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**ADDITION OF VARYING AMOUNTS OF SODIUM BICARBONATE TO
COLOSTRUM REPLACER: EFFECTS ON IgG ABSORPTION, SERUM
BICARBONATE, AND HEMATOCRIT IN NEONATAL CALVES**

By

Rosemarie G. Cabral

B.S. University of Massachusetts, December 2008

THESIS

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Master of Science

In

Animal Science

December, 2010

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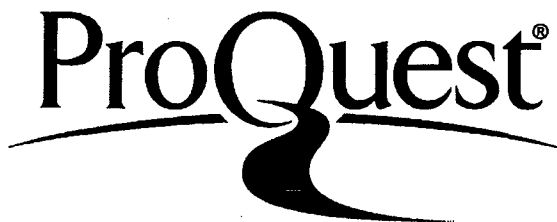
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ACKNOWLEDGEMENTS

There are so many people I would like to thank for their help on my journey through this program. I hardly know where to start.

I would like to thank my committee members:

Dr. Deborah Haines: thank you for donating the colostrum replacer, assays, and for all your helpful suggestions along the way. Thank you also for inviting me into your home and allowing me to work in your lab even though my lab skills needed some work.

Dr. Andre Brito: I feel bad that I did not get to talk to you more. Thank you for laughing at my jokes even if they were lost in translation.

Dr. Drew Conroy: thanks for listening to me vent on my bad days and for giving me the chance to be a part of CREAM.

I would also like to thank Nancy Whitehouse for all her help with running my statistics. I am almost positive that I would still be trying to figure out how to load my data into SAS if you hadn't helped me. Thanks for all those hugs too, they always brightened my day!

To the barn crew: Jon, John, Roger, and Sandy, thank you for always calling me when my cows were calving and sometimes even moving them for me. This project definitely would not have happened if you weren't there to help. I still owe all of you a huge plate of cookies.

To my fellow DNRC grad students: Scott, Shara, Colleen, and Megan. Thank you all for listening to me vent, cry, scream, laugh, and giggle through this project. You all helped me get through this.

Scott: Thanks for always being the voice of reason. I can't wait to read your book about grad school.

Shara: I can't believe you never lost your ring in the molasses. Thank you for always listening to me complain when things got really bad. You're my favorite hippie. The End.

Megan: Thanks for laughing at my rants about life. I hope you have as much fun as I have had in this program. Remember that even though the bad days are really bad, the good days are even better.

Colleen: Thanks for being my best buddy through all of this. I don't think I could have made it through those last few calves had you not been there to help feed and take blood samples when I was so tired I couldn't remember my name.

I would like to thank my family as well. Without my Mom and Dad's constant encouragement to push myself farther and harder I don't think I would have had the strength to get this far. Thank you for always giving me a second chance when I screwed up horribly, for buying me groceries when my fridge was empty, and reminding me that it would all be worth it in the end. And Dad, I finally beat the gringos.

I need to thank Dr Mark Huyler who taught my first nutrition class. If it weren't for you I never would have fallen in love with dairy nutrition and I never would have met Pete. I can never thank you enough and I hope that I've made you proud even though I didn't do that well on my chick ration.

Finally, I'd like to thank my amazing advisor Dr. Peter Erickson. Thank you for fighting for me along the way and taking a chance on the shy girl. It has been

a tough road getting here and I thank you for never losing faith in me or my abilities. I know that I never would have gotten to this point without your encouragement and words of wisdom. I will never be able to express how grateful I am to have you as an advisor. It has been a privilege to work with you and I look forward to working with you more. Thank you for listening to me cry when things weren't going well, buying me coffee when I needed a break, and taking me to lunch when I was broke. I hope this thesis makes you proud. It has been "unfluffed" to the best of my ability. Pedro, you have been a second father to me, and you have gone above and beyond to help me succeed. I hope that someday I can inspire students the way you have inspired me.

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List of Abbreviations

CR = colostrum replacer

MR = milk replacer

AEA = apparent efficiency of absorption

AUC = area under the curve

IgG = immunoglobulin G

IgM = immunoglobulin M

IgA = immunoglobulin A

E. Coli = Escherichia Coli

ABSTRACT

ADDITION OF VARYING AMOUNTS OF SODIUM BICARBONATE TO COLOSTRUM REPLACER: EFFECTS ON IgG ABSORPTION, SERUM BICARBONATE, AND HEMATOCRIT IN NEONATAL CALVES

by

Rosemarie G. Cabral

University of New Hampshire, December 2010

Fifty-two calves were assigned to a randomized complete block design and were randomly assigned by block to one of four treatments: colostrum replacer with no sodium bicarbonate, colostrum replacer with 15g of sodium bicarbonate, colostrum replacer with 30g of sodium bicarbonate, and colostrum replacer with 45g of sodium bicarbonate to test for linear and quadratic effects. Calves were fed colostrum replacer (≥ 200 g of IgG) in one feeding at 0 h and 2L milk replacer at 12, 24, 36, and 48 h. Only calves from multiparous cows, without dystocia, and born in calving pens were used. Sodium bicarbonate had a negative effect on IgG absorption, apparent efficiency of absorption, and area under the curve especially when the 45g treatment was fed. Sodium bicarbonate treatments had no effect on hematocrit. Serum bicarbonate concentrations and

area under the curve for sodium bicarbonate increased with amount of sodium bicarbonate fed.

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

CHAPTER I

REVIEW OF LITERATURE

Introduction

Calves are the future of the dairy herd. Their health and vitality are crucial to the longevity of the operation. For this reason, calves need special attention to make sure that their health is in no way compromised so they may survive to breeding age. Calves are born agammaglobulinemic, which means they have essentially no immunoglobulins at birth (Lopez et al., 1988). This is due to the type of placenta that all ruminants have. The synepithelialchorial placenta does not allow for immunoglobulins (Ig) to pass to the neonate. This leaves them susceptible to many serious health problems that could potentially damage their future productivity levels or even be fatal.

Colostrum is essential to calf health and nutrition. It is this first mammary secretion that provides Ig, such as IgG, that are essential to the health and well being of the animal. Immunoglobulin G is the primary Ig for passive immunization of the neonate (Butler, 1969). Through colostrum, the calf receives not only IgG but also fat, lactose, minerals, vitamins, and water. Upon birth, calves should receive at least 4L of colostrum within the first 24 h when macromolecular transport is at its peak for Ig absorption, it is within this time frame that passive immunity can be established through the absorption of IgG from colostrum

(Jaster, 2005). The efficiency of IgG absorption from 12 h to 24 h was only 31 % indicating that most absorption occurs from the initial feeding at or near birth (Shea et al., 2009). Achievement of passive transfer is indicated by serum IgG levels being ≥ 10.0 g/L (NAHMS, 2007), anything less than that would indicate failure of passive transfer. By enhancing of IgG absorption during the initial feeding there should be a health benefit to the calf, which will be reflected in the serum IgG concentration. Inadequate transfer of colostral IgG 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). It is apparent that intake of colostrum is not only important for the calf's initial survival but for its future productive life.

Part I: Colostrum

Colostrum is the first mammary secretion that provides many nutrients and Ig that are essential to the initial survival of the neonate. The proper feeding of high quality colostrum is directly correlated to calf health and vitality. Proper feeding entails the calf receiving sufficient amounts of high quality colostrum soon after birth. Colostrum provides a concentrated source of nutrients such as fat, lactose, protein, water, vitamins, and minerals along with the Ig. These colostrum components affect the gastrointestinal tract, produce transient systemic metabolism and endocrine changes and have long lasting effects on immunoprotection as well as the nutritional status of the newborn calf (Bösze, 2008).

Colostrogenesis, the production of colostrum, is defined as the prepartum transfer of Ig from maternal circulation into mammary secretions, which occurs in a distinct stage of mammary gland development (Barrington et al., 2001). The transfer of Ig begins several weeks prepartum and halts just prior to parturition (Brandon et al., 1971). Amounts from several hundred grams to 3 kilograms of Ig are moved to the mammary gland during this period (Larson, 1980). It is the high concentrations of Ig, especially IgG₁ that distinguishes colostrum from milk.

The mechanisms that control colostrogenesis are still being investigated, but it is recognized that lactogenic hormones, such as prolactin, do contribute to its regulation (Barrington et al., 2001). It is also known that local mechanisms affect colostrogenesis. It has been shown that the composition of colostrum varied among individual quarters on the same udder (Guidry et al., 1980).

Mammary Ig secretions originate from both humoral and local production. Humorally they arise from the blood stream and are transferred to the lacteal secretions. Locally, Ig are produced by plasmacytes, located adjacent to the secretory epithelium in the mammary gland (Larson et. al., 1980). There are 3 types of Ig present in bovine colostrum, IgA, IgM, and IgG. These Ig provide immunity against common disease organisms as they are a concentrated source of serum antibodies (Larson et. al., 1980).

The transport of IgG from the blood across the mammary barrier into the colostrum occurs via a specific transport mechanism within the secretory cells. This mechanism involves the Ig first being bound to surface receptors, which aggregate on one side of the cell. This is followed by pinocytotic engulfment of these areas. It is presumed that the Ig attached to the inner membrane of the vesicles then travel through the cell to be discharged into the luminal secretions (Larson, 1980).

While IgG makes up the largest portion of Ig present, IgA and IgM are also present. These two types of Ig are produced locally in the mammary gland by plasmacytes (Larson, 1980); however, smaller amounts are transported from the blood (McGuirk and Collins, 2004).

Composition of Colostrum

A calf's health and vitality is not solely facilitated by colostrum Ig, it is also the many other nutrients present that contribute to the overall well being of the neonate. The fat, lactose, and protein all serve as fuel for the calf while the vitamins and minerals are essential as cofactors for enzymatic functions (NRC,

2001). Colostrum is beneficial because it provides a highly concentrated source of these nutrients compared to milk (Table 1).

Proteins

Proteins are divided into 2 main classes, the whey and the casein portion, which include the lactoglobulins and lactalbumins. Large amounts of protein are needed for the development of the digestive tract during the immediate post-natal period. Most of the amino acids necessary for digestive tract development are provided by colostrum. Yvon et al. (1993) found that α -lactalbumin was crucial early on due to its low retention time in the abomasum and rapid degradation in the intestine. β -lactoglobulin, like α -lactalbumin, also has a short retention time in the abomasum and a rapid degradation in the small intestine. This makes it a major source of amino acids during the first several hours post-partum (Yvon et al., 1993). Casein, however, is a delayed source of amino acids due to its coagulation in the abomasum.

