Spring 2007

Serum IgG concentrations of neonatal calves fed colostrum replacer supplemented with lactoferrin

Erin C. Shea

University of New Hampshire, Durham

Follow this and additional works at: https://scholars.unh.edu/thesis

Recommended Citation
https://scholars.unh.edu/thesis/281

This Thesis is brought to you for free and open access by the Student Scholarship at University of New Hampshire Scholars' Repository. It has been accepted for inclusion in Master's Theses and Capstones by an authorized administrator of University of New Hampshire Scholars' Repository. For more information, please contact nicole.hentz@unh.edu.
Serum IgG concentrations of neonatal calves fed colostrum replacer supplemented with lactoferrin

Abstract
The objective of this experiment was to determine the effect of various levels of lactoferrin supplementation in colostrum replacer fed as 1 or 2 doses on apparent efficiency of IgG absorption, serum IgG concentrations, and intestinal development in neonatal Holstein bull calves. Eighty Holstein bull calves were assigned to a 2 x 4 factorial arrangement of treatments in a completely randomized block design. Bull calves were collected at two locations, Fairchild Teaching and Research Center at the University of New Hampshire (n = 48) and a commercial dairy (n = 32). All calves were fed colostrum replacer (CR) according to manufacturer's recommendations within 90 min of birth with or without lactoferrin treatment. Lactoferrin treatments were 0 (control), 0.5, 1, and 2 g/d. At 12 h of age calves were fed either a second feeding of CR with or without Lf or 2 L of milk replacer (MR) with or without Lf. Calves continued to be fed MR with or without Lf in 12 h increments throughout the duration of study. Calves from the commercial dairy were removed from study at 48 h, while calves born at the university were removed from the study at 72 h. Blood samples were collected for IgG at 0, 6, 12, 18, 24, and 48 h. On average, all calves attained blood serum IgG concentration ≥ 10 mg/mL resulting in successful passive transfer, except for calves fed 1 dose of CR 1 or 2 g/d of supplemental Lf. However, there was an effect on CR feeding with calves fed 2 doses of CR having significantly higher blood serum IgG concentrations. There was no effect of Lf supplementation on plasma IgG and serum protein. There was a decrease in apparent efficiency of absorption (AEA) of IgG when 2 doses of CR were fed versus 1 dose of CR. Lactoferrin supplementation resulted in a suppressing quadratic effect on AEA of IgG. At the sixth feeding in which calves were 60 +/- 2 h of age, D-xylose (0.5 g/kg BW) was mixed with the MR with or without supplemental Lf (UNH calves) to determine xylose absorption by the small intestine. Blood samples were collected before xylose was fed at 0, and 2, 4, 6, 8, and 12 h after feeding xylose, for measurement of plasma glucose and xylose concentrations. Xylose means and area under the curved showed Lf to have a quadratic effect on xylose absorption with 0.5 and 1 g/d of supplemental Lf having lower plasma xylose concentrations then calves fed 0 and 2 g/d of supplemental Lf. There was no effect of CR feeding or supplemental Lf on plasma glucose concentrations. This study indicates supplementing Lf to a CR has a negative effect on both serum IgG and xylose absorption, demonstrating supplemental Lf is not beneficial to the neonatal calf.

Keywords
Agriculture, Animal Culture and Nutrition

This thesis is available at University of New Hampshire Scholars' Repository: https://scholars.unh.edu/thesis/281
SERUM IgG CONCENTRATIONS OF NEONATAL CALVES FED COLOSTRUM REPLACER SUPPLEMENTED WITH LACTOFERRIN

BY

ERIN C. SHEA
Bachelor of Science, University of Massachusetts-Amherst, 2005

THESIS

Submitted to the University of New Hampshire in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Animal Science

May, 2007
INFORMATION TO USERS

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleed-through, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.
This thesis has been approved and examined.

Thesis Director, Dr. Peter S. Erickson
Associate Professor of Animal and Nutritional Sciences

Dr. Charles G. Schwab
Professor of Animal and Nutritional Sciences

Dr. Hugh Chester-Jones
Associate Professor Dairy and Beef Production Systems
University of Minnesota, Waseca, MN

5/4/07
Date
ACKNOWLEDGMENTS

First I would like to thank the Department of Animal and Nutritional Sciences and the University of New Hampshire for providing me with an assistantship, providing me with the opportunity to teach and conduct research.

I would like to thank my advisor, Dr. Peter Erickson for taking me on as a graduate student and increasing my knowledge of dairy science. He was always available to answer a question and give his advice. I am grateful for his support and faith in my future success.

Dr. Schwab: For always having a friendly smile and taking time out of his busy schedule to answer any question.

Dr. Hugh Chester Jones: For taking time to come to UNH and assisting me in my research.

Nancy Whitehouse: For providing me with her guidance and her friendship throughout my time at UNH. She was always there to help me with my study and provided me with the opportunity to assist with other experiments. I will miss your candy dish and our Thursday lunches. I enjoyed getting to know her family and I always felt welcome. Thanks for everything little lady!

Jon Whitehouse: Thanks for keeping my bull calves around, even when the calf barn was full of them. I always knew I could walk down to the barn and get a good laugh from one of your jokes, even if I was the joke©.
To the rest of the staff at the Fairchild Teaching and Research Center: Thank you for calling me and letting me know when a bull was born. Also, for moving the calf away from its mother if I could not get there right away.

To my fellow graduate students Sue Marston, Sarah Boucher, and Kim Morrill: Thanks for always giving your advice and assistance. You would all take time out of your day to review a paper I was writing or let me practice my seminars. I have learned a lot from you all and wish you all the success and happiness in whatever path you choose.

To Holli, my fellow calf researcher: You were always willing to help, from taking a blood sample to giving me food and letting me sleep on your couch. I know you will be a great vet someday.

To Russ and Tom at ATH-MOR Holsteins thanks for allowing me to use your bull calves for my study. Thanks for taking the time to call me and letting me know when a calf was born. I still might be bleeding calves if I did not used 32 of your bulls.

To Debbie Haines at University of Saskatchewan for providing me with the colostrum replacer and analyzing all my blood samples for IgG.

To my undergraduate professor Dr. Huyler for introducing me to the topic of animal nutrition and for recommending Dr. Erickson take me on as a graduate student.

To Jeff Doherty and Ray Putnam at MPAL thank you for introducing me to research. I would not be where I am today if you had not hired me on as your dishwasher and provide me with the opportunity to conduct research.
Last but not least to my family for always giving me their love and encouragement. Knowing that I could always count on you all for anything, gave me the support I needed to accomplish my goals. Being the youngest has allowed me to watch and learn from all your success and mistakes. I have five excellent role models and could never have done this without each and every one of you.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS.................................................................iii

LIST OF TABLES..............................................................................ix

LIST OF FIGURES..............................................................................x

LIST OF ABBREVIATIONS..............................................................xi

ABSTRACT......................................................................................xii

CHAPTER PAGE

I. REVIEW OF LITERATURE

Part 1. Nutritional and Immunological needs of the calf.............................1

   Introduction..................................................................................1

   Placental Transfer.........................................................................2

   Characteristics of Neonatal Calf.....................................................3

   Immunity Overview......................................................................4

   Immunoglobulin Structure and Function...........................................5

   Colostrum...................................................................................8

   Colostral Nutrients......................................................................10

   Passive Transfer........................................................................12

   Factors Effecting Passive Transfer................................................15

   Disease Risks Associated with Colostrum Feeding...........................18

   Colostrum Replacer and Supplements............................................20

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
LIST OF TABLES

Table 1. Nutrient analysis of dried colostrum powder and milk replacer........................50

Table 2. Overall means for IgG, serum protein, xylose, and glucose. Apparent efficiency of absorption and area under the curve for IgG, xylose, and glucose.............51
LIST OF FIGURES

Figure 1. Serum IgG concentrations (mg/mL) of calves fed either 1 or 2 doses of colostrum replacer with varying levels of lactoferrin over the first 48 h of life.................52

Figure 2. Serum protein concentrations (mg/mL) of calves fed either 1 or 2 doses of colostrum replacer with varying levels of lactoferrin over the first 48 h of life.................53

Figure 3. Plasma xylose concentrations (mg/mL) of calves fed either 1 or 2 doses of colostrum replacer with varying levels of lactoferrin over the first 48 h of life.................54

