severe acid-base abnormalities in calves with acidosis (blood pH < 7.2) (Coskun et al., 2010).

Ayers and Besser (1992) evaluated the effects of alkalizing agents, administered prior to feeding colostrum, on IgG absorption, blood-gas, and acid-base values. One treatment was sodium bicarbonate that was administered at 3 mEq/kg of body weight intravenously, which showed increases in blood pH (+0.04 units), PCO_2 (+4.1 mm of Hg), and HCO_3 concentrations (+4.4 mEq/L). Absorption of IgG was not affected by sodium bicarbonate treatment or by the alteration of the blood acid/base pH status.

Sodium bicarbonate has also been added to acidified and fermented colostrum to act as a buffer (Foley et al., 1978). Foley et al. (1978) evaluated the effects of pH and fermentation on availability of colostral Ig for absorption by

newborn calves. Researchers observed that adding sodium bicarbonate enhanced absorption of Ig from fermented colostrum in the newborn calf. The addition of sodium bicarbonate to fermented colostrum (Jenny et al., 1983) and to acidified colostrum (Eppard et al., 1981) has shown improvements in feed intake. Foley et al. (1978) suggest that the addition of 7.3 g of sodium bicarbonate per kg of fermented colostrum can improve absorption of colostral Ig.

Sodium bicarbonate has also been shown to function by inhibiting growth of certain bacteria such as *Escherichia coli* 0111 (*E. coli*) in human milk and bovine colostrum (Griffiths and Humphreys, 1977). Griffiths and Humphreys (1977) observed that adjusting the pH to 7.4 with sodium bicarbonate resulted in the development of bacteriostatic activity, while adjusting the pH to 7.4 with NaOH was ineffective (Griffiths and Humphreys, 1977). This suggests that the response is not a pH effect as adjustment with bicarbonate only, and not with other buffers, made the colostrum inhibitory (Muller and Kilmer, 1979). The bacteriostatic action of bicarbonate appears to be related to its requirement by lactoferrin for the binding of iron. Although bovine colostrum contains sufficient lactoferrin, it does not inhibit the bacterial growth at its natural pH (Muller and Kilmer, 1979). This is due to a high concentration of citrate from both milk and colostrum, which is antagonistic to lactoferrin and competes with lactoferrin for iron, making it available for bacterial growth (Griffiths and Humphreys, 1977). Adding sodium bicarbonate to colostrum would raise the pH and could reduce the risk of *E. coli* infection in the calf. Another possibility is the effect of sodium. Since it takes less

sodium hydroxide to raise the pH compared to sodium bicarbonate, the amount of sodium added could be the cause for the effect.

Morrill et al. (2010) has shown the benefits of adding sodium bicarbonate to colostrum replacers in increasing the absorption of IgG. They fed calves CR with or without 30 g of supplemental sodium bicarbonate over 2 feedings. The researchers observed higher serum IgG levels at 24 h postpartum for calves receiving 30 g of supplemental sodium bicarbonate in CR compared to control calves (16.3 vs. 13.2 g/L). However, Cabral et al. (2011) fed varying amount of sodium bicarbonate to CR (0 g, 15 g, 30 g, and 45 g) and showed that 45 g of sodium bicarbonate had the numerically lowest intestinal absorption of IgG while 30 g of sodium bicarbonate had the numerically highest serum IgG concentrations at 24 h after birth. Serum IgG concentrations at 24 h showed a negative linear trend ($P < 0.08$).

Conclusion

Various studies have been conducted to evaluate calf management and the factors affecting IgG absorption in the neonatal calf. However, there is little research available on the effects of supplemental sodium bicarbonate to colostrum replacer and maternal colostrum on IgG absorption in the neonatal calf. The work involving colostrum replacer with the addition of sodium bicarbonate has shown some positive results in IgG uptake in newborn calves, however, the results have not been consistent and more research needs to be conducted. The outcomes of this research could lean to an inexpensive and

effective way of attaining successful passive transfer in newborn calves to improve calf health and survival.

CHAPTER II

ADDITION OF SODIUM BICARBONATE TO MATERNAL COLostrum: EFFECTS ON IgG ABSORPTION AND HEMATOCRIT IN NEONATAL CALVES

Introduction

Raising healthy calves is one of the most important factors in maintaining a productive and profitable dairy operation. Calves are born agammaglobulinemic due to the synepitheliochorial placenta of the cow, which prevents the transfer of Ig from the dam to the fetus. Consequently, newborn calves have an immature immune system with little protection against pathogens, which risks their health and future production.