Immunoglobulins are also a type of protein. During the first 24-48h of life, Ig are poorly hydrolyzed by the proteases in both the abomasum and intestine. They are mostly absorbed intact. They are minimally digested due to the limited acid secretion and decreased proteolytic activity of the neonatal digestive tract (Davis and Drackley, 1998). Immunoglobulins are susceptible to trypsin, which is present at this time; however, trypsin inhibitor is found in very high concentrations in colostrum. After the first milking, the concentration of trypsin inhibitor drops rapidly (Piñeiro et al., 1978). This is beneficial to the calf because

Table 1: Composition of Holstein Colostrum versus Milk

Variable	Colostrum	Milk
Fat (%)	6.7	3.6
Total Protein (%)	14.0	3.2
Ig (%)	6.0	0.09
IgG (g/100mL)	3.2	0.06
Calcium (%)	0.26	0.13
Zinc (mg/100mL)	1.22	0.30
Iron (mg/100mL)	0.2	0.05
Vitamin A (mg/100mL)	295	34
Vitamin E (mg/g fat)	84	15
Vitamin B12 (mg/100mL)	4.9	0.6
Folic Acid (mg/100mL)	0.8	0.2

(Adapted from Foley and Otterby, 1978)

the intact Ig that is absorbed provides them with their initial immunity until their own immune system develops.

Immunoglobulins

The term immunoglobulin refers to a family of high molecular weight proteins that have common physio-chemical characteristics and antigenic determinants (Butler, 1969).

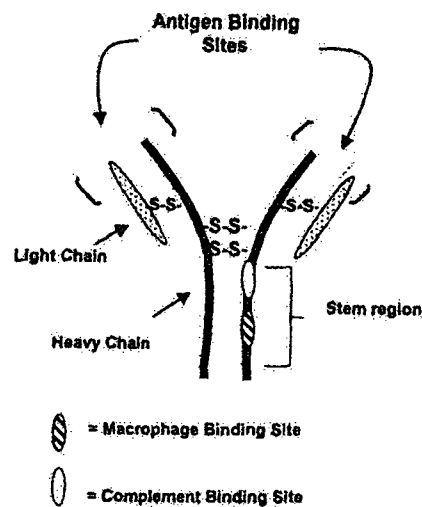


Figure 1: Structure of an antibody. (Adapted from Akers 2002)

All Ig share the same basic structure. Each is a monomer or polymer comprised of four-chain molecules made up by two heavy polypeptide chains and two light polypeptide chains (Butler, 1969). This protein family includes molecules with antibody activity along with chemically related normal or pathological proteins. Antibody specificity is associated with certain Ig types; however, they are not classified in this manner. Instead they are distinguished by their physio-chemical and antigenic properties (Butler, 1969). All three types (IgG, IgA, and IgM) are key in the prevention of disease in the calf. If isolated, the individual Ig's do not

provide the immune protection is effectively as colostrum when all three Igs are present (Logan et al. 1974).

Immunoglobulin G

Immunoglobulin G is the most abundant class of Ig comprising 85-90% of the serum and whey Ig (Klaus et al., 1969). Immunoglobulin G can be further subdivided into IgG₁ and IgG₂. The IgG₁ class is selectively transported to the lacteal secretions of the udder from the circulation. The IgG₂ class is considered more basic than IgG₁. The IgG₂ class is found in high concentrations in serum and lower in milk, colostrum, and saliva (Butler, 1969). Under normal circumstances, there are no appreciable differences in serum concentrations between IgG₁ and IgG₂; however, IgG₁ is the primary Ig of lacteal and salivary secretions (Butler, 1969). Although IgG₁ is concentrated in the mammary gland, the calf's gut has no selectivity between the IgG types (Butler, 1969).

Immunoglobulin M

Immunoglobulin M makes up less than 10% of the colostrum and serum immunoglobulins (Klaus et al., 1969). This type of Ig is important for the primary immune response, complement fixation (enhances inflammatory response and stimulates direct destruction of bacteria), and as an agglutinating antibody of serum (Butler, 1969). Not only does IgM contribute to the initial immune defense of the calf but it also assists in intestinal immunity against enteric pathogens (Logan et al., 1974).

Immunoglobulin A

Immunoglobulin A occurs in the lowest concentrations (about 5%; Larson et al., 1980). It is mainly found in milk and colostrum and is more plentiful in whey than serum. Immunoglobulin A is locally synthesized in the mammary lymphoid and epithelial tissues (Butler, 1969). Many of the functions that would normally be performed by IgA in other species are instead carried out by IgG and IgM in the calf (Roy, 1980; Butler, 1986).

Fat

Neonatal calves only have about 3% body fat compared to 16% body fat found in human infants (Faulkner, 1983). It is critical that calves receive a feeding of colostrum soon after birth since 3% body fat will only sustain them for 18 hours (Okamoto et al., 1986). In Holstein cattle, colostrum has 6.7% fat compared to 3.6% in milk (Table 1; Foley and Otterby, 1978). This increased fat percentage is crucial at this time. Gross energy content of colostrum can be calculated using kcal/g values for lactose, non-Ig protein, and milk fat. According to Davis and Drackley (1998) the result of this calculation is 1.16 kcal/g for colostrum and 0.69 kcal/g for milk. The increase in gross energy is critical for the calf at this time. The fat and lactose found in colostrum is necessary for thermogenesis and maintaining body temperature (Davis and Drackley, 1998).

Vitamins and Minerals

Colostrum is a concentrated source of the major and trace minerals and vitamins as compared to milk (Table 1). This is crucial for the development of the newborn's digestive system and the initiation of its own metabolism (Davis and

Drackley, 1998). Zinc, iron, folic acid, choline, and riboflavin are examples of some minerals and vitamins that are more concentrated in colostrum than milk (Akers, 2002). Vitamins A, D, and E do not cross the placental barrier in cows (Quigley and Drewry, 1998). Therefore, colostrum will be the calf's only source of these vitamins. For this reason, it is also important that the dam is supplemented with these vitamins to ensure that there are adequate amounts passed into the lacteal secretions.

Water

At birth, it is crucial that the calf is fed with colostrum in order to ensure that it does not dehydrate. Diarrhea is the leading cause of calf death in the U.S., however, it is not the diarrhea that kills the calf but the dehydration caused by this massive loss of liquid. Providing water, not only from colostrum and milk, but free choice as well, is critical in the survival of the calf.

Factors Affecting Colostrum Quality

Primarily Ig content, more importantly the IgG content, is used to evaluate colostrum quality. Knowing the content provides a basis to determine the amount the calf will consume and potentially absorb. Passive transfer is dependent on the IgG concentration of the colostrum (Nocek et al., 1984). High quality colostrum contains at least 50 g/L of IgG (Godden, 2008). There are several factors that can influence nutrient composition and Ig content in colostrum including parity, breed, management practices and environment.

Parity

Parity is believed to affect colostrum quality due to the difference in pathogen exposure between older and younger dams. The theory is that primiparturient heifers have not been exposed to as many antigens as older cows. Therefore, the antibodies against antigens are not present to be transferred into the colostrum. Gulliksen et al. (2007) reported that there was an increase in IgG concentration between cows of first or second parity in comparison to those with at least four parities, which corroborates other studies (Tyler et al., 1999; Moore et al., 2005).

Work done by Devery-Pocius and Larson (1983) indicated that total IgG₁ reached peak concentration (nearly double) in the third and fourth lactations. Concentrations of IgG₂ also increased with lactation number however these results were similar except for lactations 5-8. The IgM and IgA levels did not share this trend. Because IgG transport into the mammary gland is by a specialized transport mechanism, it can be hypothesized that this system may not be fully functional until later lactations.

Quantity

Quantity of colostrum produced at the first milking can influence the IgG concentration. Increased milk weight at first milking was negatively correlated with IgG₁ concentration (Pritchett et al., 1991). This is most likely due to a dilution effect of the increased milk volume.

Breed

Guy et al. (1994) used 15 beef (Charolais x Hereford) and 13 dairy (Holstein) cattle to compare IgG₁ concentrations in colostrum. The overall colostrum IgG₁ concentrations were greater for beef cows at 113.4 g/L compared to 42.7 g/L in dairy cows. While more IgG₁ was transferred from maternal circulation to the mammary gland of the dairy cows compared to the beef cows, final colostrum IgG₁ concentration was still greater in the beef cows. This reduction in IgG concentration is most likely due to a dilution effect associated with greater lactogenic activity of dairy cows.

Concentration of total Ig also varies between individual dairy breeds. Muller and Ellinger (1981) compared colostrum from 5 breeds of dairy cattle. Total Ig concentration of colostrum was lowest in Holstein cows (55.9 g/L). Numerically, Ig concentration for Holstein cows was lower than Guernsey (63.1 g/L) and Brown Swiss cows (65.7 g/L). Holstein cow colostrum concentrations were lower than Ayrshire (80.8 g/L) and Jersey cows (90.4 g/L) ($P < 0.05$). Jerseys were consistently highest ($P < 0.05$) in not only IgG₁ (66.5 g/L) but IgA (18.6 g/L) and IgM (5.3 g/L) as well. Genetics and/or dilution effects may be attributed to these breed variations.

Climate/Environment

As cows are exposed to temperatures outside their thermal neutral zone many of their normal bodily functions are compromised. Respiration rate, intake, fluid consumption, and metabolism are all affected. These all have an impact on colostrumogenesis and the efficacy of Ig transfer into the mammary gland. Cows

exposed to elevated environmental temperatures show a decrease in colostrum casein, lactalbumin, and reduced concentrations of IgG and IgA, however IgM and lactoglobulin were unaffected (Nardone et al., 1997). These researchers (Nardone et al., 1997) suggest that the decreased IgG transfer to the lacteal secretions is due to reduced blood flow to the mammary gland because of increased air temperature. Immunoglobulin A is thought to decrease due to the immune reactivity of the mammary plasmacytes, which are, decreased thus inhibiting IgA production (Nardone et al., 1997).

Management Factors

Management factors affecting colostrum quality relate to the care and health of the dam prepartum. One health concern is mastitis. Mastitic infections during the dry period can result in lower IgG content. New mastitic infections during the dry period can be avoided by proper care of the dry cow. This includes not only the use of antibiotics at dry off and teat sealants but also proper maintenance of the dry pens. Using antibiotics and teat sealants can prevent infection as well as reducing the ability for bacteria and other pathogenic organisms from entering the mammary gland. Maintaining dry pens also help in reducing the growth of bacteria. By keeping the pens clean and manure-free, the chances of bacterial or viral growth diminishes decreasing the chances of contracting environmental mastitis and other diseases.

Prepartum milking of heifers and cows has increased in popularity as a method to reduce udder edema. While this will relieve edema and benefit the dam, it also reduces colostrum quality. Immunoglobulin G transfer into the

mammary gland is nearly completed in the few weeks leading up to parturition. Pre-milking cows or excessive leaking from the teat prior to parturition results in the loss of IgG thus decreasing the quality of the colostrum (Kruse, 1970).

Other management factors that can potentially affect colostrum quality and composition include, diet, body condition score prior to calving (Odde, 1988) and the vaccination protocol for dry cows. Deficiencies in either energy or protein have been shown to negatively affect colostrum quality. Vaccinations can be beneficial not only to the dam but to the calf as well. When vaccinations are given they can increase specific IgG transfer to the colostrum, providing the calf with an immune boost (Davis and Drackley, 1998).