Figure 4. Plasma glucose concentrations (mg/mL) of calves fed either 1 or 2 doses of colostrum replacer with varying levels of lactoferrin over the first 48 h of life.................55
LIST OF ABBREVIATION

CR = colostrum replacer
MR = milk replacer
FPT = failure of passive transfer
Lf = lactoferrin
BW = body weight
DM = dry matter
AEA = apparent efficiency absorption
AUC = area under the curve
d = day
h = hour
kg = kilogram
mL = milliliter
dL = deciliter
ABSTRACT

SERUM IgG CONCENTRATIONS OF NEONATAL CALVES FED COLOSTRUM REPLACER SUPPLEMENTED WITH LACTOFERRIN

by

Erin C. Shea

University of New Hampshire, May 2007

The objective of this experiment was to determine the effect of various levels of lactoferrin supplementation in colostrum replacer fed as 1 or 2 doses on apparent efficiency of IgG absorption, serum IgG concentrations, and intestinal development in neonatal Holstein bull calves. Eighty-Holstein bull calves were assigned to a 2 x 4 factorial arrangement of treatments in a completely randomized block design. Bull calves were collected at two locations, Fairchild Teaching and Research Center at the University of New Hampshire (n = 48) and a commercial dairy (n = 32). All calves were fed colostrum replacer (CR) according to manufacturer's recommendations within 90 min of birth with or without lactoferrin treatment. Lactoferrin treatments were 0 (control), 0.5, 1, and 2 g/d. At 12 h of age calves were fed either a second feeding of CR with or without Lf or 2 L of milk replacer (MR) with or without Lf. Calves continued to be fed MR with or without Lf in 12 h increments throughout the duration of study. Calves from the commercial dairy were removed from study at 48 h, while calves born at the
university were removed from the study at 72 h. Blood samples were collected for IgG at 0, 6, 12, 18, 24, and 48 h. On average, all calves attained blood serum IgG concentration $\geq 10$ mg/mL resulting in successful passive transfer, except for calves fed 1 dose of CR 1 or 2 g/d of supplemental Lf. However, there was an effect on CR feeding with calves fed 2 doses of CR having significantly higher blood serum IgG concentrations. There was no effect of Lf supplementation on plasma IgG and serum protein. There was a decrease in apparent efficiency of absorption (AEA) of IgG when 2 doses of CR were fed versus 1 dose of CR. Lactoferrin supplementation resulted in a suppressing quadratic effect on AEA of IgG. At the sixth feeding in which calves were 60 ± 2 h of age, D-xylose (0.5g/kg BW) was mixed with the MR with or without supplemental Lf (UNH calves) to determine xylose absorption by the small intestine. Blood samples were collected before xylose was fed at 0, and 2, 4, 6, 8, and 12 h after feeding xylose, for measurement of plasma glucose and xylose concentrations. Xylose means and area under the curved showed Lf to have a quadratic effect on xylose absorption with 0.5 and 1 g/d of supplemental Lf having lower plasma xylose concentrations than calves fed 0 and 2 g/d of supplemental Lf. There was no effect of CR feeding or supplemental Lf on plasma glucose concentrations. This study indicates supplementing Lf to a CR has a negative effect on both serum IgG and xylose absorption, demonstrating supplemental Lf is not beneficial to the neonatal calf.
CHAPTER I

REVIEW OF LITERATURE

Part 1: Nutritional and Immunological needs of the Neonatal Calf

Introduction

The first step in successful heifer management begins with the first colostrum feeding. High quality maternal colostrum is designed to meet the nutritional and immunological needs of the neonatal calf. If high quality colostrum is not fed to the calf at birth, the calf will have a higher chance for morbidity and mortality. The three largest costs during the first three months of age are feed, labor, and medication (Quigley, 1996b). These costs are reduced if the calf receives high quality colostrum in the first 24 h of life. This is due to successful passive transfer of IgG, resulting in blood serum IgG concentrations ≥10mg/mL. Failure of passive transfer (<10mg/mL) will not only result in economic loss during the first 3 months but throughout the animals entire life. Calf weights at 60, 120, and 180 d have been positively correlated with serum immunoglobulins obtained by successful passive transfer (Odde, 1986). There is also a correlation between failure of passive transfer and a negative impact of future milk production and an increase in mortality among dairy cows (Porto et al., 2007).

These findings have led to research related to colostrum management and factors affecting IgG absorption. Products such as colostrum replacers and supplements have been developed that will either provide both nutritional and immunological needs of the
calf or aid in increasing the quality of maternal colostrum. Although these products may be beneficial when high quality colostrum is unavailable, they do not consistently provide. Successful passive transfer (Foster, 2006). In order for improvement of these products to occur, there needs to be a better understanding of other components found in maternal colostrum and their role in intestinal development and IgG absorption. Part 1 of this review provides an understanding of why the neonatal calf has certain nutritional and immunological needs and how these needs can be met. Part 2 of this review will focus on the bioactive protein lactoferrin, and how its biological functions may be beneficial to the neonatal calf.

**Placental Transfer**

Placentas are classified for their morphological and structural differences. The bovine placenta is morphologically classified as a cotyledonary placenta, due to the cotyledons, which attach the placenta to the uterine wall (Senger, 1999). The placenta is structurally classified as a synepitheliachorial placenta because it consists of three maternal and three fetal membranes (Senger, 1999). It is through these morphological and structural characteristics that determine what substances are allowed to pass through the placenta from dam to fetus.

The dam and fetus have completely separate blood supplies resulting in no direct blood flow from one to the other (Blowey, 1999). The cotyledons, which attach the placenta to the uterine wall, are the site of nutrient and waste transfer between the dam and fetus. At this location, blood vessels grow very close together allowing for an exchange of nutrients from dam to fetus and waste from fetus to dam (Blowey, 1999). However, only small molecules are allowed to pass between the dam and fetal blood.
vessels (Blowey, 1999). This prevents large molecules such as Ig from reaching the fetus, resulting in the calf being born hypogammaglobulinemic.

The placenta also regulates the exchange of other nutrients such as glucose and amino acids. Glucose and amino acids are transported across the placenta by facilitated diffusion using specific carrier molecules (Senger, 1999). Lipids are not able to cross through the placenta, which also prevents fat soluble vitamins (A, D, E) from passing to the fetus. This results in the neonatal calf having high nutritional needs at birth.

**Characteristics of Neonatal Calf**

**Immunological and nutritional needs.** The metabolic adjustments necessary in the transition from a fetus to a neonatal calf are dramatic. The neonatal calf has very high nutritional needs due to the selective transfer of molecules through the placenta. The neonatal calf needs immunity, energy, protein, vitamins and minerals for it to survive. At birth, the calf has only 3% body fat, which is critical for development, and therefore, not available as an energy source (Akers, 2002). It is estimated that the total fat and glycogen available to the calf at birth would be used up in about 18 h without supplemental feeding (Davis and Drackley, 1998). The limited amount of glycogen stored in the liver or other tissues is rapidly used, and gluconeogenesis is limited in the liver at this time. Mother Nature has compensated for the lack of Ig and nutrients passed through the placenta by having the first secretion from the mammary gland, known as colostrum, being an excellent source of both immunological protection and nutrients needed for survival.

**Characteristics of the gastrointestinal tract.** Another important aspect concerning the neonatal calf is that the rumen is nonfunctional. At birth, the abomasum accounts for
about 70% of the volume of the entire stomach. The rumen, reticulum, and omasum account for the other 30%. This is dramatically different from the mature animal where the forestomach (rumen, reticulum, and omasum) accounts for more than 90% and the abomasum less than 10% of the stomach (Church, 1988). This makes a neonatal calf's digestive system similar to simple stomach animals such as the pig and human.

Milk bypasses the rumen and flows directly into the abomasum through the formation of the esophageal groove. The esophageal groove is formed through the muscular folds of the reticulum (Church, 1988). Neural stimulation from suckling and milk proteins results in the formation of the groove, extending the esophagus to the abomasum. Due to the specific proteins found in milk, colostrum, and milk replacer the esophageal groove will form whether calves drink from a bottle or bucket. However, water alone will not cause the groove to form resulting in water entering the rumen (Church, 1988).