Feeding adequate amounts of high quality colostrum soon after parturition is critical for calf survival. Colostrum is the first mammary secretion that provides a concentrated source of Ig, especially IgG, which is the main contributor to passive immunity in the neonate (Butler, 1969). Not only does colostrum provide vital Ig, but it also provides significant amounts of nutrients such as fat, lactose, minerals, vitamins, and water that support the calf during the first few days of life.

Successful passive immunity is primarily affected by colostrum quality and quantity as well as age at first feeding of colostrum (Nocek et al., 1984). After birth, calves should receive 4 L of high quality colostrum within the first 24 h

when the gut is open to the transport and absorption of large macromolecules to maximize IgG absorption (Stott et al., 1979). Achievement of passive transfer is indicated by serum IgG concentrations of ≥ 10 g/L at 24 h postpartum. Other factors that influence passive transfer in the neonate include prepartum feeding of the dam (Guy et al., 1996), seasonal effects (Donovan et al., 1986), colostrum pH (Quigley et al., 2000), dystocia (Davis and Drackley, 1998), breed (Roy, 1980), and sex of the calf (Odde, 1988). Research has shown that calves that do not achieve passive transfer have reduced growth rates, increased risk of disease and death, increased risk of being culled, and decreased milk production in their first lactation (Smith and Foster, 2007).

Maternal colostrum is the best source of Ig and nutrients for the neonate, however, when good quality colostrum is unavailable, products such as CR and colostrum supplements have been developed to substitute maternal colostrum or to boost the Ig content of poor quality colostrum, respectively. Providing the neonate with early and adequate intake of high quality colostrum is essential to the survival of the neonate and future production.

Sodium bicarbonate has been added to acidified and fermented colostrum to act as a buffer. Foley et al. (1978) showed that adding sodium bicarbonate enhanced absorption of Ig from fermented colostrum in the newborn calf. The addition of sodium bicarbonate to fermented colostrum (Jenny et al., 1983) and to acidified colostrum (Eppard et al., 1981) has also shown improvements in feed intake. Griffiths and Humphreys (1977) observed that sodium bicarbonate has

bacteriostatic effect on certain bacteria species such as *Escherichia coli* 0111 (*E. coli*) found in human milk and bovine colostrum.

Recently, the addition of sodium bicarbonate to CR has been shown to increase IgG uptake (Morrill et al., 2010). Researchers fed calves CR with or without approximately 30 g of supplemental sodium bicarbonate over 2 feedings and observed higher serum IgG levels at 24 h postpartum for calves receiving 30 g of supplemental sodium bicarbonate in CR compared to control calves (16.3 vs. 13.2 g/L). However, contrary results were shown when calves were fed varying amount of sodium bicarbonate to CR (0 g, 15 g, 30 g, and 45 g; Cabral et al., 2011). This author found that 45 g of sodium bicarbonate had the numerically lowest intestinal absorption of IgG and serum IgG concentrations at 24 h postpartum showed a negative linear trend ($P < 0.08$). Sodium bicarbonate has been added to CR and has shown effects on IgG absorption in neonatal calves, however, there is no research on supplementing sodium bicarbonate to colostrum.

The objectives of this study were to determine the effects of supplemental sodium bicarbonate to colostrum on absorption of IgG and hematocrit in the neonatal calf.

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 # 100503).

Twenty-six Holstein bull calves born from primiparous and multiparous cows were used in this study. Calves were blocked by birth and assigned to 1 of 13 blocks and randomly assigned to 1 of 2 treatments within each block: 1) colostrum + 0 g of supplemental sodium bicarbonate (control; **C**) or 2) colostrum + 30 g of supplemental sodium bicarbonate (**30g**).

Calves were removed from their dam prior to nursing within 30 min after birth. Calves were then weighed on a platform scale (Salter Scales, Fairfield, NJ), had their navels dipped in 7% iodine, and were placed in a naturally ventilated, enclosed calf room. Calves were housed in individual pens (1 x 2.15m) bedded with kiln-dried sawdust where they remained for the duration of the study (48h). Calves were assigned a dystocia score of 1 to 3 based on the difficulty of calving: 1 = unassisted calving, 2 = assisted easy calving, 3 = assisted difficulty calving. Calves used for this study had a calving score of a 1 or 2.