Testing Colostrum Quality

Testing colostrum is a quick and easy practice to ensure quality maternal colostrum is being fed. The colostrometer was developed by Fleenor and Stott (1980) and is a practical field method for measuring Ig concentration in bovine colostrum. In their experiment, Fleenor and Stott (1980) collected 14 colostrum samples from Holstein cows within 24 h postpartum and determined that there was a linear relationship between colostrum specific gravity and Ig concentration. However, there were no correlations between specific gravity and fat, non-protein nitrogen, or casein. The colostrometer can be used as a qualitative assessment of colostrum or as a method to estimate the amount of Ig present (Fleenor and Stott, 1980). It is a valuable tool for the producer as it provides a way to test colostrum and avoid failure of passive transfer due to the feeding of inferior colostrum.

Improving Colostrum Quality

Quality colostrum is classified as having a minimum concentration of 50 g of IgG/L (Besser et al., 1985 and 1990). Not all colostrum produced meets this qualification. In a study using colostrum from 1,250 cows, 57.8% of colostrum samples had less than 50 g of IgG/L (Gulliksen et al., 2007). In cases when the colostrum produced is of poor quality or the dam is unable to stand for milking, is leukosis or, Johne's positive, or has mastitis, there are alternatives. In these cases, colostrum supplements and replacers have been created for supplementation of colostrum or as a total colostrum replacement.

Colostrum Supplements

Due to the number of calves that do not achieve passive transfer and the lack of quality maternal colostrum, colostrum supplements were developed. These products are designed to provide supplemental IgG to the neonate during the time of macromolecular transport (Davenport et al., 2000). Colostrum supplements provide exogenous IgG from either bovine lacteal secretions, bovine serum, or eggs. These products are intended to supplement and provide <100g of IgG/dose, but not totally replace colostrum (Quigley et al., 2001)

Santoro et al. (2003) completed a study using 48 calves fed either colostrum ± trypsin inhibitor or a colostrum supplement ± trypsin inhibitor. The serum IgG concentrations were lower in calves fed the colostrum supplement compared to maternal colostrum. Their results indicate that the colostrum supplement did not provide as much serum IgG as colostrum (4.55 g/L vs. 14.6 g/L) at 24h of age.

Hopkins and Quigley (1997) completed a study to determine if the addition of a colostrum supplement to maternal colostrum affected absorptive efficiency and serum IgG concentration. Fifty two bull and heifer calves were blocked by sex and assigned to one of three types of feeding regimens: one feeding of 3.8 L of maternal colostrum ($\geq 200\text{g}$ of IgG), 1.9 L of maternal colostrum in two feedings, or 1.9 L of maternal colostrum in 2 feedings plus 272 g (25g of IgG) of colostrum supplement at the first feeding. Blood was collected via jugular venipuncture at 0, 24, and 48 hr to be analyzed for IgG. At 24 hr postpartum, serum IgG concentrations were lowest for calves fed 2 colostrum feedings with supplement compared to 2 feedings without supplement. At 48 hr, however, serum IgG levels did not differ among any of the treatments. This suggests that supplementation is unnecessary if high quality colostrum is available or that there may be a component of the supplement that binds or inhibits absorption of IgG. Shea et al. (2007) added lactoferrin to colostrum replacer and found a decrease in absorption. Lactoferrin may enhance intestinal development or reduce pathogenic bacteria in the small intestine. The cause of this is unknown however it appears that lactoferrin also has an inhibitory effect on IgG absorption when levels are elevated above normal colostrum concentrations. The results of both these studies infer that absorption of IgG could possibly be a more complex process than passive transfer.

Bovine Serum Products

Bovine Serum products are an inexpensive colostrum supplement. These products are derived from bovine blood from slaughterhouses, which is

considered a waste product. The high levels of IgG in blood make this a theoretically viable way of attaining IgG for these products.

Arthington et al. (2000) used bovine serum products, colostrum, and 2 milk-derived IgG supplements in a study to compare absorptive efficiency of IgG. At 24 hr plasma IgG levels were 12.1 g/L for colostrum, 2.2 and 3.5 g/L for the milk-derived supplements, and 6.8 g/L for bovine serum product. Despite plasma IgG levels being highest in colostrum-fed calves, apparent efficiency of absorption (AEA) was highest in calves fed the bovine serum product. This is related to the amount of IgG that was initially fed to calves. The researchers believe that these results indicate that in the absence of maternal colostrum, bovine serum products would be an acceptable supplement to feed. However, the 24h IgG levels were 6.8 g/L which is well below the recommended serum level of 10 g/L, this would indicate that passive transfer was not achieved therefore these products are not an acceptable supplement to feed.

Though these results suggest that serum derived IgG supplements are more effective than other types they are banned in some countries where the use of animal proteins is prohibited. This ban is due to the fear of diseases such as Bovine Spongiform Encephalopathy being transferred not only animal-to-animal but animal to human as well.

Colostrum Replacer

Although colostrum provides the calf's first antibodies against disease it can also be the source of its first exposure to bacteria and other pathogens. Many serious conditions can be passed from mother to calf through colostrum

including *Escherichia coli*, leukosis, and Johne's disease. Exposure to these organisms and diseases early on can be detrimental to the health of the calf and cause significant economical problems. The creation of colostrum replacers (CR) was to provide calves with the necessary antibodies for them to thrive when quality maternal colostrum is unavailable or when the dams are carriers for certain diseases transmitted via colostrum. Fulwider et al. (2007) reported that 94.7% of dairies surveyed fed colostrum while the remainder fed a CR due to the incidence of Johne's disease.

Colostrum replacer needs to provide >100 g of IgG/dose, which is the minimum amount required to achieve passive transfer (serum IgG concentration of >10.0 g/L at 24h; Quigley et al., 2001). A CR that contains only 100g of IgG/dose will not have adequate IgG to achieve passive transfer. A CR needs to not only contain adequate amount of Ig but also the nutrients necessary for the calf to thrive which include protein, energy, vitamins, and minerals.

In a study performed by Shea et al. (2007), passive transfer was compared between calves fed lacteal-based CR with or without supplemental lactoferrin. Calf feeding protocol was either one dose of CR (one dose = 105g of IgG) within 90 min of birth or two doses (one within 90 min of birth and a second at 12 hr). Calves fed one dose had an average serum IgG of 10.7 g/L at 24 hr. Calves fed two doses had an average serum IgG of 14.4 g/L at 24 hr. All calves receiving 2 doses of CR achieved passive transfer, however, when fed 1 dose, passive transfer was not always attained. These results indicate that CR is an

adequate alternative to maternal colostrum and can be fed as a total replacement with passive transfer still being achievable.

Despite CR being an acceptable alternative to maternal colostrum, uptake of IgG is still lower in the replacer compared to maternal colostrum. Swan et al. (2007) investigated passive transfer of IgG and preweaning health in Holstein calves fed either maternal colostrum or a plasma-derived CR. Maternal colostrum had an IgG concentration of 76.7 ± 30.0 g/L. Colostrum replacer contained 125g of IgG. The mean serum IgG concentrations were higher for calves fed maternal colostrum between days one and eight (14.8 ± 7.0 g/L) compared to those fed CR (5.8 ± 3.2 g/L). Many studies like this have been completed and result in similar outcomes (Smith and Foster, 2007).

Absorption of IgG from CR may be enhanced by the addition of sodium bicarbonate. Morrill et al. (2010) fed CR in two feedings, providing 132g of IgG \pm 20g of sodium bicarbonate at the first feeding and 66g of IgG \pm 10g of sodium bicarbonate in the second feeding. The researchers observed higher serum IgG levels ($P < 0.05$) for calves receiving supplemental sodium bicarbonate in CR compared to those calves that did not (16.3 vs. 13.2 g/L).

Part II: Maximizing Passive Transfer to the Neonate

Calves are born agammaglobulinemic meaning they lack immunoglobulins indicating that they have a naïve immune system. Due to the synepitheliochorial placenta, maternal antibodies are blocked from being passed to the calf. It is imperative that passive transfer is maximized in order to give calves the best chance of survival. During the first 24 hours the gut is considered open, meaning

it can absorb large macromolecules such as Ig and other intact proteins.

Colostrum needs to be provided as soon after birth as possible in order to ensure that absorption is maximized. Gut closure begins soon after birth and once complete no more Ig can be absorbed. Inadequate transfer of colostral IgG has been shown to reduce growth rates, increase risk of disease and death, increase risk of being culled and decrease milk production in their first lactation (Smith and Foster, 2007). Therefore, intake of colostrum is not only important for the calf's survival, but for its future production abilities.

The National Animal Health Monitoring and Surveillance (NAHMS) states, "that over 40% of calves had IgG levels below 1,000 mg/dl or had failure of passive transfer" (NAHMS, 2007). Mortality rates of preruminant calves (<8 wks of age) on farms should be no greater than 8% but nationally the average is around 10.5% (NAHMS, 2002). Considering the knowledge that has been acquired through research, these data are concerning.

Passive Transfer

There are 6 layers to the synepitheliochorial placenta of the cow that inhibit the transfer of serum Ig from dam to calf. Therefore, the calf is born without immunity unless there is damage to the placenta at which point some Ig may be transferred (Redman, 1979). Because of this, all Ig absorption occurs postnatally from the antibodies found in colostrum. Once the Ig reaches the intestine it is absorbed intact through the epithelium and into the circulation of the neonate. Most of this absorption occurs within the first 24 hours and decreases

rapidly after that point. The IgG absorbed in this time frame provides immunological protection to the calf until its own immune system matures.

Transfer of IgG to the Calf

Neonatal calves are considered pseudoruminants because their rumen is immature and nonfunctional at this state. Large molecular proteins and peptides are absorbed through the intestinal wall via pinocytosis, which decreases through the seventh day after birth. At this point, protein digestion by lysosomes within enterocytes is replaced by digestion in the lumen of the gastrointestinal tract and on the borders of the enterocytes (Blum, 2006). Stott et al. (1979) suggest that IgG absorption in the small intestine occurring within the first 24-36 hours is through non-selective endocytosis of macromolecules.

In calves, absorption of macromolecules from the intestinal lumen to the blood occurs in two phases: A) uptake or internalization within intestinal epithelium and B) transport or subsequent expulsion of the macromolecules into systemic circulation (Stott et al., 1979). There is some dispute about specificity during uptake of macromolecules. Stott et al. (1979) infers that the feeding of colostrum stimulates pinocytosis in the endocytes, which is quickly followed by cessation of macromolecule internalization. Sanglid (2003) suggests that there is an existence of intestinal selectivity among the IgGs and absorption does have specificity for certain macromolecules during endocytosis.