**Immunity Overview**

The immune system is composed of two defense mechanisms, cell-mediated immunity and humoral immunity. Cell-mediated immunity involves T lymphocytes (T cells) which protect the body against cells that have been infected by viruses, parasites, or even cancer cells (Akers, 2002). Humoral immunity is comprised of B lymphocytes (B-cells) that can differentiate into plasma cells, which secrete antibodies. Antibodies also known as immunoglobulins are antigen-binding glycoproteins present on the B-cell membrane and secreted by plasma cells (Goldsby, 2003). The roles of antibodies are to bind to bacteria and their toxins or to bind to free viruses, making them inactive (Akers, 2002). The antibody-antigen complex results in the antigen being susceptible to
destruction by phagocytes or complement activation (Akers, 2002). There are five major classes of Ig: IgA, IgG, IgM, IgD, and IgE, all of which have a specific function within the immune system. The prevalence of a certain class of Ig depends on the species of animal. Ruminants have nearly all their Ig as IgG, while humans have more than 80% of their Ig as IgA (Akers, 2002).

The humoral immune system is comprised of memory B cells, which are responsible for the increase in antibody concentration when an animal is exposed to the same pathogen a second time. The memory B cells have the ability to recognize a pathogen in which the body has already been exposed, thus allowing for a faster immune response that will destroy the pathogen, preventing the animal from becoming ill.

At birth the calf's immune system is immature, resulting in its inability to make Ig and fight the pathogens it becomes exposed to at birth. The calf receives the Ig required to fight disease through the cow's colostrum it ingests. The cow has already been exposed to pathogens on the farm resulting in her immune system having specific Ig that can recognize and destroy this harmful pathogen. When the calf consumes the colostrum it is also ingesting these pre-made Ig, resulting in the calf having the Ig required to fight the pathogens it is exposed to and increasing its chances of survival.

**Immunoglobulin Structure and Function**

Each antibody molecule is comprised of two identical heavy chains and two identical light chains, which are linked together by disulfide bonds (Akers, 2002). The shape of the antibody molecule resembles the letter Y with the two heavy chains making up both the arms and stem. The light chains overlap portions of the heavy chains in the arms, and are joined to the heavy chains by a disulfide bond and by noncovalent
interactions such as salt linkages, hydrogen bonds, and hydrophobic bonds to form a heterodimer (Goldsby, 2003). The two identical heavy chains forming the stem of the Y are also linked together by noncovalent interactions and disulfide bonds (Goldsby, 2003). The two arms are classified as the Fab portion and the stem is considered the Fc domain (Rojas and Apodaca, 2002). The sites for binding of the antibody to the antigen are found at the two top ends of the Fab portion. These sites are known as the V regions (variable region) due to their highly variable sequence of 110 or so amino acids. The sequence of these amino acids determines the Ig specificity to an antigen (Goldsby, 2003). One Ig molecule is capable of binding at least two antigen molecules making the Ig divalent (Akers, 2002). The Fc domain interacts with molecules such as complement or Fc receptors, marking the antibody-antigen complex for destruction by, either neutralization, precipitation, agglutination, or opsonization (Akers, 2002).

**Structure of an IgG Molecule**
**Immunoglobulin G.** IgG has a molecular weight of 180,000 (Anderson et al., 1985) and is the most abundant class of antibodies found in blood serum and lymph. In all animals, IgG works to fight against bacteria, fungi, viruses, and foreign particles. It is the smallest Ig, which allows it to leave the blood stream and enter body pools where it can fight antigens. Immunoglobulin G is divided into subclasses IgG1 and IgG2, with IgG1 being the most prevalent in the bovine serum. The structural characteristics that distinguish these subclasses from one another are the size of the hinge region and the number and position of the interchain disulfide bonds between the heavy chains (Goldsby, 2003).

**Immunoglobulin M.** IgM is the class of antibodies found in circulating body fluids. It has a molecular weight of 900,000 and is expressed as a membrane bound antibody on B cells (Anderson, 1985; Goldsby 2003). It is comprised of five monomer units held together by disulfide bonds, resulting in its pentamer shape. This distinguishing characteristic allows IgM to be much more efficient than IgG when neutralizing a viral infection or activating the complement system. Immunoglobulin M can bind 10 small molecules or 5 larger molecules where IgG can only bind two at the most (Goldsby, 2003). Immunoglobulin M is the first antibody to appear in response to an initial exposure to an antigen (Goldsby, 2003). Due to the large size of IgM, it does not diffuse well and therefore found in very low concentrations in the intracellular tissue fluids; however, it does play an important accessory role to IgA as a secretory molecule (Goldsby 2003).
Immunoglobulin A. Immunoglobulin A has a molecular weight of 380,000 (Anderson et al, 1985) and is an antibody whose main function is to fight ingested antigens. It is found in mucosal and exocrine gland secretions (Rojas and Apodaca, 2002). The highest level of IgA secreted is by the intestine due to the constant interaction with endogenous flora and external microbes (Didierlaurent et al, 2002). To compensate for the lack of a fully functional immune system in the neonatal calf colostrum contains IgA, which coats the intestinal lining, preventing pathogens from attaching and causing disease. Immunoglobulin A binds antigens such as bacteria, viruses, parasites, and toxins. This Ig works by preventing the attachment and invasion of external pathogens into the mucosal surface by hindering their motility. For example, IgA can inhibit flagella function, induce microbial aggregation, compete with pathogens for adhesion sites, and to neutralize viruses (Williams and Gibbons, 1972). Immunoglobulin A and antigen complexes are entrapped in mucus and then eliminated by the ciliated epithelial cell of the respiratory tract or by peristalsis of the gut (Goldsby, 2003).

Structure of IgA molecule

Colostrum

Colostrum is the first secretion from the mammary gland after calving and is designed to meet the nutritional needs of the neonatal calf. Bovine colostrum is composed of 14% protein, 6.7% fat, and 2.7% lactose, while milk, which is classified as about the sixth milking or the third day after calving, has 3.1% protein, 3.5% fat, and 5%
lactose (Akers, 2003). As for Ig, bovine colostrum is composed of 75% IgG, 12% IgM, and 12% IgA while bovine milk contains only about 0.6% IgG and even lower levels of IgM and IgA (Foley and Otterby, 1978). Colostrum supplies the newborn with its first line of defense, nutrients, and growth factors.

**Colostrogenesis.** Colostrum is formed through the process known as colostrogenesis. Colostrogenesis is a process during late gestation, which results in the transfer of serum IgG into the mammary gland (Holland, 1990). Blood IgG enters lacteal secretions by a specific intracellular transport mechanism (Anderson et al., 1985). Immunoglobulin G is divided into two subclasses, IgG1 and IgG2. Immunoglobulin G1 is the most prevalent immunoglobulin found in bovine colostrum. It occurs in concentrations 5-20 times that of IgG2 due to the high prevalence of IgG1 specific receptors on the secretory cells (Anderson et al., 1985). IgG1 is transported from the serum to the colostrum, by first diffusing across the vascular epithelium and then selectively transported by mammary epithelial cells (Butler, 1983). Immunoglobulin G then specifically binds via Fc-specific receptors located on the basal membrane of the secretory epithelium (Butler, 1983). The basal membrane then engulfs the IgG molecule, which will then be released into the colostrum (Holland, 1990). The onset of lactogenesis rapidly decreases IgG transport, suggesting that the hormones responsible for alveolar cell differentiation may also suppress colostrogenesis (Akers, 2003). During the transfer of IgG from the serum to the colostrum, there is a significant decrease in maternal serum IgG and constant increase in colostrum IgG (Butler, 1983).

Immunoglobulin M and IgA are less prevalent in bovine colostrum and rather then coming directly from the maternal blood serum they are synthesized in the
mammary gland. They are synthesized in plasma cells lying adjacent to the secretory epithelium (Anderson et al., 1985). Following synthesis IgA binds to a protein receptor known as the secretory component, forming a complex called secretory IgA (sIgA), which consist of two IgA molecules, the J chain, and the secretory component (Anderson et al., 1985). Secretory IgA then moves through the secretory cell to the apical surface and is discharged into the colostrum (Anderson et al., 1985). The pathway of IgM is believed to be similar to IgA.

Colostrum Quality. The quality of colostrum is classified according to its IgG concentration; low quality < 22 mg of IgG/mL, medium quality 22 to 50 mg of IgG/mL, and high quality > 50 mg of IgG/mL. The IgG concentration is often measured indirectly using a colostrometer. The colostrometer measures the specific gravity of the colostrum, which equates to the Ig level. The higher the specific gravity the higher the colostrum quality.