Nine batches of colostrum that tested greater than 50 g/L of IgG with a colostrometer were collected, pooled, placed in freezer bags, and stored at -20°C until needed. A 5 mL sample of colostrum from each batch was collected and stored in -20°C until analyzed for IgG by radial immunoassay (Triple J Farms; Bellingham, WA). Calves on the C treatment received 2.68 L of colostrum + 0 g of supplemental sodium bicarbonate at 0 h (within 75 minutes of birth) and 1.32 L of colostrum + 0 g of supplemental sodium bicarbonate at 6 h after birth. Calves on the 30g treatment received 2.68 L of colostrum + 20 g of sodium bicarbonate at 0 h (within 75 min of birth) and 1.32 L of colostrum + 10 g of sodium

bicarbonate at 6 h after birth. Another 5 mL sample of colostrum was taken before each feeding and stored in -20°C until analyzed for IgG by radial immunoassay (Triple J Farms; Bellingham, WA). The pH of the maternal colostrum with or without the addition of sodium bicarbonate was measured using a pH meter (Orion 230A pH Meter, Thermo Fisher Scientific Inc., Beverly, MA) before it was fed to the calves. If the calves did not consume the feedings from the bottle within 30 min, they were fed via esophageal tube.

Three hundred and forty grams of non-medicated milk replacer (Blue Seal Feed, Inc.; Londonderry, NH) was reconstituted with 2L of water and fed to calves at 24, 36, and 48h after birth. A sample of milk replacer was sent to Agri-King (Fulton, IL) for nutrient analysis. The sample was then analyzed for CP (method 99.03; AOAC 2002) and minerals (Ca, P, Mg, K, Na, and Fe; method 985.01; AOAC, 2002). Concentration of total fatty acids were determined by saponification with KOH in ethyl alcohol. Fatty acids were then released from the soaps with HCL and extracted with petroleum ether (AOAC, 1995). Lactose was determined using the method 984.22 (AOAC, 2002) with these modifications: flow rates through HPLC (Beckman, Fullerton, CA) were 1 mL/min, melibiose was the internal standard, and evaporative light scattering detection was used instead of a refractive index. Nutrient analysis of the milk replacer is presented in Table 3.

Blood Collection for Immunoglobulin G and Hematocrit

Blood samples were collected in 7-mL vacutainer tubes (Kendall, Mansfield, MA) via jugular venipuncture using a 22-gauge needle before the first feeding of colostrum (within 45 min of birth, referred to as 0 h) and at 6, 12, 24, and 48 h after birth. Three capillary tubes of blood were subsampled and centrifuged (Haematokrit 210; Andreas Hettich GmbH & Co; Germany) at 16,060 *g* at 25°C for 5min. The remaining blood was allowed to clot at room temperature then centrifuged (CentraMP4R; International Equipment Company; Needham HTS, MA) at 1,310 x *g* at 25°C for 20 min. Serum samples were stored at -20°C until analyzed for IgG by radial immunoassay (Triple J Farms; Bellingham, WA).

Apparent efficiency of IgG absorption (AEA) at 24h of age was estimated using the equation: (plasma IgG [g/L] x body weight [kg] x 0.092/IgG (g) intake) x 100% (Quigley et al., 1998). The IgG concentration at 6, 12, 24, and 48h were also analyzed. Area under the curve (AUC) was analyzed using the trapezoidal rule with the equation: 0.5 x difference in time x different in IgG concentration (Phillips and Taylor, 1973).

Statistical Analysis

Calf serum IgG concentrations and hematocrit were analyzed as a randomized complete block design using the repeated measures determined in the MIXED procedure of SAS® (SAS Institute, 2001) according to the following model:

$$Y_{ijk} = \mu + B_i + S_j + \beta X_{ij} + H_k + E_{ijk}$$

Where:

Y_{ijk} = the dependent variable,
 μ = the overall mean,
 B_i = the random effect of block ($i = 1, \dots, 13$),
 S_j = the fixed effect of the j^{th} sodium bicarbonate level ($j = 0, 30$),
 β = the regression (covariate coefficient),
 X_{ij} = the covariate measurement,
 H_k = is the fixed effect of hour of the experiment ($k = 0, 6, 12, 24, 48$),
 E_{ijk} = the residual error $\sim N(0, \sigma^2 e)$

In this model, the random effect of calf within treatment subclass is used as the error term for the effect of sodium bicarbonate. Residual errors, which refer to errors within calf across time and represent errors from repeated measurement in the experimental units (calf) were modeled using an unstructured covariance structure. Degrees of freedom were calculated using the Kenward-Rogers option of the MIXED procedure of SAS® (SAS Institute, 2001). A covariate term was included in the model to reduce the variance due to calf within treatment subclasses. The covariate variable was initial body weight. Block was found to be not significant ($P > 0.25$) and removed from the model for all variables. Least square means were determined for treatment. Treatment responses with $P \leq 0.05$ were considered to be statistically significant. The PDIF option in SAS® was used to test treatment differences among least square means.