Using pig and lamb fetuses, Sanglid (2003) conducted a study evaluating the uptake of macromolecules by the intestine of the fetus during the last weeks in utero. He found that macromolecule uptake is present in the fetus but is

markedly increased in the neonate. This observation indicates that the ability to uptake and transfer intact proteins from epithelium to circulation is a specific process that develops near the time of parturition and is not solely a result of immaturity of the intestine. Sanglid (2003) also suggests that the degree of humoral immunological protection depends not only on the amount and timing of colostrum feeding, but also Ig quality, concentration and intestinal capacity of the calf to absorb Ig.

The cessation of colostral Ig permeability in the intestine was originally thought to occur spontaneously and increasing in rate of closure at 12h postpartum. Mean closure time was found to be near 24h (Stott et al., 1979). However, more recent research has shown that gut closure is a more energy dependant process considering that both hypoxia at birth (Tyler and Ramsey, 1991) and insulin-mediated hypoglycemia (Tyler and Ramsey, 1993) both delay gut closure. As the neonate increases in age, epithelial cells progressively lose their capability to perform pinocytotic activities, which reduces colostral uptake and transmission of colostral components into the circulation (Stott et al., 1979). Once IgG is absorbed it enters the lymphatic system and on into the peripheral circulation. This allows for IgG to remain effective in immune protection until the calf's own immune system matures (Redman, 1979).

While prolonging the absorption period may be beneficial in helping maximize the amount of Ig absorbed, it could also be detrimental due to the increase in ability for bacteria and other pathogens to be absorbed directly into

the blood stream of the calf. Shortening the period of absorption may help in decreasing the likelihood of bacterial uptake.

Rate of Absorption

Rate of absorption can be affected by age at first feeding of colostrum and amount of colostrum fed. These factors will determine the final serum IgG₁ concentration of the neonate. Shea et al. (2009) found that most of the IgG absorbed occurs before 12h after birth with highest rates occurring before 6h (Figure 2). They also found that feeding 2 feedings of colostrum 1 at 0h and another 12h later increased IgG uptake. In a survey issued by the USDA (2007), age at first feeding for all operations averaged around 3.3h postpartum (Table 2).

Amount of Absorption

To achieve successful passive transfer, it is recommended that a 43 kg Holstein calf be fed at least 100 g of IgG in the first feeding of colostrum (Davis and Drackley, 1998). However, because many producers do not test colostrum before feeding, it has been suggested that it be fed at 10-12% of the calf's body weight (Godden, 2008). Theoretically, this should ensure that adequate IgG is fed to achieve passive transfer.

Testing for Passive Transfer

Passive transfer can be determined in 2 manners: measuring serum IgG concentration or measuring serum protein levels. Serum IgG concentrations are considered to be the most accurate method of determining status of passive transfer. Serum IgG concentrations of ≥ 10 g/L is considered successful passive transfer in the calf. According to the USDA (2007), 45.2% of large dairies (>500

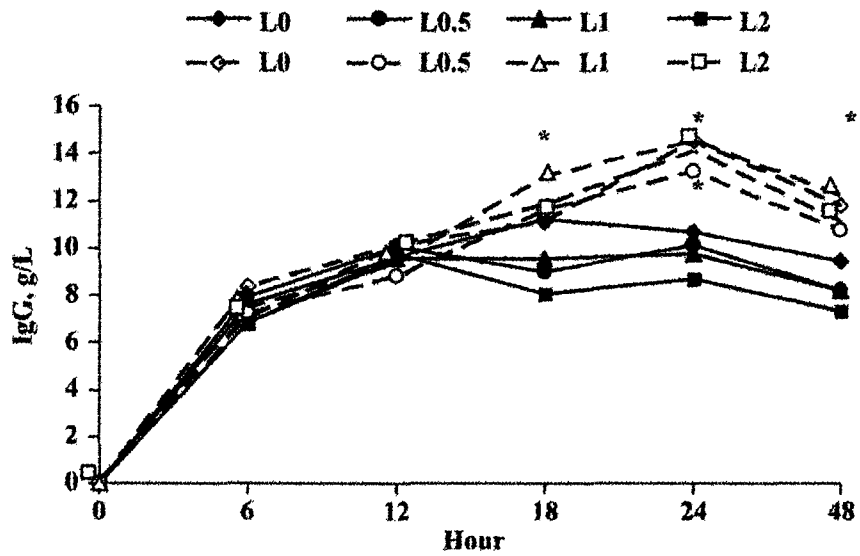


Figure 2. Serum IgG concentration (g/L) of calves fed 1 (closed symbol) or 2 (open symbol) doses of colostrum replacer with varying amounts of lactoferrin over the first 48 h of life. L0 = 0 g of lactoferrin, L0.5 = 0.5 g of lactoferrin, L1 = 1 g of lactoferrin, L2 = 2 g of lactoferrin. The largest SEM 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)

Table 2. Colostrum management practices by herd size

	Small (<100)	Medium (100-499)	Large (>500)	All Operations
Age at first feeding (h)	3.4	3.3	2.8	3.3
Estimate IgG levels (%)¹	7.6	19.8	45.2	13
Monitor serum IgG (%)²	1.1	2.4	14.5	2.1

(Adapted from USDA, 2007)

¹ Percent of herds that estimate IgG levels by herd size.

² Percent of herds that estimate serum IgG concentrations by herd size.

head) estimate IgG levels where only 7.6% of small dairies estimate IgG levels. Testing these levels 24-48h after birth can give the producer a way of evaluating individual calf health status (Hancock, 1985). Management procedures can also be evaluated by monitoring serum IgG concentrations when calf morbidity is elevated.

Serum protein levels can also be used as a determinant of passive transfer. Though less accurate, it does give producers a general idea of serum IgG concentrations. In this method, a serum protein concentration of at least 5.5 g/dL is desired. According to the USDA (2007), only 2.1% of U.S. dairy herds routinely monitor serum protein levels in their heifer calves. Larger dairies (>500 head, 14.5%) are leading the industry by checking serum protein levels regularly compared to small herds (<100 head) where only 1.1% routinely check serum protein concentrations (Table 2)

Factors that Affect Passive Transfer

Passive transfer is primarily affected by colostrum quality and quantity, and age at feeding (Nocek et al., 1984). Other factors that affect passive transfer include prepartum feeding of the dam, environment, calving ease (Stott et al., 1975), sex of calf (Donovan, 1986), and colostral pH (Quigley et al., 2000).

Colostral Ig Concentration

Besser et al. (1985) found a negative correlation between absorptive efficiency and mass of Ig, both IgG ($P < 0.001$) and IgM ($P < 0.01$), fed. In their study, calves that were fed a lower amount of IgG and IgM in colostrum absorbed a higher proportion of IgG and IgM than those fed a higher mass of IgG and IgM.

These results indicate that the transport system for IgG and IgM has a point at which it becomes saturated when overloaded. This may also indicate that there is a specific receptor for IgG and IgM since passive transfer, theoretically, should not reach a saturation point.

The apparent efficiency of absorption (AEA) may be decreased when a higher mass of IgG is fed. However, higher concentrations yield higher serum IgG concentrations. Jaster (2005) evaluated the quality, quantity, and timing of the colostrum feeding on IgG₁ absorption in Jersey calves. It was observed that higher concentrations of total IgG₁ ingested resulted in higher serum IgG₁ concentrations compared to calves fed lower concentrations. It was also noted that calves receiving 2 feedings of 2L of high IgG₁ colostrum at birth and at 12 h had higher AEA compared to calves receiving either 4L of high IgG₁ colostrum in 1 feeding or 2 feedings of 2L of low IgG₁ colostrum at birth and at 12h. High IgG colostrum contained 84 mg/mL and low IgG colostrum contained 31.2 mg/mL. Data indicated that IgG absorption was maximized in calves receiving 2 separate feedings of high quality colostrum.

Prepartum Feeding of Anionic Salts

Prepartum feeding of anionic salts to cows is a way to prevent metabolic disorders during the transition period. Unfortunately, while this is beneficial to the cow, it has been found that calves born from cows who have been fed anionic salts prepartum are born with respiratory and metabolic acidosis (Guy et al., 1996). Respiratory acidosis is caused when the lungs do not expel carbon dioxide sufficiently and therefore blood pH drops <7.2 (Bleul et al., 2007). This

condition can last for 24h postpartum. Guy et al. (1996) found that calves born from cows fed a cationic diet (+445 mEq/kg of DM) had higher serum IgG₁ levels at 24h postpartum than calves from cows fed anionic diets (+75 mEq/kg of DM).

Respiratory and metabolic acidosis has been shown to decrease AEA and the ability of the calf to achieve passive transfer (Quigley and Drewry, 1998) due to reduced IgG absorption (Boyd, 1989). Morrill et al. (2010) observed no effect on IgG absorption or AEA in calves born from dams fed anionic salts compared to calves born from dams not fed anionic salts. Morrill et al. (2010) fed control cows (no anionic salts) a +77 mEq/kg diet and treated cows (anionic salts) a -100 mEq/kg diet. As stated previously, Guy et al. (1996) fed diets that were +445 mEq/kg and +75 mEq/kg. The difference between the studies (Morrill et al., 2010; Guy et al., 1996) may have been dissimilar because of the difference in magnitude of the dietary cation/anion balance.

Vitamin C, Vitamin E, and Selenium

Kamada et al. (2007) found that addition of selenium (Se) to colostrum increased IgG absorption. When calves were fed 1.0 mg/kg of Se in each of 4 colostrum feedings 24h plasma IgG concentrations increased 20%, from 100 to approximately 120 (relative amount of IgG with no addition of Se = 100). Addition of 3.0 mg/kg of Se in 1 feeding of colostrum caused an increase of 42% compared to the average (100 to approximately 140). This increased concentrations of IgG lasted for 2 weeks postpartum. Kamada et al. (2007) believe that the increase was not caused by a nutritional but pharmacological

effect and hypothesize that the increase is due to the activation of intestinal epithelial cell pinocytosis.

When Se is fed prepartum, calves showed an increase in liver, blood and plasma Se concentrations (Abdelrahman and Kincaid, 1995). There is also an increase in Se found in the colostrum of dams fed Se during the last 60d prepartum. While Se helps with immune function, Abdelrahman and Kincaid (1995) did not measure IgG concentration however; immunity may have been improved due to Se influences on the immune response of animals.

Vitamin C and E are both antioxidants. Neither has shown to have a significant effect on IgG uptake. In a study done by Hidirolou et al. (1995), IgG uptake was unchanged by the supplementation of either vitamin. However, there was a trend for IgM to increase compared to control calves when both vitamins were supplemented.