The quality varies between breeds and the parity of the cow. Jerseys have higher quality colostrum because they produce colostrum that has a higher percentage of solids then a Holstein cow (Akers, 2003). In addition, cows that produce less milk normally have higher IgG concentrations due to the milk being more concentrated. A multiparous cow has higher quality colostrum, because it has been exposed to more pathogens and thus has built up a stronger immune system then a primiparous cow. If the calf is fed higher quality colostrum, they will have the amount of IgG required to fight disease...

Colostral Nutrients

Protein. Colostrum is composed of 14% protein. Proteins are synthesized from amino acids derived either from the blood stream or from amino acids synthesized by the
secretory cells (Akers, 2002). Consumption of large amounts of amino acids is critical for the stimulation of protein metabolism in the neonatal calf (Quigley and Drewry, 1998). The neonate begins protein synthesis in the visceral organs, brain, lungs, and skeletal muscle, within a few hours after birth (Quigley and Drewry, 1998).

The primary milk protein casein, coagulates in the abomasum forming a clot of milk. Casein is then slowly digested, providing a source of amino acids between meals (Quigley and Drewry, 1998). Other proteins such as β-lactoglobulin and α-lactalbumin are simply degraded in the abomasum and quickly hydrolyzed into amino acids (Quigley and Drewry, 1998). The large mass of Ig in colostrum provides an important source of amino acids even though they are more slowly digested (Quigley and Drewry, 1998). The neonate will establish homeostasis once enough amino acids are available for protein synthesis and gluconeogenesis (Quigley and Drewry, 1998).

Other proteins such as lactoferrin (Lf) and transferrin have a bioactive role in the nonspecific defense to a bacterial infection. They have the ability to inhibit growth of Gram-positive and Gram-negative bacteria because of their ability to sequester iron and limit its use by the bacteria (Akers, 2002). Properties of Lf will be discussed further in part 2.

Energy. The high fat content of colostrum (6.7%) is important to the neonate immediately after birth. The neonatal calf only has 3% body fat resulting in the neonate having poor insulation (Akers, 2002). The total fat and glycogen available to the calf at birth would be used up in about 18 h without supplemental feeding (Davis and Drackly, 1998). The colostral energy content may affect the thermoregulation and fatty acid oxidation that are necessary to sustain gluconeogenesis (Quigley and Drewry, 1998). The
faster the calf reaches homeostasis the higher its chances for survival (Santoro, 2001).

Heat production was increased by 18 and 9% in the first and second hour after colostrum consumption in calves maintained in a 10°C environment (Vermorel et al., 1983)

**Vitamins and Minerals.** Fat soluble vitamins A, D, and E do not cross the placental barrier, resulting in colostrum being the primary source of these nutrients for the calf after birth (Quigley and Drewry, 1998). Supplementation of Vitamin A and E (α-tocopherol) to the dry cow diet is important to have adequate amounts of these nutrients in colostrum (Quigley and Drewry, 1998). Vitamins A and E are not present in the calves’ blood stream at birth, while Vitamin D may be synthesized by precursors in the calf’s tissue (Santoro et al., 2004). Nemec et al. (1994) showed that Vitamin E improved the development of cellular immunity in neonatal pigs. Vitamin A plays a role in maintaining a healthy epithelium, growth, bone formation, immune function, and protein synthesis (Rufibach et al., 2006; Franklin et al., 1998; De Luca et al., 1969).

Selenium crosses the placenta and may accumulate in fetal tissues, such as the liver. Dietary selenium (120mg of Se/kg) has been reported to increase colostral IgG and it absorption by the neonatal calf (Swecker et al., 1995). Colostrum is also more concentrated than milk in zinc, iron, folic acid, choline, and riboflavin (Akers, 2002).

**Passive Transfer**

The transfer of IgG from mother to neonatal calf through colostrum is known as passive transfer. This provides the neonate with a form of passive immunization, which is the acquisition of immunity by a receipt of preformed Ig rather than by active production of Ig after exposure to antigens (Goldsby, 2003). The absorption of IgG across the small intestine appears to be species specific. In a number of newborn animal
species, selectivity has evolved to the exclusion of the transport of nearly all proteins, such as man, guinea pig, and rabbit, whereas in others, such as the pig and the ruminant selectivity appears to be poorly developed. There is also an intermediate group of selectivity, which includes the rat, mouse, and perhaps carnivores (Staley and Bush, 1985).

In the small intestine of neonatal mice and rats, the FcRn receptor is expressed at high levels by intestinal epithelial cells and mediates absorption of IgG by receptor-mediated transcytosis (Mayer et al., 2002). FcRn is a major histocompatibility complex class 1-related protein that mediates bidirectional IgG transcytosis in a variety of tissues (Rojas and Apodaca, 2002). Receptors are adapted to fit their attachment sites onto the Fc-portion of Ig (Staley and Bush, 1985). It appears the binding of IgG to the FcRn receptors is pH-dependent (Jones and Waldman, 1972; Rodewald, 1976), occurring at a pH of 6.0 to 6.5, which is similar the pH of the luminal content of the small intestine (Staley and Bush, 1985). The FcRn expression is developmentally down-regulated, resulting in almost complete loss of intestinal FcRn at the time of weaning in mice (Mayer et al., 2002).

The mechanism of IgG absorption across the small intestine of ruminants is less complex. Previous studies have shown the possibility of the absorptive epithelial cells within the jejunum of the neonatal calf possessing receptors, such as mice and rats, which mediate the binding and cellular transport of Ig (Butler, 1983; Widdowson, 1985). Staley and Bush (1985) introduced IgG conjugated to ferritin, and ferritin suspended in boiled bovine colostral whey into the jejunum and observed the jejunal cells did not take up the ferritin, but ferritin conjugated to IgG was observed in the apical tubular complex. The
conjugated ferritin was not transported into the vacuolar system of the cell and did not exit the cell. Cells in the ileum, however were the most active in the uptake of ferritin-IgG or ferritin. This study indicates the calves may have some selectivity in absorption, based on the particular protein rather then molecular weight in the jejunum. However, selectivity was not shown in the ileum in which both ferritin-IgG or ferritin were found (Staley and Bush, 1985).

It appears in more recent studies that absorption of IgG in ruminants occurs non-selectively during the first 24 h of life by pinocytosis (Mayer et al., 2002). Pinocytosis is a type of endocytosis in which a cell ingests extracellular fluid and the material dissolved in it (Goldsby, 2003). Mayer et al. (2002) were not able to detect FcRn in enterocytes, which are the cells responsible for the initial absorption of intestinal IgG. This would support other studies that found Ig uptake to be a non-specific event. However, FcRn was expressed by the crypt cells of the newborn lamb, which may be responsible for IgG1 being the major Ig in the secretions of the small intestine (Mayer et al., 2002). This indicates that FcRn is responsible for IgG being brought back into the mucosa from the neonate’s blood stream to fight infection along with IgA. No matter how IgG is absorbed across the small intestine, the passive humoral immunity supplied to the neonate at this time is essential for its survival.

Successful passive transfer is considered to be achieved when the serum IgG concentration is equal to or greater then 10g/L at 24 h after birth. The national dairy heifer evaluation project found that 40% of all calves sampled between 24 and 48 h had an IgG concentration of < 10g/L and over 25% < 6.2g/L (Wells et al., 1996). The economic impact of failure of passive transfer (< 10g/L of IgG) at 24 h after birth is
substantial. Management procedures and products that improve passive immunity in calves are crucial to the dairy industry.

**Gut Closure.** Gut closure is the necessary phenomenon that allows for the maturation of the digestive system, resulting in the cessation of absorption of macromolecules from the small intestine by about 24 h after birth (Stott et al., 1979). The maturation process of the small intestine includes multiple events, such as intestinal cell turnover, increasing abomasum acidity, development of intestinal secretions, and appearance of intra-epithelial digestive vacuoles.