Apparent efficiency of absorption and AUC were analyzed as a randomized complete block design using the MIXED procedure of SAS® (SAS

Institute, 2001) according to the following model:

$$Y_{ijk} = \mu + B_i + S_j + E_{ijk}$$

Where:

Y_{ijk} = the dependent variable,

μ = the overall mean,

B_i = the random effect of block ($i = 1, \dots, 13$),

S_j = the fixed effect of the j^{th} sodium bicarbonate level ($j = 0, 30$),

E_{ijk} = the residual error $\sim N(0, \sigma^2_e)$

In this model, the random effect of calf within treatment subclasses was used as the error term for the effect of sodium bicarbonate. Degrees of freedom were calculated using the Kenward-Rogers option of the MIXED procedure of SAS® (SAS Institute, 2001). Block was found to be not significant ($P > 0.25$) and removed from the model for all characteristics. Least square means were determined for treatment. Treatment responses with $P \leq 0.05$ were considered to be statistically significant.

Results

Average body weight (mean \pm SD) of all calves was 43.75 ± 6.13 kg. The average IgG concentration (mean \pm SD) of the nine pooled maternal colostrum batches was 82.05 ± 8.45 g/L and the average IgG fed amount (mean \pm SD) was 329.89 ± 34.56 g. The pH of the maternal colostrum treatments were 5.97 ± 0.27 (C) and 6.64 ± 0.20 (30g). At birth, the average serum IgG concentration for all calves was 0.49 ± 0.96 g/L.

There was not a significant difference in serum IgG concentrations at 24 h between treatments ($P < 0.32$). Calves on C had serum IgG concentrations of

32.59 g/L at 24 h and calves on treatment 30 g had serum IgG concentrations of 31.73 g/L at 24 h (Table 4). Of the 26 calves, all attained passive transfer of ≥ 10 g/L at 24 h. During the initial feeding, 4 calves were fed via esophageal feeder with 2 calves being on C and 2 calves on 30 g. These 4 calves and 6 others were fed via esophageal feeder at the 6 h feeding with 2 calves being on treatment C and 4 calves on treatment 30 g.

Area under the curve for IgG and AEA for IgG was not affected by the addition of sodium bicarbonate (Table 4). There were also no differences in hematocrit between the 2 treatments over the 48 h (Table 4 and Figure 7).

Discussion

Serum IgG concentrations at 0, 6, 12, 24, and 48 h postpartum were not different between calves with or without 30 g of supplemented sodium bicarbonate (Figure 6). These results differed from a previous study by Morrill et al. (2010) who reported that calves fed 30 g of supplemental sodium bicarbonate to CR had higher serum IgG concentrations at 24 h ($P < 0.05$) compared to control calves (16.3 g/L vs. 13.2 g/L). The difference in results could be due to the use of maternal colostrum in this study and CR in Morrill et al. (2010). Studies have shown that CR has been found to be an acceptable alternative to maternal colostrum; however, absorption of IgG is lower in CR fed calves compared to maternal colostrum fed calves (Smith and Foster, 2007). Researchers have studied the effects of various factors, including source of IgG, method of IgG fractionation, amount and type of non-IgG protein, and the presence of fat and

lactose, on the efficiency of IgG absorption in calves fed CR products and supplements (Arthington et al., 2000; Davenport et al., 2000). Davenport et al. (2000) observed that large amounts of casein in colostrum supplements significantly reduced the efficiency of IgG absorption and Hopkins and Quigley (1997) found that the addition of some colostrum supplements reduced the absorption of IgG from colostrum.