Seasonal Effects

Immunoglobulin absorption is affected by season due to differences in environmental temperatures. In temperate climates it has been reported that average monthly serum IgG₁ concentrations are lowest in winter but increase during the spring and early summer peaking in September (Boyd, 1972; Gay et al., 1965; Gay et al., 1983). Donovan et al. (1986), observed that Ig absorption was highest in February and March and dropped due to the elevated temperatures during the summer months. This difference in outcome is most likely due to the Donovan et al. (1986) experiment being performed in a subtropical climate. It appears that heat stress may be the cause for these

results, however, the exact means by which environment affects the Ig and serum protein concentration is unknown (Donovan et al., 1986).

Colostrum pH

The average pH of maternal colostrum is 6.17 (Tsioulpas et al., 2007). The chemical composition of CR and supplements may have an effect on the efficiency of IgG absorption in the calf (Quigley et al., 2000). The AEA may differ among these replacers and supplements because of variation in ingredient composition and chemical characteristics such as pH and IgG concentration.

Quigley et al. (2000) studied the effect of differing pH levels in colostrum supplements on AEA. In this study, Calf Nutritional Colostrum Supplement (American Protein Corporation, Ames, IA), which is a product derived from bovine serum, was reconstituted with water and fed to calves. The final pH of this product was 7.5. Sodium citrate was used to lower the pH of the colostrum supplement from 7.5 to 7.0, 6.0, and 5.0. Blood samples were taken at 0 and 24h for IgG analysis. The 24 h plasma IgG levels for pH treatments 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% for the respective treatments suggesting that a change in pH between 5.0-7.5 in colostrum supplements will not alter IgG absorption. Though Quigley et al. (2000) did not see a significant change in IgG absorption when the pH was between 5.0 and 7.5, Morrill et al. (2010) reported a change. Using sodium bicarbonate to raise the pH of CR to approximately 7.0, they observed a 25% increase in IgG absorption. The difference in results was likely caused by the origin of the colostrum supplement/replacer. Quigley et al.

(2000) fed a serum-based product while Morrill et al. (2010) used a lacteal-based CR. The substance used to raise pH was also different between studies. Morrill et al. (2010) used sodium bicarbonate to increase the pH of the CR and Quigley et al. (2000) used sodium citrate to lower the pH. The bicarbonate may have provided a bacteriostatic effect that the sodium citrate could not. This will be discussed further in Part III.

Dystocia

Calves resulting from difficult births do not absorb Ig as well as calves resulting from normal births (Donovan et al., 1986). These calves may have some degree of hypoxia and acidosis due to a difficult calving, which could impede Ig uptake (Davis and Drackley, 1998).

Both Donovan et al. (1986) and Odde (1988) observed a decrease in neonatal serum protein concentrations in dystocia calves. Odde (1988) studied the effect of body condition at calving and difficulty of calving on calf vigor and Ig concentrations. Each calf was assigned a calving difficulty score between 1 and 3. Calves with a score of 1, meaning unassisted delivery, had higher IgG₁ and IgM serum concentrations compared to those calves with scores of 2 or 3. The cause of the lower serum IgG levels in dystocia calves could be tied to the increase in endogenous corticosteroid release and its subsequent effect on closure of the intestinal wall. The lower levels may also be more simply related to the fact that dystocia calves often lack vigor, therefore; their ability to nurse may be compromised. In order to help prevent failure of passive transfer in these

calves, managers should use an esophageal tube to feed any animals that do not or cannot suckle.

Sex of the Calf

Odde (1988) found that heifer calves had higher serum IgG₁ levels compared to bull calves (23.6 g/L and 20.4 g/L). This may be due to the increased likelihood of a difficult calving in bull calves since they tend to be larger (Odde, 1988).

Failure of Passive Transfer

Failing to achieve passive transfer can result in an increased risk of septicemia and mortality in the neonatal calf. It has been determined that a certain frequency of hypogammaglobulinemia cannot be avoided under practical farm conditions. This is due to the variations in birth weight, Ig concentration in colostrum, dose of colostrum, age at first feeding, and possibly due to a genetic predisposition for Ig absorption ability (Bush, 1980). Treatment with plasma (at a dosage of 20 mL/kg I.V.) can be given to calves that do not achieve passive transfer (Weaver, 2000). If this is not an option, the calf can still survive, if it is kept in a clean environment, with minimal exposure to any pathogens (Weaver, 2000). It is crucial to remember that even though the calf may have achieved passive transfer they should still be kept in a clean environment. If placed in a sullied area or where it could be exposed to bacteria and other pathogens, it still can become quite ill.

Part III: Sodium Bicarbonate

Sodium Bicarbonate (NaHCO_3) has been successfully used in correcting acidosis ($\text{pH} < 7.2$) in calves when injected intravenously (Bleul et al., 2005; Koch & Kaske, 2008; Michan et al., 1996).

Ayers and Besser (1992) evaluated the addition of alkalinizing agents and their effects on IgG absorption, blood-gas, and acid-base values. When alkalinizing agents were given prior to feeding colostrum, increases in blood pH (+0.04 units), PCO_2 (+4.1 mm of Hg), and HCO_3 concentrations (+4.4 mEq/L) were detected. Immunoglobulin G absorption was not affected by sodium bicarbonate treatment or by the alteration of the blood gas/acid base status.

Sodium bicarbonate has also been added to acidified and fermented colostrum to act as a buffer. Neonatal calves that were fed fermented colostrum had lower IgG concentrations compared to those that were fed buffered colostrum (Foley et al., 1978). Feed intake was improved in colostrum that had additional sodium bicarbonate whether it was acidified (Eppard et al., 1981) or fermented (Jenny et al., 1983). Some studies also suggest that sodium bicarbonate may help to improve performance of calves fed high concentrate diets pre- and post weaning (Kellaway et al., 1997 and Emerick, 1976).

Other than having feed intake effects, sodium bicarbonate has also shown to have an inhibitory effect on the growth of *Escherichia coli* 0111 (*E. coli*) in human milk and bovine colostrum (Bullen et al., 1972 and Griffiths and Humphreys, 1977). Bullen et al. (1972) found that sodium bicarbonate was bacteriostatic when added to increase the pH of human milk and bovine

colostrum (7.2 and 7.5 respectively). However, if amounts were added that only brought the pH up to 6.8 and 6.95 this effect was not seen. Griffiths and Humphreys (1997) discovered that it was the sodium bicarbonate itself, and not the pH change, that caused the bacteriostatic effect. This was done by altering the pH with either sodium bicarbonate or sodium hydroxide. Both samples of colostrum were treated until a pH of 7.4 is reached, however, only the sodium bicarbonate containing sample showed a bacteriostatic effect. The bacteriostatic effect is believed to be caused by the combined action of both antibody and lactoferrin and is dependent on sodium bicarbonate to counteract citrate, which is present in both milk and colostrum. Citrate has an iron-mobilizing effect that sodium bicarbonate stops (Griffiths and Humphreys, 1977). Because bacteria need iron to proliferate, preventing mobilization of iron would theoretically inhibit bacterial growth. If sodium bicarbonate was added to colostrum or CR in an amount that raised the pH to 7.2 it could reduce the risk of E. coli infection in the neonatal calf. It is also possible that the effect is due to the amount of sodium added. It takes much less sodium hydroxide to raise the pH compared to the sodium bicarbonate; therefore the amount of sodium itself could be the underlying cause for this difference.

Morrill et al (2010) fed calves CR with either no sodium bicarbonate or supplemented with 30 g of sodium bicarbonate fed over two feedings. Their study evaluated the effect of supplemental sodium bicarbonate on respiratory and/or metabolic acidosis in calves caused by the prepartum feeding of anionic salts to their dams. The results showed a higher serum IgG concentration at 24h

postpartum in the 30g supplemented calves compared to 0g (16.3 g/L vs. 13.2 g/L).

Conclusion

Vast amounts of research concerning calf health and management have been completed however, there has been little research done on the supplementation of sodium bicarbonate to the neonatal calf. The studies that have been completed show promising results in the improvement of calf health in terms of IgG uptake. Further investigation into this area could lead to an inexpensive, simple method of increasing IgG uptake and consequently increase calf health and survival.

CHAPTER II

ADDITION OF VARYING AMOUNTS OF SODIUM BICARBONATE TO COLOSTRUM REPLACER: EFFECTS ON IgG ABSORPTION, SERUM BICARBONATE, AND HEMATOCRIT IN NEONATAL CALVES

Introduction

Calves are necessary for the longevity of the dairy farm. They provide replacements for dairy cattle that are no longer productive. For this reason, calves need special attention to make sure that their health is not compromised so they may survive to breeding age and through their numerous lactations. Calves are born agammaglobulinemic, meaning they lack immunoglobulins indicating that they have a naïve immune system (Lopez et al., 1988). This is due to the type of placenta that all ruminants have. The synepithelialchorial placenta does not allow for Ig to pass to the fetus. The neonate is therefore susceptible to many health problems that can damage their future productivity or even be fatal.

It is critical to the health of the calf that they are provided adequate amounts of high quality colostrum. Colostrum is this first mammary secretion that provides Ig, such as IgG, which are essential to the health and well being of the animal. Immunoglobulin G is the primary Ig for passive immunization of the

neonate (Butler, 1969). Through colostrum, the calf receives not only IgG but also fat, lactose, minerals, vitamins, and water. Without these nutrients the calf would not be able to survive longer than 18h postpartum (Okamoto et al., 1986).

It is crucial that the calf is fed colostrum as soon as possible after birth in order to maximize passive transfer of IgG. Upon birth, calves should receive at least 4 L of colostrum within the first 24 h when the gut is open to absorb the Ig, it is within this time frame that passive immunity can be established through the absorption of IgG from the colostrum (Jaster, 2005). The efficiency of IgG absorption from 12 h to 24 h was only 31 % indicating that most absorption occurs from the initial feeding at or near birth (Shea et al., 2009).

Achievement of passive transfer is indicated by serum IgG concentrations being ≥ 10.0 g/L, anything less than that would indicate failure of passive transfer. By enhancing IgG absorption during the initial feeding there should be a health benefit to the calf, which will be reflected in the serum IgG concentration. Inadequate transfer of colostral IgG in neonatal calves, 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). It is apparent that intake of colostrum is not only important for the calf's initial survival but for its future production abilities.

Sodium bicarbonate has been added to acidified and fermented colostrum to act as a buffer. Neonatal calves that were fed fermented colostrum had lower serum IgG concentrations compared to those that were fed buffered colostrum (Foley et al., 1978). Feed intake was improved in colostrum that had additional

sodium bicarbonate whether it was acidified (Eppard et al., 1981) or fermented (Jenny et al., 1983). Some studies also suggest that sodium bicarbonate may help to improve performance of calves fed high concentrate diets pre- and post weaning (Kellaway et al. 1997 and Emerick, 1976). Sodium bicarbonate has also shown to have a bacteriostatic effect and specifically it has had inhibitory effects on the growth of *Escherichia coli* 0111 (*E. coli*) in human milk and bovine colostrum (Bullen et al., 1972 and Griffiths and Humphreys, 1977). A study performed by Morrill et al. (2010) indicated that feeding CR with supplemental sodium bicarbonate can increase IgG uptake. The researchers fed CR in 2 feedings, providing 132g of IgG ± 19.5g of sodium bicarbonate at the first feeding and 66g of IgG ± 9.75g of sodium bicarbonate in the second feeding. Morrill et al. (2010) observed higher serum IgG levels ($P < 0.05$) for calves receiving supplemental sodium bicarbonate compared to those that did not (16.3 vs. 13.2 g/L respectively).