**Factors Effecting Passive Transfer**

**Time of First Feeding.** Many factors affect whether or not the calf will receive successful passive transfer. Age of the calf at first colostrum feeding has a large impact on IgG absorption. An experiment by Haines et al. (1993) showed that serum IgG declined from over 14g/L when fed at birth to less than 10g/L when fed 6 h after birth. Only calves fed colostrum before 6 h had serum IgG levels greater than 10g/L. This is due to the maturation of the intestinal tract, resulting in the loss of absorptive sites in the small intestine. At birth there is also a limited amount of enzyme secretions, allowing IgG to pass to the small intestine where it can be absorbed as a whole molecule. By 12 h after birth enzyme secretions become more active, decreasing the chance of the IgG molecule to reach circulation. Quigley et al. (1995) showed that serum IgG and IgM concentration could be increased by adding 1g of soybean trypsin inhibitor to colostrum. The soybean trypsin inhibitor reduced the activity of trypsin in the small intestine allowing more of the Ig molecules to be absorbed.
Delaying the first colostrum feeding also enhances the risk of bacterial colonization of the intestine. Bacteria can compete with IgG molecules for absorption sites, thus decreasing the amount of IgG absorbed. The ingestion of bacteria also may accelerate the gut closure process. Logen et al. (1977) challenged calves with *Escherichia coli* either before or after the first colostrum feeding. Seventy-five percent of the calves receiving *E. coli* before colostrum feeding died while none of the calves that received colostrum first died.

**Quantity and Apparent Efficiency of Absorption.** In addition to the timing of colostrum ingestion, serum IgG concentration depends on the mass of IgG consumed (Kruse, 1970; Stott et al., 1979; Stott and Fellah, 1983). The mass of IgG consumed depends on the volume of colostrum fed, IgG concentration of the colostrum, and the efficiency of absorption of IgG by the calf (Morin et al., 1997).

The apparent efficiency of absorption (AEA) is equal to

\[
\text{[Plasma IgG at 24 h (g/L) x BW(kg)x0.092]/IgG intake(g)},
\]

with 0.092 representing the average blood volume (9.2% of BW) (Quigley et al., 1998). Research data suggest that the average AEA of IgG from maternal colostrum is about 35% (Quigley and Drewry, 1998). This data indicates that an average newborn weighing 40 kg must consume at least 103 g of IgG by 24 h to achieve a blood serum concentration of 10g/L (Quigley et al., 2002). In order to consume at least 103 g of IgG, the calf must ingest a minimum of 2.1 L of high quality colostrum (50g/L) within 2 hours after birth, for successful passive transfer to occur.

Levieux and Oilier (1999) estimated that 18% of dairy cows produce colostrum yielding less then the required amount of 100 g of IgG, for successful passive transfer.
Do to this variability in colostrum quality and variability in calf birth weight, it is recommended that the calf receive 4 L of high quality colostrum within the first 12 h of life. The main practice on dairy farms involves feeding 2 L at birth and another 2 L at 12 h later. If a total of 4 L of high quality colostrum (>50mg/ml of IgG) is fed either in one feeding or split into two feedings then the calf should receive the desired amount of 103g of IgG within the first 12 hours of life (Kruse, 1970).

Method of Feeding. The method of feeding colostrum also has an effect the calf’s ability to receive passive transfer. There are three management practices used on the dairy farm to administer the first colostrum feeding. The first method is to allow the calf to nurse from the dam. However, having the calf nurse from the dam increases its risk of failure of passive transfer. The calf may have difficulty finding the udder and ingest bedding and feces, which may contain harmful bacteria. In addition, when nursing from the dam one cannot determine the amount of colostrum consumed. Without consuming sufficient quantities of colostrum, the calf will not be able to obtain the required amount of IgG to prevent failure of passive transfer. The last concern when allowing the calf to nurse from the dam is the delay of standing which results in a delay of consumption.

The other methods of feeding are through the use of a bottle or an esophageal feeder. They both allow the farmer to control of the time and quantity in which the calf consumes the colostrum. However, studies show a slight increase in IgG absorption in calves feed by a bottle rather then the esophageal feeder. This is due to the different path colostrum takes though the gastrointestinal tract when an esophageal feeder is used. When a calf nurses from either the dam or the bottle, the suckling reflex and milk proteins cause the formation of the esophageal groove. The formation of the esophageal
groove results in the milk by passing the rumen and flowing directly into the abomasum.

In the method of using an esophageal feeder the esophageal groove does not form, resulting in the colostrum being deposited into the nonfunctional rumen. It then takes the milk two to four hours to leave the rumen, which may result in lower AEA due to the decrease in absorption sites over time.

**Disease risk associated with colostrum feeding**

**Johne’s Disease.** Johne’s Disease is a contagious disease of the intestinal tract caused by the bacteria *Mycobacterium Paratuberculosis*. The ileum becomes thick due to an inflammatory response caused by the infection. This prevents the intestine from functioning normally. The animal will continue to eat normally but will be unable to absorb nutrients resulting in the animal becoming anorexic and eventually dying.

Johne’s has an incubation time of 2 to 5 years, in which time the animal may not be showing signs of the disease but still shedding the bacteria. Johne’s is age dependent, meaning it takes a smaller dose to infect a calf then it would to infect an adult cow. This results in calves less than 6 months of age being the most susceptible. The bacterium is spread through manure, milk, and colostrum. The probability of calves becoming infected by drinking milk from infected cows is a direct function of time spent with the cow. If the calf remains with the cow for an extended period of time, she will have nursed more often thus having more exposure to the bacteria, either through ingestion of the colostrum or of manure on teats.

It is estimated that >22% of United States dairies are infected with Johne’s disease and that it costs the producers $245 per cow/yr and the United States’ dairy
industry $200-250 million annually (NAHMS, 2002). The high costs are due to a 2-19% reduction in milk yield, increased cull rate, and greater mortality.

**Bovine Leukosis Virus.** Bovine Leukosis Virus (BLV) is a virus that survives in the lymphocytes and once the animal is infected, it is infected for life. NAHMS (2002) reported that 89% of United States dairy operations have cattle infected seropositive for BLV. The virus survives in the lymphocytes, which are found in blood, milk, and colostrum along with other body fluids. If the calf ingests the BLV containing colostrum or milk, it will become infected. There is also a 4-8% chance of BLV crossing the placenta and infecting the fetus. However, only 5% of the cows infected with the virus ever develop the clinical diseases because it does not show up until later in life. In addition, it may remain dormant until brought on by stress.

Bovine Leukosis Virus causes a reduction in milk production and reproductive performance. The reduced performance is caused by tumors, which may develop in the abomasum resulting in loss of appetite, and tumors developing on the uterus. The largest economic impact of BLV is the number of carcasses condemned at slaughter plants. The number of condemned carcasses at slaughter has tripled since 1975. This is based on the number of lymph node tumors, indicating that a stronger elimination program needs to be established. In addition, purebred breeders suffer the biggest economic losses, because buyers do not want to bring the virus into their herd. The virus can also be spread through the semen, so many major artificial insemination companies will only accept semen from bulls that test negative.

There are other diseases such as BVD and salmonella that can be transferred to the calf through the ingestion of colostrum. Both can be detrimental to the calf's health.
and increase their chances of morbidity and mortality. Management procedures need to be implemented on dairies, which minimize the spread of disease through colostrum feeding. This will then result in an overall healthier and more productive herd.