The results from the current study were similar to those of Cabral et al. (2011), who found that the addition of 30 g of sodium bicarbonate to CR had similar serum IgG concentrations at 24 h compared to no addition of sodium bicarbonate (17.16 g/L vs. 16.85 g/L). This could have been due to the large amount of IgG fed at the initial feeding, which may have saturated the calf's intestine and IgG receptors. Cabral et al. (2011) also observed a linear reduction in IgG absorption with increasing levels of sodium bicarbonate, likely caused by the 45 g of sodium bicarbonate negatively affecting IgG absorption when supplemented with CR (14.52 g/L; Cabral et al., 2011). The difference in results between Morrill et al. (2010) and Cabral et al. (2011) was possibly related to differences in feeding protocols. While Morrill et al. (2010) fed CR in 2 feedings providing 132 g of IgG \pm 19.5 g of sodium bicarbonate at the first feeding (0 h) and 66 g of IgG \pm 9.75 g of sodium bicarbonate at the second feeding (6 h), Cabral et al. (2011) fed 211.6 g or 216.8 g of IgG (variation due to 2 lots of CR) with the addition of sodium bicarbonate (0 g, 15 g, 30 g, or 45 g) at a single feeding.

Calves on C had slightly higher (32.59 g/L) serum IgG concentrations at 24 h than calves on 30 g (31.73 g/L) at 24 h; however, at 48 h, the serum IgG concentrations were identical (28.11 g/L vs. 28.12 g/L; Figure 6). The lack of sodium bicarbonate effect may be explained by the amount of IgG fed (329.89 ± 34.56 g) between 0 h and 6 h. Researchers recommend feeding newborn calves 4 L of colostrum with greater than 50 g/L of IgG (~200 g of IgG) within the first 6 to 8 h of life (McGuirk and Collins, 2004). In this study, calves were fed, on average, more than 100 g of IgG above what is considered the minimum recommended amount, which may have caused a saturation of the macromolecular transport mechanism across the calf intestinal epithelium or the serum IgG concentration reached a threshold level (Besser et al., 1985).

It has been observed from other studies that the greater the amount of Ig fed at the initial feeding of colostrum, the higher the serum Ig concentrations at 24 h in calves (Besser et al., 1985; Stott et al., 1979). Stott et al. (1979) found that calves fed 2 L of colostrum at birth had mean serum concentrations of IgG nearly double those fed 1 L (14.9 g/L vs. 8.5 g/L). Calves in the present study received 2.68 L of 82.05 ± 8.45 g/L at the initial feeding and another 1.32 L at 6 h. Also, these studies were feeding lower amounts of IgG fed (~100 g of IgG), while in the current study, the mean IgG fed amount was 329.89 ± 34.56 g, which could have saturated the receptors in the intestines for IgG and could have masked the effect of sodium bicarbonate. In fact, at 6 h postpartum, the serum IgG concentrations averaged 12.02 g/L for C and 12.21 for 30 g, indicating that

calves reached passive transfer even before the second feeding of colostrum, possibly saturating the IgG receptors (Figure 6).

Researchers have found that when a higher mass of IgG is fed, the serum IgG concentrations increased, but AEA decreases (Besser et al., 1985; Jaster, 2005). Apparent efficiency of absorption of IgG at 24 h was not improved by addition of sodium bicarbonate, which disagrees with data from Morrill et al. (2010). Morrill et al. (2010) observed an increase in AEA in calves supplemented with 30 g of sodium bicarbonate from 26.1 to 31.2% respectively ($P < 0.05$). However, Cabral et al. (2011) showed a negative linear response with the 45 g treatment showing the lowest AEA (27.18%). The AEA measured in the current study averaged 35.02% for calves receiving treatment C and 32.33% for those in treatment 30 g, indicating values greater than the previous studies but agrees with the mean AEA (20 to 35%) from maternal colostrum (Quigley and Drewry, 1998). Although the current study's AEA values are larger than previous studies with sodium bicarbonate, the discrepancy could be due to the fact that colostrum was used instead of colostrum replacer and the serum IgG concentrations at 24 h for calves receiving 30 g of sodium bicarbonate was twice as high for the current study than for Morrill et al. (2010) and Cabral et al. (2011).

Area under the curve for IgG was also not affected by sodium bicarbonate, which is dissimilar to Morrill et al. (2010) who observed an increase in AUC with calves fed supplemental sodium bicarbonate. Cabral et al. (2011) found a negative linear response with increasing levels of sodium bicarbonate. The

dissimilar results may have been due to the differences in the feeding protocols (amounts of IgG fed and colostrum versus colostrum replacer).