The objectives of this study were to determine if the supplementation of 0, 15, 30, or 45g of sodium bicarbonate to CR would enhance the absorption of IgG 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 # 090702).

Fifty-two Holstein calves born from multiparous cows were used on this study. Calves were blocked by birth, and within each block, randomly assigned to

1 of 4 treatments within that block. The treatments were: 1) CR + 0g of sodium bicarbonate (control; **C**); 2) CR + 15g of sodium bicarbonate (**15g**); 3) CR+ 30g of sodium bicarbonate (**30g**); 4) CR + 45g of sodium bicarbonate (**45g**).

Calves were removed from their dams within 30 minutes after birth. Calves were weighed, had navels dipped in 7% iodine, and then placed in a naturally ventilated, enclosed calf room. Calves were individually housed in pens bedded with kiln-dried sawdust where they remained for the duration of the study (48h). Calves were also assigned a dystocia score of 1 to 3 based on the difficulty of the calving: 1 = unassisted calving, 2 = assisted easy calving, 3 = assisted difficult calving. Only calves with a calving score of 1 or 2 were used on this study. All calves received 1 feeding of Land O Lakes Colostrum Replacer (Table 3, Saskatoon Colostrum Company; Saskatoon, SK, Canada) at 0h (within 45m). Feeding method was requested by Saskatoon Colostrum Company. If this first feeding was not consumed, colostrum was kept warm and introduced again 1 hour later. If the remaining colostrum was not consumed in the second feeding, it was then fed via esophageal tube to the calf. Three hundred and forty grams of non-medicated milk replacer (Blue Seal Feeds, Inc.; Londonderry, NH) was reconstituted with 2L of water and fed to calves at 12, 24, 36, and 48h. Samples of both CR and milk replacer were sent to Agri-King (Fulton, IL) for nutrient analysis. Both samples were analyzed for crude protein (method 990.03; AOAC 2002), fat, lactose, Ca, P, Mg, K, Na, and Fe (Table 3). Fatty acids were determined by saponification with KOH in ethyl alcohol. Fatty acids were then liberated from the soaps with HCl and extracted with petroleum ether (AOAC,

1995). Lactose was determined using method 984.22 (AOAC,2002) with these modifications: flow rates though HPLC (Beckman, Fullerton, CA) was 1 mL, melibiose was the internal standard, and evaporative light scattering detection was used instead of a refractive index. Minerals analyzed were determined as described above (AOAC, 2002).

Blood Collection for Immunoglobulin G, Sodium Bicarbonate Assay, and Hematocrit

Blood samples were collected via jugular venipuncture before the initial feeding of colostrum replacer (within 45 min postpartum, referred to as 0h) and at 6, 12, 24, and 48h after birth. Samples were collected in 7 mL tubes. Three capillary tubes of blood were subsampled from the 7 mL sample tube. The remainder of the blood was allowed to clot at room temperature for no longer than 1h. Capillary tubes were centrifuged (Haematokrit 210; Andreas Hettich GmbH & Co; Germany) at 13000 rpm at 25°C for 5 min. The remaining blood sample was then centrifuged (CentraMP4R; International Equipment Company; Needham HTS, MA) at 3300 rpm at 25°C for 20 min. Serum samples were stored at -20°C until analyzed for IgG by radial immunoassay and bicarbonate concentrations (Stockham and Scott, 2008) using a Hitachi analyzer (Hitachi 912 Automatic Analyzer; Roche Diagnostics; Quebec, Canada).

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

(Phillips and Taylor, 1973) with the equation: 0.5 x difference in time x difference in IgG concentration.

Statistical Analysis

Calf IgG, bicarbonate, and hematocrit were analyzed using the MIXED procedure of SAS® (SAS Institute, 2001). The 24 h calf IgG, bicarbonate, hematocrit, AEA of IgG, AUC of IgG, and AUC of bicarbonate data were analyzed as a randomized complete block design using the MIXED procedure of SAS® (SAS Institute, 2001) according to the following model:

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

Where:

Y = the dependent variable

μ = the overall mean

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

S_j = the fixed effect of the j^{th} sodium bicarbonate level ($j = 0, 15, 30, 45$)

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

Degrees of freedom were calculated using the Kenward-Rogers option of the MIXED procedure of SAS® (SAS Institute, 2001). Single degree of freedom contrasts for linear and quadratic effects were determined. Significant treatment effects were noted at $P \leq 0.05$. Block was found to be not significant ($P > 0.25$) and removed from the model for all characteristics.

Results

Average body weight of all calves was 44.16 ± 5.48 kg. The pH of the CR treatments were C (6.1 ± 0.34), 15g (6.6 ± 0.09), 30g (6.9 ± 0.08), and 45g (7.1 ± 0.21). At birth the average serum IgG concentration for all calves was 0.49 ± 0.73 g/L. Serum IgG concentrations at 24 h had a negative linear trend ($P < 0.08$). Calves on the 30g treatment or on C had the highest 24h serum IgG

concentrations at 17.42 and 17.03 g/L respectively. Calves on the 15g treatment had 24 h serum IgG concentrations of 16.04 g/L, while calves on the 45g treatment had the lowest 24h serum IgG concentrations at 14.36 g/L (Table 4 and Figure 3). Of the 52 calves, 51 attained serum IgG concentration ≥ 10 g/L indicating successful passive transfer. The calf that did not achieve passive transfer was on the 45 g treatment and had a serum IgG concentration of 7.7 g/L at 24h. During the initial feeding: 4 calves were fed via esophageal feeder and 1 given 2 feedings of CR on C treatment, 1 calf was given 2 feedings on the 15g treatment, 3 calves were given 2 feedings on the 30g treatment, and 1 calf on the 45g treatment was fed via esophageal feeder.

Area under the curve for IgG indicated a negative linear response ($P < 0.03$). The AUC of IgG was lowest for those calves on the 45g treatment. Apparent efficiency of absorption of IgG had a negative linear response as well ($P < 0.05$). Calves on the 45g treatment had AEA values lower than the other 3 treatments. The negative linear results for the 24 h serum IgG concentrations, AUC, and AEA were likely a result of the 45g having such a profound negative effect on IgG absorption.

Serum bicarbonate concentrations numerically increased with increasing dosage (Table 4 and Figure 4). Area under the curve for serum bicarbonate had a positive linear response and was lowest in calves on the C treatment and highest in calves on the 45g treatments ($P < 0.0001$). Area under the curve for serum bicarbonate increased linearly ($P < 0.0001$) and quadratically ($P < 0.03$) with

increasing dosage . There were no differences in hematocrit among treatments (Table 4 and Figure 5).

Discussion

Serum IgG concentrations at 24 h were numerically higher for calves on C or 30g treatments (Table 4 and Figure 4). These results differ from Morrill et al. (2010) who observed higher serum IgG levels ($P < 0.05$) for calves receiving supplemental sodium bicarbonate compared to those that did not (16.3 vs. 13.2 g/L). The difference in serum IgG levels may have been due to the difference in feeding protocol. Morrill et al. (2010) fed CR in 2 feedings, providing 132g of IgG \pm 19.5g of sodium bicarbonate at the first feeding and 66g of IgG \pm 9.75g of sodium bicarbonate in the second feeding. In the present study, 211.6 or 216.8g of IgG (variation due to 2 lots of CR) were fed \pm sodium bicarbonate treatment at the 0h feeding. Jaster (2005) observed that feeding calves 2 feedings consisting of 2L of colostrum rather than one 4L feeding led to higher AEA levels and increased IgG absorption. This discrepancy may be due to a saturation of the system allowing for no further absorption to occur. Similarly, if sodium bicarbonate has any effect on IgG uptake the large amount of colostrum fed at the initial feeding (>200g of IgG) may have masked the effects of the treatments. This is in contrast to Morrill et al. (2010) who fed approximately 200g of IgG in 2 feedings.

Apparent efficiency of absorption of IgG at 24h indicated a negative linear response. The AEA of calves on the 45g treatment was lowest overall indicating that absorption was severely compromised in these calves. These results differ

from Morrill et al. (2010). They observed an increase in AEA in calves supplemented with 30g of sodium bicarbonate compared to those that were not fed sodium bicarbonate (31.2% vs. 26.1% respectively; $P < 0.05$). Because the AEA of the 45g treatment was lower than other treatments a negative linear response was observed. The difference between the results of Morrill et al. (2010) and the current study is most likely due to the feeding protocol.

Area under the curve for IgG showed a negative linear response. Serum IgG concentrations were lowest in the 45g treatment thus resulting in a low AUC value (Table 4 and Figure 3). This is contrary to data collected by Morrill et al. (2010) who reported an increase in AUC in calves that were supplemented with sodium bicarbonate.

The data from the 24 h serum IgG concentrations, AEA, and AUC all show a negative linear effect ($P < 0.05$) or trend ($P < 0.10$) (Table 4). This effect was caused by the 45g treatment having a profound negative impact on absorption. If the 45 g treatment is excluded then a numeric positive response can be seen (Figure 6 and 7). The data from the 30 g treatment shows the mean 24 h IgG concentration of 17.16 g/L compared to 16.85 g/L for C and 16.04 g/L for 15g treatment. The highest serum IgG concentration was 23.10 g/L on 30g treatment, which is higher than the concentrations for either C or 15g, or 45g treatments (20.10, 18.50 and 18.8 g/L respectively). There may be a positive response to the 30g treatment however the amount of CR fed at one time may be masking this effect. The results clearly indicate that 45g of sodium bicarbonate is detrimental to IgG absorption. This suggests that at 45g of sodium bicarbonate,

the calf may be experiencing some type of alkalosis causing a decrease in the calf's ability to absorb IgG.

The serum bicarbonate levels were similar regardless of sodium bicarbonate dosage (Table 4 and Figure 4). Data collected from the University of Saskatchewan (Saskatoon, Saskatchewan, Canada) indicated that the normal bicarbonate range for dairy cattle 3-7 years old is 17-33 mmol/L. Calves in this study peaked at 33 mmol/L despite increasing sodium bicarbonate dosage. This peak could be due to the body's physiological response to elevated bicarbonate levels. High levels of serum bicarbonate can be associated with metabolic alkalosis or as a compensation for acidosis (Stockham and Scott, 2008). Serum bicarbonate levels will be raised in an effort to return the body to normal physiological pH in cases of acidosis. Typically, acidosis is associated with difficult calvings (Davis and Drackley, 1998). Acidosis as the cause is unlikely in this study because only calves with a calving ease score of 1 or 2 were used. Metabolic alkalosis may have been attained at the 45g treatment.