**Colostrum replacer and supplements**

The recognition of high rates of failure of passive transfer (FPT) in calves and the risk of transferring disease to calves has lead to areas of research which would provide additional IgG or an alternative source of maternal colostrum. Dairy producers now have the option of feeding a colostrum replacer or colostrum supplement when high quality maternal colostrum is not available. Colostrum replacements may also be used as a part of biosecurity programs to prevent transmission of disease-causing organisms such as *Salmonella* spp, *Mycobacterium paratuberculosis*, bovine leukemia virus, and bovine viral diarrhea virus (Foster et al., 2006). Colostrum replacer is defined as having > 100 g IgG/dose and providing other nutrients required by the calf (Quigley et al., 2002b). A colostrum supplement has < 100g IgG/dose and is not formulated to replace maternal colostrum but rather be fed with it (Quigley et al., 2002b). Supplements do not provide the necessary energy, amino acids, or vitamins and minerals necessary for the calf's development. Colostrum replacers and supplements were introduced into the market in the mid to late 1980's and have become an important class of products for producers. Annually more then 500,000 calves in the U.S. are treated with colostrum products as a means of improving success of passive transfer and reducing cost associated with neonatal disease. Three sources of IgG that may be used in formulating colostrum supplements and replacers are lacteal secretions (colostrum and milk), blood, and eggs.
Lacteal Secretions. Colostrum supplements or replacers from lacteal secretions are derived from dried colostrum or whey secretions. Though these products maybe convenient and have been shown to protect calves under some circumstances, the Ig content is highly variable. Colostrum supplements have been found to range from 0.3 grams to 17.8 grams of IgG per dose, resulting in the calf being at a risk of FPT if only fed a colostrum supplement (Haines et al., 1990; Hunt and Hunt, 1991). The production of an effective replacer for bovine colostrum is dependent upon processing only colostrum containing high Ig concentrations and upon having an efficient method of dehydration, which preserves Ig function (Chelack et al. 1993). In a study conducted by Chelack et al. (1993), only colostrum collected from the first two postpartum milkings of multiparous cows contained sufficient immunoglobulin mass to be suitable for processing into a colostrum replacer. Chelack et al. (1993) also tested three dehydration methods of colostrum; freeze-dried, microwave vacuum evaporation, and spray-dried colostrum. Freeze-drying was found to be the most effective method for maintenance of Ig function and recovery of total solids. However, the lengthy processing time, high energy consumption, severely limited processing capacity, and high maintenance costs resulted in freeze-drying of large volumes of colostrum not being economical. The spray-drying method was the fastest and most energy efficient, costing 2.5 times less than either freeze-drying or microwave vacuum evaporation (Chelack et al, 1993). Spay-drying involves having atomized droplets of colostrum contact a countercurrent of hot air, resulting in heat transfer which promotes dehydration (Chelack et al. 1993). The spray-dried colostrum retained 94% of the Ig mass with minimal loss of Ig function; however, there was a 25% loss in total solids (Chelack et al, 1993). The end result was a product
that provided 126 g of IgG when fed to calves. The serum IgG at 48 h of age was 11.6 and 10.6 g/L for calves fed the colostrum and spray-dried colostrum, respectively. However, even with these developments of processing colostrum products that contain the required amount of 100 g of IgG/dose, no milk- or colostrum-based replacer products have been shown to routinely result in serum IgG concentrations ≥ 10 g/L (Foster et al., 2006).

Mee et al. (1996) conducted a study in which a whey protein concentrate (WPC) was fed as a colostrum substitute and compared to maternal colostrum. Calves fed the WPC alone had a 24 hour serum IgG concentration of only 3.0 mg/mL compared to calves fed maternal colostrum which had a serum IgG concentration of 17.8 mg/mL. This resulted in calves fed the WPC having a higher mortality rate ($P < 0.05$). The WPC was derived from ultrafiltrated and freeze-dried cheese whey collected from thousands of cows in North America. This processing method alone would result in high variability in IgG and nutrients within the product itself. Other studies have compared commercially available colostrum-based replacement products and evaluated their effect on serum IgG. These studies (Foster et al., 2006; Garry et al., 1996) found a high variability in colostrum replacement products alone, with some colostrum based products providing sufficient IgG for passive transfer while other products did not, even if all products provided the required amount of >100 g of IgG/dose. It is estimated that the AEA of colostrum replacer based on lacteal secretions ranges from 5 to 30% (Mee et al., 1996; Arthington et al., 2000). The variability of AEA among products and within the product itself results in the high variability of whether a calf will receive passive transfer when fed a lacteal secretion based colostrum replacer. In addition, with maternal colostrum
having an AEA of 35% would also explain why calves fed maternal colostrum have a higher percentage rate of successful passive transfer. The reason for the lower AEA in colostrum products is yet to be fully understood.

**Bovine Serum Extracts.** Colostrum replacer/supplements derived from serum protein generally have an AEA of 25-30%, which is higher then lacteal secretion based products and similar to maternal colostrum (Arthington et al., 2000; Quigley et al., 1998). It is also the least expensive source since bovine blood is plentiful and considered a waste product from slaughter houses. It has high levels of IgG with little IgM and IgA.

Bovine blood is collected from sources that are under USDA inspection and approved for human consumption. Bovine serum powder can be prepared by removing fibrin by addition of excess Ca, and the resulting serum is concentrated by filtration and spray-dried to produce a light tan powder (Quigley et al., 2001). The bovine serum powder is then mixed with other ingredients so it will provide the calf with both its immunological and nutritional needs.

Quigley at al. (2001) demonstrated that the method used for fractionation of bovine plasma to manufacture CR influenced absorption of IgG. The results of this study showed that when polyethylene glycol was used in the fractionation process, plasma IgG concentrations in the calf were reduced. This may have been caused by altered absorption kinetics, equilibration of IgG with nonvascular IgG pools or metabolism of IgG by the body within the first 24 h Quigley at al. (2001). Polyethylene glycol is used widely as a method for purifying IgG from plasma, lacteal secretions, and eggs (Akita and Nakai, 1993; Svendsen et al., 1995; Uemura et al., 1989) and may be the result of lower AEA in colostrum products compared to maternal colostrum.
Although, bovine serum based colostrum products are the most effective in assuring successful passive transfer when compared to other products, it is no longer allowed in countries where the use of animal proteins has been banned. This is due to the concern of diseases such as BSE being transferred from animals to humans during consumption.

**Chicken Egg.** Hens are hyperimmunized to produce IgY, which is the chicken form of IgG. The resulting eggs have high titers against the target antigen (Ikemori et al., 1997). The eggs are concentrated and sprayed-dried. Egg IgY is used as a source of oral Ig, which only provides local protection in the small intestine. The IgY is not well absorbed across the small intestines (Kuroki et al., 1993; Ikemori et al., 1997; Hennig-Pauka et al., 2003).

**Limitations to colostrum products:** There are many limitations to the use of colostrum supplements and replacers. For example, the profile of antibody specificity in a colostrum product may vary markedly from the antigenic reservoir on a dairy farm. Although the calf may absorb significant IgG, they may provide little protection if they are specific for the wrong pathogens. Biosecurity is also another potential risk when feeding an animal protein from an unknown source. Colostrum products may be exposed to such things as pasteurization and irradiation for the purpose of eliminating pathogens, however the risk of transmission of disease is not completely ameliorated. Another negative aspect of colostrum products is that the processing steps such as pasteurization and spray-drying will reduce the activity of important colostral components such as colostral lymphocytes and heat labile proteins. Lastly, the cost of feeding a colostrum supplement or replacer is expensive.
**Part 2: Lactoferrin**

**Introduction**

Lactoferrin is an iron binding glycoprotein of the transferrin family. It is widely distributed in the physiological fluids of mammals (Saito et al., 1991). Unlike transferrin, which can be found in species as distant as insects, Lf can only be found in mammals, suggesting that its emergence had to do with infant nutrition and protection (Baker and Baker, 2005). Lactoferrin is believed to have evolved through the duplication of the transferrin gene at the time of emergence of mammals, with subsequent divergence in its amino acid sequence and functional properties (Baker and Baker, 2005). It can be found in fluids such as tears (Kiljistra et al., 1983), bronchial mucus (Fahy, 1993), secondary granules (PMN's), (Bennett and Kokocinski, 1978), cerebrospinal fluid (Talukder, 2003), and milk (Molenaar et al., 1996).

Lactoferrin was first discovered in the whey fraction of cow's milk (Sorensen and Sorensen, 1939). Its most striking characteristic was its intense red color when incubated in the presence of Fe$^{3+}$ ions (Baker and Baker, 2005). The site of Lf synthesis is assumed to be the epithelia from which it is secreted (Sanchez et al., 1992).

The concentration of Lf in milk varies between species and the stage of lactation. Humans have the highest concentration at 4 mg of Lf/mL of milk, while rats and rabbits have no Lf (Sanchez, 1992), and in the cow, goat, and sow milk, the Lf concentration is between (20-200ug/mL) (Cowles et al, 2005). The concentrations of this protein have also been well documented for cows during colostrogenesis, lactation, and involution (Smith and Schanbacher, 1977). Lactoferrin is elevated in colostrum (1 to 2 mg/mL) but is low during lactation (0.01 to 1mg/mL); Lf increases to between 20 and 100mg/mL in
the secretion that is produced during involution (Molenaar et al., 1996). There are also breed and parity effects, with dairy breeds having higher concentrations of Lf than beef breeds and multiparous cows having higher amounts than primiparous cows (Tsuji et al., 1990). The health of the mammary gland also plays a role in the amount of Lf found in the milk. As the somatic cell count increases, so does the level of Lf (Harmon et al., 1977). It is believed that the Lf produced in the mammary gland may play a role in both protecting the mammary gland of the cow and the intestinal tract of the newborn from infection.