Hematocrit data showed no effects of sodium bicarbonate treatment (Figure 7). Hematocrit is a measure of packed cell volume, indicating hydration status. The increase of sodium in the blood due to the increasing levels of sodium bicarbonate was thought to have caused the calf to become more hydrated and be able to absorb more. All calves showed a numerical decrease over the 48 h period, which shows calves becoming more hydrated. There was not an effect of sodium bicarbonate, which could be attributed to hydration being affected by other bodily mechanisms, and the fast growing development of the neonate.

At parturition, calves are normally born without any IgG in their blood unless there was some damaged placentation, which can cause some transfer of IgG from the dam to the fetus (Redman, 1979). However, 5 calves in this experiment had above normal 0 h serum IgG levels of ≥ 1 g/L and there were no signs of retained placentas in their dams. The cause of the abnormal serum IgG level at 0 h is unknown, but there could have been some damage to the placenta that was unnoticed. The mean IgG concentrations at 0 h in this study was 0.49 ± 0.96 g/L and ranged from 0.00 to 4.18 g/L, which was very similar to Cabral et al. (2011) who observed serum IgG concentrations at 0 h to be 0.49 ± 0.73 g/L. Chigerwe et al. (2008) reported that calves in utero exposed to infection had higher serum IgG concentrations at birth. The elevated serum IgG levels in the 5

calves may have been due to infection prepartum, but no signs of illness were detected in the dams.

Conclusion

The addition of sodium bicarbonate had no effect on IgG absorption in Holstein bull calves. Area under the curve for IgG and AEA for IgG were also not affected by supplementing colostrum with sodium bicarbonate. More research needs to be studied to determine the effects of the amount of IgG fed and colostrum versus colostrum replacer with or without supplemental bicarbonate on IgG absorption. Also, studying the effects of the different components in maternal bovine colostrum that affect intestinal development and absorption of IgG to help explain the dissimilar results of supplemental sodium bicarbonate.

Table 3. Nutrient analysis of milk replacer (DM basis)

Item	MR ¹
DM, %	
CP, %	21.40
Fat, %	20.47
Lactose, %	41.11
Ca, %	1.01
P, %	0.76
K, %	1.75
Mg, %	0.15
Na, %	0.87
Zn, mg/kg	113

¹ Milk Replacer, non-medicated. Blue Seal Feeds. Londonderry, NH.

Table 4. Initial body weight, serum IgG concentrations, area under the curve (IgG), apparent efficiency of absorption (IgG), and 24 h hematocrit of calves with or without the addition of sodium bicarbonate.

Item	Treatments ¹		SE ²	P ³
	C	30		
Initial BW, kg ± SD	45.31 ± 5.70	42.19 ± 6.15		
Serum, IgG g/L 24 h	32.59	31.73	1.42	0.55
AUC ⁴ , IgG g/L*h	1270.04	1188.41	45.21	0.29
AEA ⁵ , %	35.02	32.33	2.24	0.20
Hematocrit, % 24 h	34.32	32.44	2.00	0.36

¹ Treatments: C = 0g of sodium bicarbonate; 30 = 30g of sodium bicarbonate.

² Standard Error.

³ P = the probability that C is different than 30.

⁴ AUC = Area under the curve; 0.5 x difference in time x difference in IgG concentration (Phillips and Taylor, 1973).

⁵ AEA = Apparent efficiency of absorption; (plasma IgG [g/L] x body weight [kg] x 0.092/IgG intake) x 100% (Quigley et al., 1998).