Area under the curve for serum bicarbonate levels showed a linear effect and were highest for 30 and 45g treatments compared to C and 15g treatments (Table 4 and Figure 4). High levels of serum bicarbonate can be associated with metabolic alkalosis or as a compensation for acidosis (Stockham and Scott, 2008) Over the experimental period, serum bicarbonate levels were raised in an effort to return the body to normal physiological as reflected by AUC

Hematocrit data showed no treatment effect. This may be attributed to the body itself being dynamic. Hydration status can be affected by several bodily

functions and mechanisms that tracking a treatment effect is very difficult. However, through the 48 h study all calves showed a numerical decrease in hematocrit (Figure 5). Hematocrit is a measure of packed cell volume, which is an indicator of hydration status. Though no treatment effect was seen, all hematocrit readings decreased over the 48h period. This result indicates that that the calves became more hydrated during the study. Hematocrit levels tend to be increased in calves within the first few days postpartum before decreasing to an average of 0.23 – 0.31 L/L for adult cattle. Brun-Hansen et al. (2006) observed that calves' hematocrit was similar to adult cattle 3 – 4 days postpartum.

Several calves had above normal 0 h IgG levels. Typically, calves are born without any IgG present in their blood. However, 8 calves were born with 0 h serum IgG levels ≥ 1 g/L. The cause of this is unknown. However, abnormal or damaged placentation can cause some transfer of maternal IgG to the calf in utero (Redman, 1979). Though the dams of these calves did not have retained placentas there may have been some damage to the placenta that was undetected. Chigerwe et al. (2008) reported that 52.9% of 170 Holsteins calves used had ≥ 0.16 g/L IgG before ingestion of colostrum. The mean IgG concentrations detected in their study was 0.64 ± 0.05 g/L however the concentrations detected ranged from 0.16 to 2.34 g/L. In the present study 0h IgG was 0.49 ± 0.73 g/L and ranged from 0.1 to 3.8 g/L. This would indicate that there is some type of transplacental transfer, or production of IgG by the calf after transplacental or transcervical exposure. Calves in utero exposed to infection have shown increases in serum IgG and IgM (Chigerwe et al. 2008). This may be

the cause of the elevated IgG in the current study's calves however no illness was noted in the dams of calves with elevated 0 h IgG concentrations.

Conclusion

Addition of sodium bicarbonate to CR had a negative effect on IgG absorption in high doses. Addition of 45g of sodium bicarbonate to CR resulted in reduced IgG absorption and efficiency of absorption of IgG. Area under the curve for serum bicarbonate increased with increasing amounts of sodium bicarbonate fed which would be expected with increasing doses. Future research in this area should focus on feeding protocols with or without addition of sodium bicarbonate and their effects on IgG absorption. This would help to elucidate if sodium bicarbonate is an effective supplement in different feeding regimens.

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

Item	CR ¹	MR ²
DM, %		
CP, %	55.32	21.40
Fat, %	20.90	20.47
Lactose, %	12.48	41.11
Ca, %	0.95	1.01
P, %	0.80	0.76
K, %	0.68	1.75
Mg, %	0.13	0.15
Na, %	0.28	0.87
Zn, mg/kg	75	113
IgG (g/dose)	211.6 ³ /216.8 ⁴	---

¹ Land O Lakes Colostrum Replacer. Saskatoon Colostrum Company; Saskatoon, SK, Canada

² Milk Replacer, non-medicated. Blue Seal Feeds. Londonderry, NH.

³ Calves 1-7 were fed this concentration

⁴ Calves 8-52 were fed this concentration

Table 4. Mean body weight, 24 h serum IgG concentrations, area under the curve (IgG), apparent efficiency of absorption (IgG), 24 h serum bicarbonate concentrations, area under the curve (serum bicarbonate), and 24 h hematocrit of calves fed varying amount of sodium bicarbonate.

Item	C	Treatments ¹			SE ²	Contrast	
		15	30	45		Linear	Quadratic
Initial BW	42.82 ± 4.40	45.36 ± 5.40	42.41 ± 5.03	44.81 ± 5.94			
Serum IgG g/L	16.85	16.04	17.16	14.52	0.75	0.08	0.22
	13.30	12.00	12.00	7.70			
High	20.10	18.50	23.10	18.80			
AUC ³ IgG g/L*h	679.92	622.15	681.99	561.72	29.06	0.03	0.29
AEA ⁴ , %	32.09	30.34	31.34	27.18	1.51	0.05	0.43
Serum Bicarbonate mmol/L (24 h)	28.97	31.67	33.03	33.10	4.67	0.28	0.49
AUC ³ Bicarbonate mmol/L*h	1277.31	1405.15	1465.62	1475.23	25.58	<0.0001	0.03
Hematocrit, %	30.60	32.53	31.58	30.19	1.84	0.79	0.36

¹ Treatments: C = 0g of sodium bicarbonate; 15 = 15g sodium bicarbonate; 30 = 30g sodium bicarbonate; 45 = 45 g sodium bicarbonate.

² Standard error.

³ AUC = Area under the curve; 0.5 x difference in time x difference in IgG concentration (Phillips and Taylor, 1973).

⁴ AEA = Apparent efficiency of absorption; (plasma IgG [g/L] x body weight [kg] x 0.09/IgG intake) x 100% (Quigley et al., 1998).

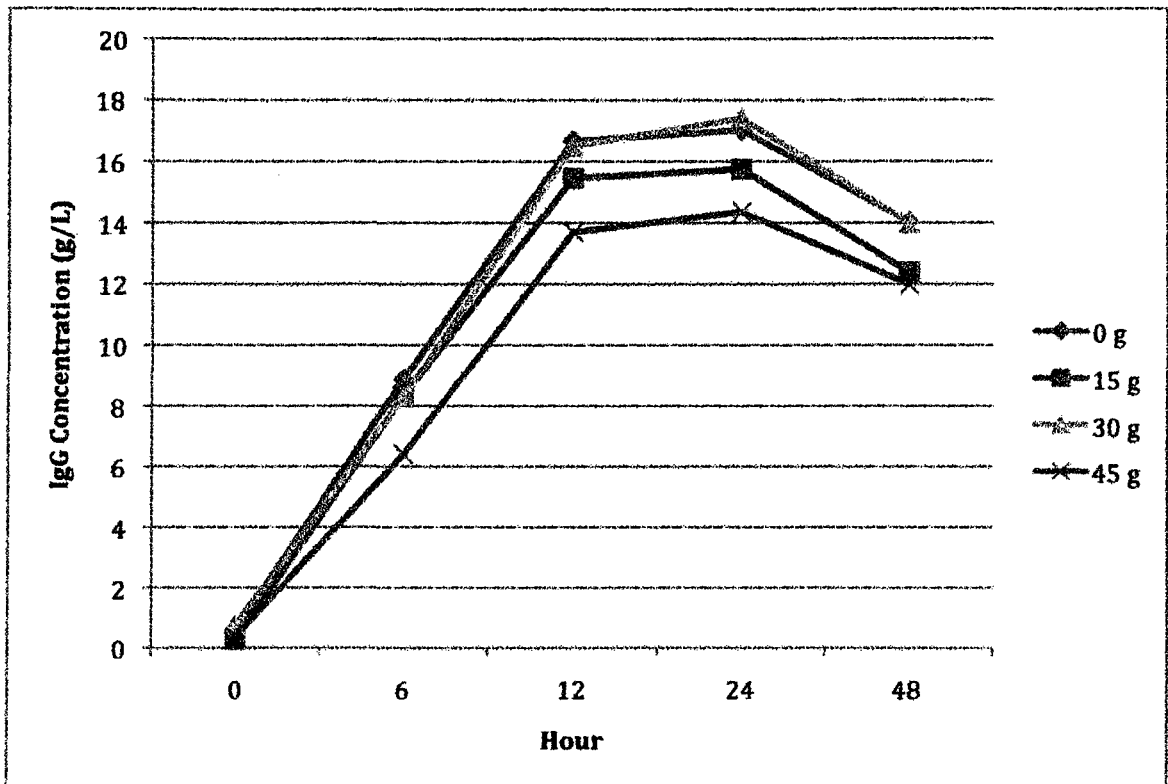


Figure 3: Serum IgG concentration for calves receiving 0, 15, 30, or 45g of sodium bicarbonate added to the colostrum replacer. Calves receiving 0 or 30g of sodium bicarbonate added to colostrum replacer had the highest (numerically) 24h serum IgG concentrations.

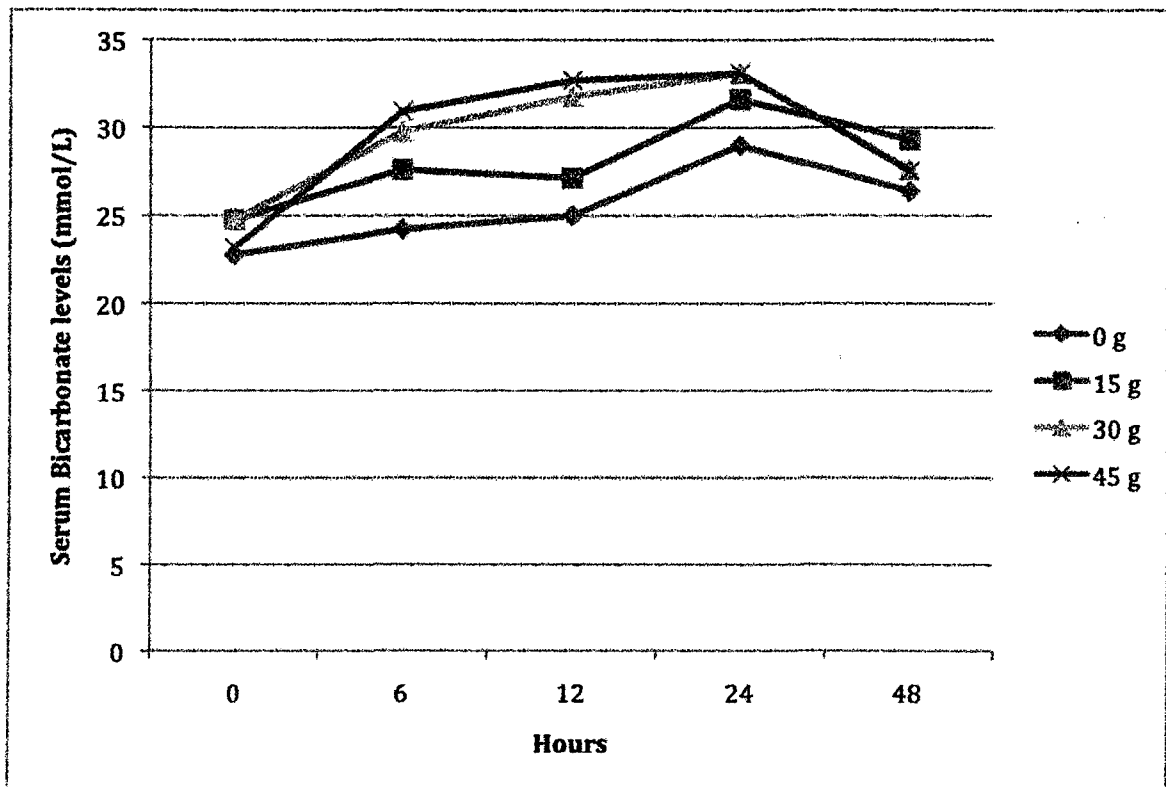


Figure 4: Serum bicarbonate levels for calves fed 0, 15, 30, or 45g of sodium bicarbonate added to colostrum replacer. As sodium bicarbonate was increased serum bicarbonate AUC increased linearly ($P < 0.0001$) and quadratically ($P < 0.03$).