Lactoferrin plays a role in the innate immune system, which works as the first line of defense against infection. It has many biological functions such as antibacterial activity (Law and Reiter, 1977; Nonnecke and Smith, 1984; Reiter, 1983; Spik et al., 1978), regulation of Fe absorption (Fransson et al., 1983 and Kawakami et al., 1988), growth promotion of lymphocytes (Hashizume et al., 1983), product regulation of macrophage, and granulocyte and neutrophil leukocytes (Ambruso et al., 1981; Broxmeyer et al., 1980 and Hurlen et al., 1984), It could also control the development of intestine by promoting cellular growth (Sanchez et al., 1992).

**Structure of Lactoferrin**

Lactoferrin is a monomeric, 80-kDa glycoprotein with a single polypeptide chain of about 690 amino acid residues. It has a three dimensional structure comprised of two folded globular lobes, known as the N-lobe and the C-lobe. The N-lobe and the C-lobe share an internal amino acid homology of about 40% (Nuijens et al., 1996). A short α-helix peptide connects the lobes, and is the feature which readily distinguishes Lf from
transferrin. It is helical in Lf, but irregular and flexible in transferrin, containing several proline residues (Baker and Baker, 2005).

Both the N-lobe and the C-lobe are divided into two regions. In each lobe, two $\alpha/\beta$ domains referred to as N1 and N2, or C1 and C2, enclose a deep cleft where the iron binding site is located (Baker and Baker, 2005). The binding sites are comprised of four protein ligands (2 Tyr, 1 Asp, 1 His) that provide three negative charges, resulting in the binding site being highly anionic (Baker and Baker, 2005). This results in their strong affinity for cations such as Fe$^{3+}$, with the three negative charges in the binding site balancing for the three positive charges of Fe$^{3+}$. The two binding sites found on the Lf molecule allow for one Lf molecule to bind to two Fe$^{3+}$ molecules.

It is suggested that the binding of a carbonate anion occurs first, which allows for the neutralization of some positive charge near the iron binding site (Cowles et al., 2006; Robblee et al., 2002). The helix N-termini face the interdomain cleft, making it somewhat positively charged; H5 from N2 or C2 domain, serves as the binding site for essential carbonate anion at the metal binding site (Baker and Baker, 2005). This non-protein ligand (CO$_3^{2-}$ ion), is responsible for determining the pH in which iron is released (Baker and Baker, 2005). At a pH of 3-4 protonation of the carbonate ion occurs, causing a weakening of the Lf- Fe$^{3+}$ bond, resulting in the iron release (Baker and Baker, 2005).

Lactoferrin can be found in two forms, holo-Lf (iron bond) or apo-Lf (iron free). The Lf found in secretions is almost entirely found in the apo-Lf state, with the capability of binding iron. The binding of iron results in the conformational change of the molecule becoming more compact. There is a 55° rotation of the N-lobe, and a 20° rotation of the C-lobe (Baker et al., 1998). The two domains of each lobe close over the bound metal.
and open to release it, resulting in the Lf-Fe$^{3+}$ bond being reversible (Baker and Baker, 2005). The apo form is more flexible because it does not have two metal ions to lock the lobes in place. The conformational changes associated with Fe$^{3+}$ binding reduces the interaction between the bactericidal domain of Lf and the bacterial surface, thereby abolishing its antibacterial activity (Dionysius et al., 1993). The bactericidal domain, concentrated into a cluster of positively charged residues near the N-terminus of the Lf molecule (Vorland et al., 1998; Elass-Rochard et al., 1998; Nibbering et al., 2001), becomes less exposed in the holo-Lf configuration as opposed to the apo-Lf configuration.

There are also structural differences between species, with bovine lactoferrin sharing only 68% and 64% of amino acid residues with the human and murine lactoferrin, respectively (Nuijens et al., 1996). Depending on species the N- and C- lobes differ significantly in their relative orientations (Moore et al., 1997). In addition, all Lf appears to glycosylated, though with varying degrees from protein to protein, with human Lf having two glycosylation sites and bovine having four glycosylation sites (Baker and Baker, 2005). The structural differences of Lf between species could result in some activities of Lf being species specific.

**Lactoferrin Receptors**

The multiple biological functions of Lf depend on its target cells and on the presence of specific receptors (LfRs) at their surface (Suzuki et al., 2005). The differences between tissues and cell types results in differences in LfRs. Lactoferrin receptors of the small intestine in several neonatal species have been observed. However, in order for LfRs to function in the small intestine, Lf must pass through the
gastrointestinal tract without proteolytic degradation (Suzuki et al., 2005). The relatively high pH and low enzyme secretion found in the neonate’s small intestine provide the environment for Lf to pass through (Suzuki et al., 2005). The elevated Lf concentrations found in colostrum and the environment of the neonate’s gastrointestinal system, indicate Lf may have a role in intestinal development. There is a variety of suggested functions of the small intestine LfR such as facilitating iron absorption, enhancement of the immune system, and stimulating mucosal differentiation. However, results from studies are controversial and the mechanism is yet to be determined.

The binding of Lf to the LfRs on the brush border membrane vesicles (BBMV) appears to be time dependent, saturable, and specific (Davidson et al., 1988; Hue et al., 1988). However, it appears in some species such as the adult bovine that transferrin can compete with Lf to bind to the LfRs, thus reducing the percentage of the Lf that becomes bound. (Talukder et al., 2003). Competitive binding experiments showed that holo-lactoferrin was more effective than less Fe-saturated forms of lactoferrin with regard to receptor binding (Lonnderdal, 1995).

**Antibacterial Properties of Lactoferrin**

Lactoferrin has been shown to inhibit the growth of many bacterial species, due to both its bacteriostatic and bactericidal properties. Its bacteriostatic activity is related to its ability to withhold iron while the bactericidal activity is not due to iron withholding. Iron is essential for bacterial growth; when Lf is present it binds the iron making it unavailable for the bacteria, resulting in the inhibition of bacterial growth (Dionysius et al., 1993). The apo-Lf found in the extracellular compartments within the host body or host mucosal surfaces binds iron, resulting in the level of iron being below the required amount to
support microbial growth (Finkelstien et al., 1983). However, most pathogenic bacteria have developed ways in which they can overcome Lf-generated iron deprivation by acquiring iron by means of two principal systems: either secreting small iron chelators (siderophores) or acquiring iron directly from host transferrin and Lf (Valenti and Antonini, 2005). The siderophores compete with Lf for iron, which then reduces the growth-inhibiting effect of the protein. In the case of acquiring iron directly from Lf, this involves binding Lf to bacterial receptors found on the bacteria (Gray-Owen et al., 1996). The Lf-bacteria binding causes a confirmation change in Lf, which lowers the affinity of Lf the C-lobe for iron (Ekins et al., 2004).

Bactericidal properties have also been shown in which there is a direct interaction between Lf and bacterial surfaces (Arnold et al., 1977). A non-specific binding between Lf and bacterial surfaces, of both Gram-negative and Gram-positive species, are caused by the high density of charges found on the surface of the Lf molecule (Baker et al., 2002). Lactoferrin binds to the porins present on the outer membrane of Gram-negative bacteria (Gado et al., 1991), resulting in the release of lipopolysaccharides. This will enhance the susceptibility of the bacteria to osmotic shock, lysozymes, and other antibacterial molecules (Leitch and Willcox, 1998). In the case of Gram-positive bacteria its effect is likely to be similar to that of cationic and amphipathic antibacterial peptides (Valenti and Antonini, 2005). The positively charge Lf molecule binds to the negatively charge lipid matrix found in the membrane of Gram-positive bacteria through electrostatic interactions while the amphipathic residues disturb the non-polar membrane interior due to hydrophobic interactions (Giangaspero et al., 2001; Wessolowski et al., 2004).
Lactoferrin from several mammalian species have been shown to inhibit the growth of a number of bacteria, including human and/or animal pathogenic strains of *E. coli*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Listeria monocytogenes*, *Streptococcus* spp., *Vibrio cholerae*, *Legionella pneumophila*, *Klebsiella pneumonia*, *Enterococcus* spp., *Staphylococcus* spp., *Bacillus stearothermophilus* and *Bacillus subtilis* (Valenti and Antonini, 2005). Lactoferrin reduces the growth of harmful bacteria in the gastrointestinal tract, while promoting growth of bacteria such as *Lactobacillus* and *Bifidobacteria* which are believed to be beneficial to the animal (Valenti and Antonini, 2005).