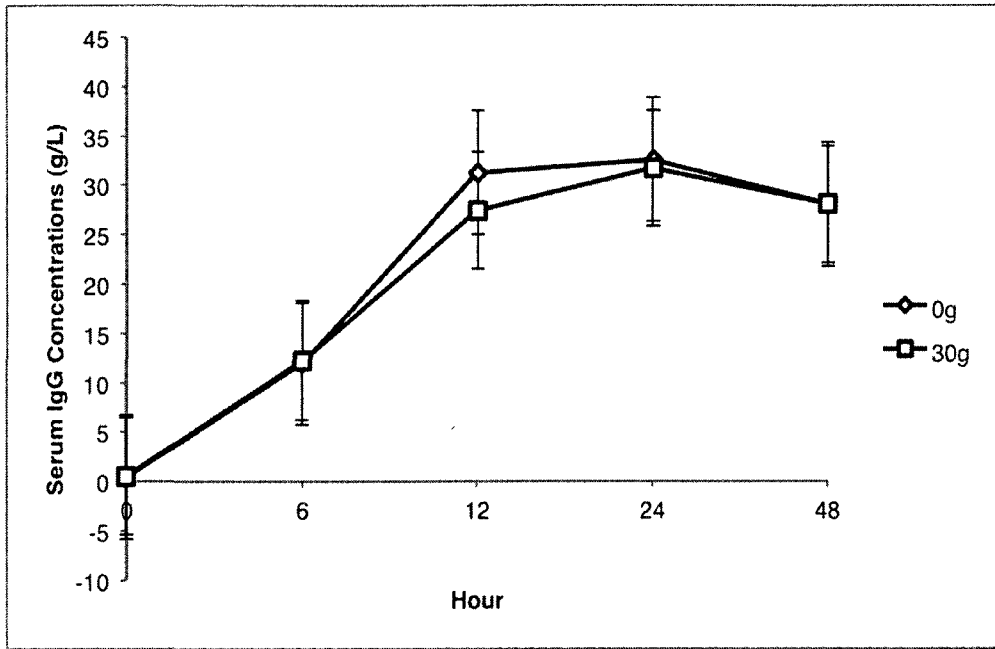


Figure 6: Serum IgG concentration \pm SEM for calves receiving 0 or 30 g of sodium bicarbonate added to colostrum.

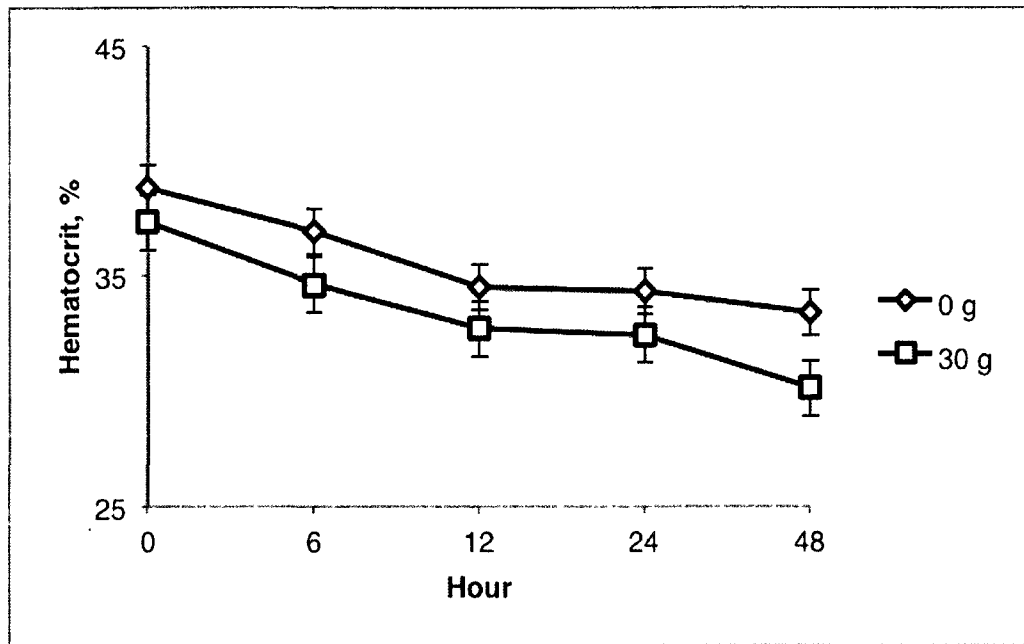


Figure 7: Hematocrit \pm SEM for calves receiving 0 or 30 g of sodium bicarbonate added to colostrum.

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APPENDICES

APPENDIX A

University of New Hampshire

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

28-Jun-2010

Erickson, Peter S
Biological Sciences
Dairy Nutrition Research Center
30 O'Kane Road
Durham, NH 03824

IACUC #: 100503

Project: Does Sodium Bicarbonate increase IgG absorption in maternal colostrum?

Category: C

Approval Date: 16-Jun-2010

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category C on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *Animal use activities that involve either no pain or potentially involve momentary, slight pain, discomfort or stress not requiring the use of pain relieving drugs or methods.*

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 Dean Elder at 862 4629 or Julie Simpson at 862-2003.

For the IACUC,



Dean Elder, D.V.M.
Vice Chair

cc: File
Whitehouse, Nancy