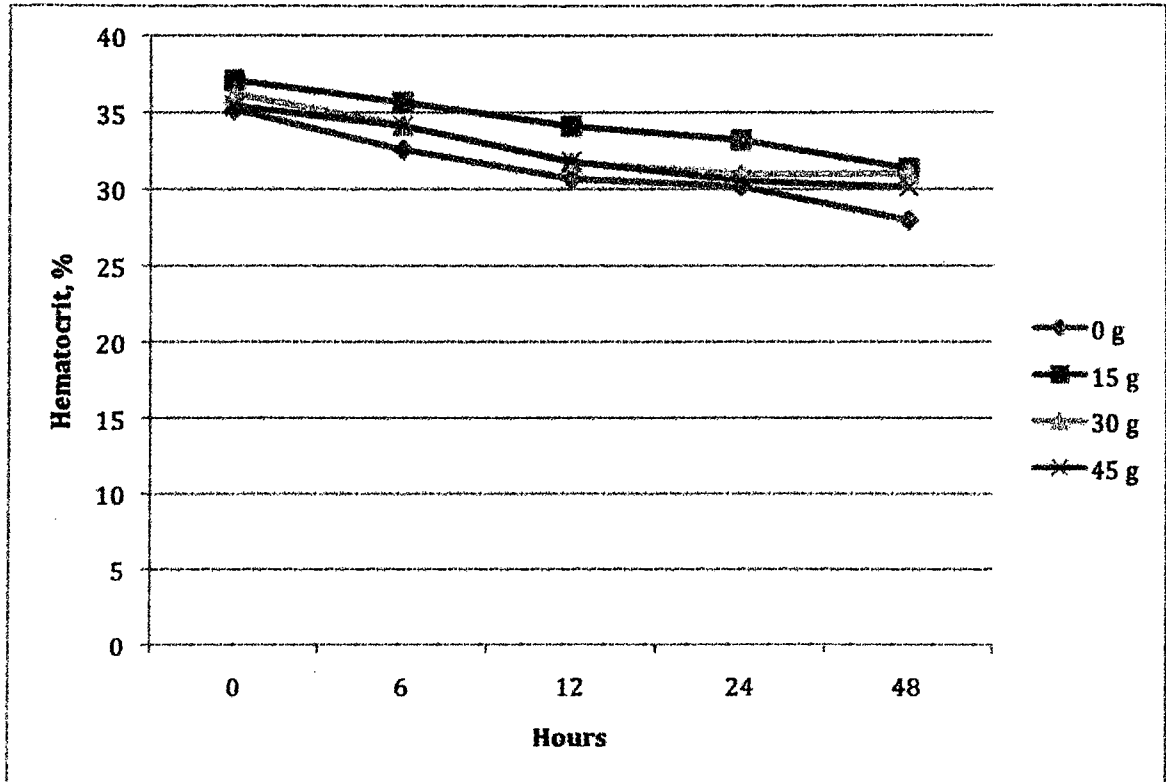


Figure 5: Hematocrit for calves fed 0, 15, 30, or 45g of sodium bicarbonate added to colostrum replacer.

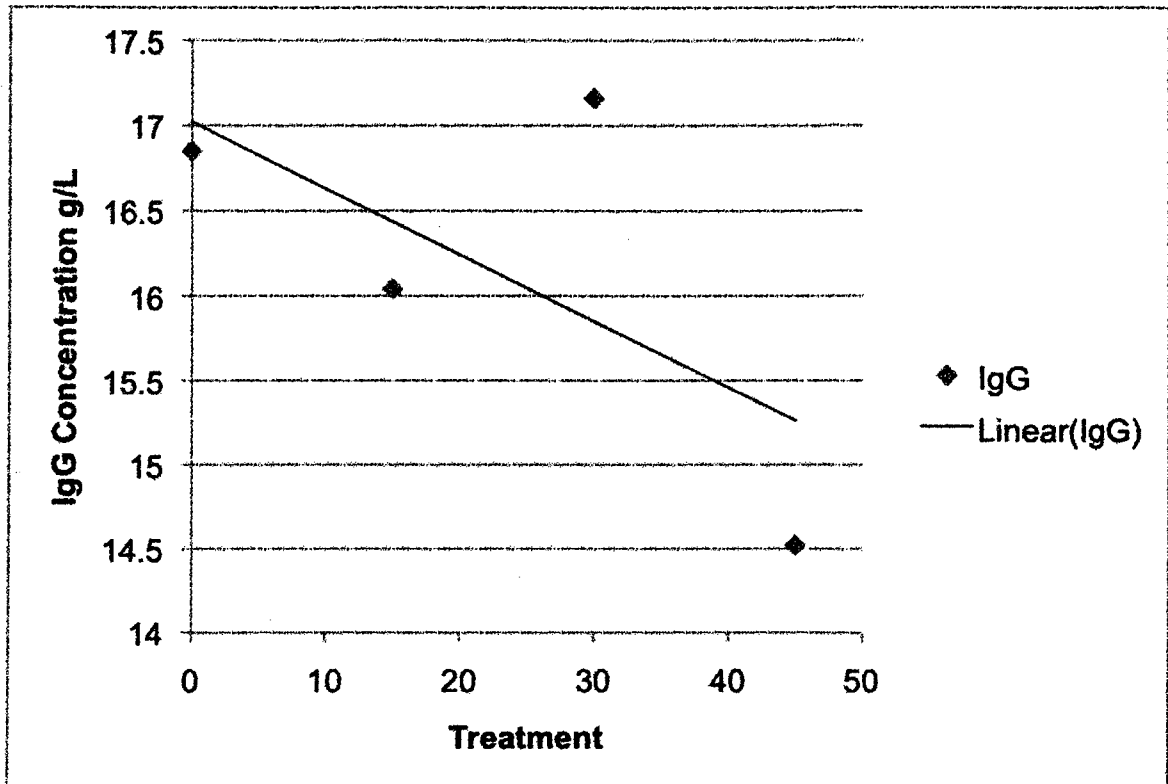


Figure 6: IgG trend line with 45g treatment included. The 45g treatment is profoundly negative causing the trend line to slope negatively.

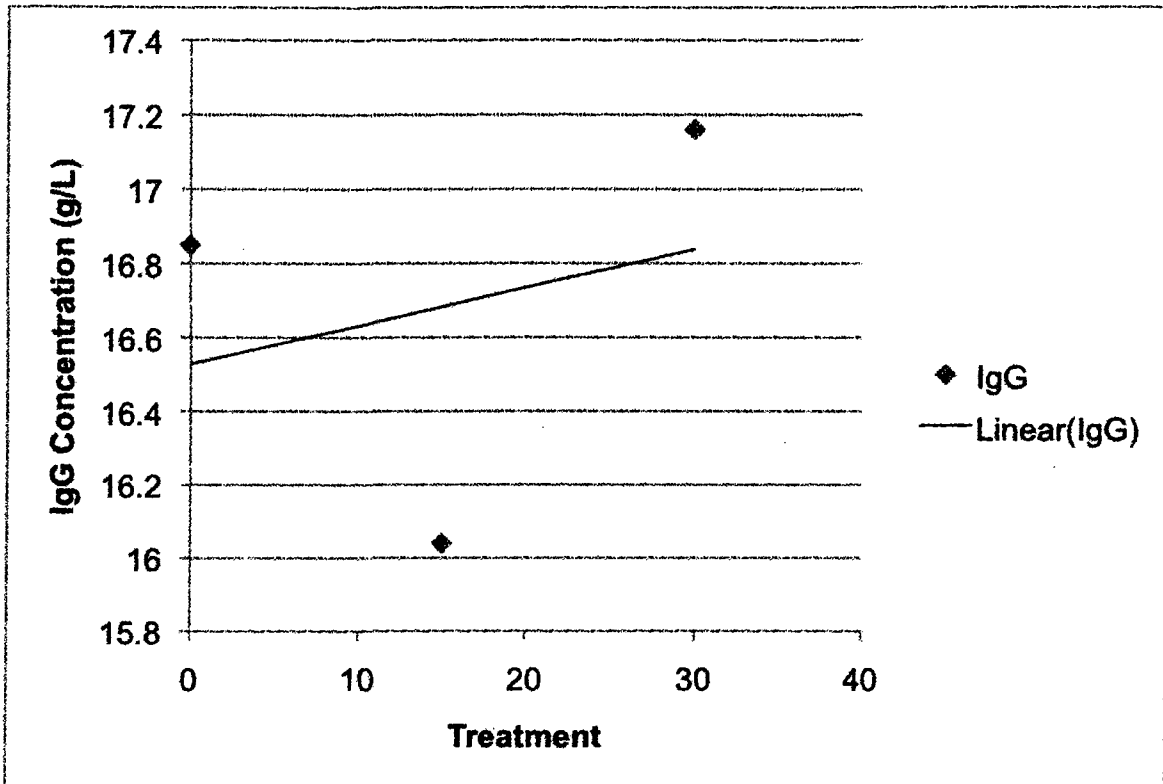


Figure 7: IgG trend line without 45g treatment included. Without the 45g treatment the trend line has a positive slope which is in agreement with work previously done by Morrill et al. (2010).

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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

25-Aug-2009

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

IACUC #: 090702

Project: Does adding sodium bicarbonate to colostrum replacer increase IgG uptake?

Category: C

Approval Date: 15-Jul-2009

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

APPENDIX B

Newborn Calf Experimental Protocol

1. Use only calves born in the maternity pen
2. Use calves with a dystocia score of 1 or 2.
3. Remove calf and weigh on calf scale before placing in calf pen.
4. Dip navel with 7% iodine.
5. Use 22-gauge needle when taking blood from the calf's jugular vein.
6. Always use alcohol wipes to prevent infection.
7. Feed recommended amount of colostrum or colostrum replacer to provide adequate IgG.
8. Try to feed within 1 hour postpartum, use esophageal feeder if calf does not drink voluntarily.
9. Keep bottles clean.
10. Keep calf pens clean to prevent spread of disease.