**Antiviral Activity of Lactoferrin**

Since 1994, potent antiviral activity of human Lf and bovine Lf has been demonstrated against both enveloped and naked viruses (Valenti and Antonini, 2005). Lactoferrin has been shown to have an effect on a variety of viruses *in vitro* such as cytomegalovirus, herpes simplex virus, human immunodeficiency virus, human hepatitis C and B viruses, rotavirus, and adenovirus (Valenti and Antonini, 2005). Lactoferrin may exert its effects by inhibition of viral fusion and entry into the cell, rather than stimulation of the immune system. Lactoferrin has been shown to inhibit antigen synthesis by the virus, it can interfere with the earlier stage of the virus (Superti F et al., 1997). The antiviral activity of Lf is the result of its strong affinity for heparin sulfate and glycosaminoglycan, carbohydrates that are the typical viral binding sites on the cell membrane (Andersen et al., 2001).
Antifungal and Anti-parasitic Activity of Lactoferrin

The effect of Lf on fungus is believed to be caused from adsorption to the cell surface rather than iron deprivation (Valenti et al., 1986). In vitro studies have shown that human Lf, bovine Lf, and lactoferricin are able to fight pathogenic fungi (Valenti and Antonini, 2005). The *Candida* species of fungi has been the most exclusively studied in Lf studies. The Lf growth-inhibitory effect appears to be much higher then those of most antifungal drugs currently available (Valanti and Antonni, 2005). The peptide lactoferricin either has two antifungal mechanisms, direct fungicidal activity or up regulation of the host defense (Gifford et al., 2005).

Anti-parasitic activities are complex and at this date unclear. In some species such as *Pneumocytis carini* Lf appears to interfere with iron acquisition, while in other species such as Tritrichomonas foetus it appears to act as an iron donor, thus enhancing infection (Tachezy et al., 1996). In the case of lactoferricin, evidence indicates it may be attracted to the surface, resulting in the disruption of the membrane or the release of parasitic structural components that subsequently activate host defense systems (Gifford et al., 2005).

Antitumor Activity of Lactoferrin

Lactoferrin and lactoferricin have both been shown to have anti-tumor effects against leukemia (Yoo et al., 1997; Yoo et al., 1997), fibrosarcomas, melanomas and coloncarcinomas (Eliassen et al., 2002), without affecting normal fibroblasts and erythrocytes (Gifford et al., 2005). The changes, which occur in the membrane, such as exposure of phosphatidylserine, of a tumor, cell results in Lfcin targeting the cell (Fadok et al., 2001). The bond of Lfcin to the cancer cell disrupts the membrane marking,
resulting in apoptosis (Aramaki et al., 2000). Lactoferrin itself has been shown to regulate natural killer cells (Damiens et al., 1998; Shau et al., 1992; Bezault et al., 1994), G1 protein modulation (Damiens et al., 1999), and inhibition of vascular endothelial growth factor (VEGF)-mediated angiogenesis (Norrby et al., 2001).

**Anti-Inflammatory Activity of Lactoferrin**

Lactoferrin has the ability to inhibit an inflammatory response through a variety of ways. First, Lf can bind free iron that has accumulated in inflamed tissues and catalyzes the production of tissue-toxic hydroxyl radicals (Legrand et al., 2005). The PMN’s release of the apo-Lf at the inflamed site, because of its iron-binding stability at low pH, it can precipitate in iron homeostasis and detoxification (Legrand et al., 2005). Other anti-inflammatory properties involve its ability to inhibit the release of cytokines, which induce recruitment and activation of immune cells at inflammatory sites. It is able to reduce the release of inflammatory cytokines by binding to the lipopolyscharide membrane of bacteria, through its lactoferricirin domain (Appelmelk et al., 1994; Brandenburg, 2001; Elass-Rochard et al., 1995), resulting in the neutralization of LPS activity (Mattsby-Baltzer et al., 1996; Zhang et al., 1999). Lactoferrin is also considered a marker of inflammatory disease because its concentration increases during inflammatory diseases such as septicemia (Bennet et al., 1978), rheumatoid synovitis (Caccavo et al., 1999), bowel disease (Kane et al., 2003), and neurodegenerative disease (Fillebeen et al., 2001).

**Lactoferrin Effect on Intestinal Development**

Previous research with other species such as the human infant indicates that LF has a stimulatory effect on cell proliferation, increased cell size and growth of gut-
associated lymphatic follicles (Artym and Zimecki, 2005). The weight of the small intestine of neonatal mice has been shown to increase by about 27% when they consumed milk containing 12 mg/mL of human lactoferrin (Zhang et al., 2001). In the same study by Zhang et al. (2001), the intestinal length increased only by about 6.5% indicating Lf enhances mucosal growth. In a study by Humphrey et al. (2002), greater villous height and fewer leukocytes were observed in the lamina propria in chicks when 5% Lf (0.125g/kg) and 10% lysozyme (0.3g/kg) were added to the diet. The increased villous height and surface area resulted in better nutrient digestion and absorption, thus improving feed efficiency. The fewer leukocytes were the result of a decrease of microbial challenges, thus improving intestinal health. The 5% Lf alone had no effect, while 10% lysozyme only had a slight increase on intestinal development. The combination of Lf and lysozymes resulted in a synergistic effect, by Lf causing the release of LPS, which enhances the bacteria’s susceptibility to lysis by lysozymes (Humphrey et al., 2002). In calves, the data of Lf having a role on intestinal development is controversial. Joslin et al. (2001) and Robblee et al. (2002) found feed efficiency to increase when Lf was supplemented to milk replacer, while Cowles et al. (2006) observed no effect of Lf on xylose and glucose absorption.

**Lactoferrin in the Mammary Gland**

The biological roles of Lf to modulate the control of macrophage, lymphocytes, and neutrophil function and its antibacterial properties aid in the immunity of the mammary gland (Sanchez and Watts, 1999). Lactoferrin concentrations increase in mammary secretions of dairy cattle during mastitis and mammary involution (Harmon et al., 1976; Rejman et al., 1989; Welty et al., 1976). Mammary involution is a critical time
for restoring mammary health. It is estimated that mastitis costs the dairy industry two billion dollars per year. Incidence of mastitis is particularly high in dairy cows during early involution, but the gland becomes more resistant to infection in the mid dry period (Oliver and Sordillo, 1989). The concentration of Lf increases from 0.4 mg/mL at drying off to 20 to 30 mg/mL by 30 d of involution (Smith and Oliver, 1981; Smith and Schanbacher, 1977), and by 30 fold during acute bovine mastitis (Schanbacher et al., 1993,1997), indicating Lf may play a role in protecting against intramammary infection.

Cows are commonly treated at dry-off with a combination of penicillin and novobiocin to protect against intramammary infection (Sanchez and Watts, 1999). Novobiocin is able to inhibit the growth of Gram positive bacteria but not Gram negative bacteria due to its inability to penetrate the lipopolysaccharide membrane (Oram et al 1996). However, Lf has been shown to damage the outer membrane of Gram negative bacteria by causing the release of LPS molecules from the outer membrane. The increase of Lf during involution may result in Lf disruption of the membrane of bacteria such as *E. coli*. This would result in novobiocin attacking Gram negative bacteria and destroying them. Although Sanchez and Watts (1999) showed that there was a synergistic effect between Lf and novobiocin in on *E. coli* bacteria in vitro, there was no reduction in bacterial counts in vivo.

Diarra et al. (2001) compared the effect of Lf in combination with penicillin on the morphology and the physiology of *Staphylococcus aureus* isolated from bovine mastitis. *Staphylococcus aureus* is an important human and animal pathogen that is the most common cause of bovine mastitis, causing a loss of milk production (Forsmen et al., 1997). Diarra et al., (2001) found that combining penicillin with relatively low
concentrations of bovine lactoferrin lead to the synergistic increase of the antibacterial activity of this antibiotic to Staphlococci strains isolated from clinical bovine mastitis. There was no effect when penicillin was used as the only treatment due to the bacteria’s resistance to the antibiotic and Lf alone only had a small inhibitory effect. Although the two combined in vitro can induce destruction of the bacteria, the mechanism of action is not yet established (Diarra et al., 2001).

The loss of the synergistic effect in vivo may be due to decreased activity of antibiotics, or the Lf present in bovine mammary secretions during involution exist as complexes with other macromolecules, which may alter Lf antibacterial activity (Wang and Hurley, 1998). Lactoferrin commonly binds to macromolecules, such as Ig, casein, secretory components, albumin, lysozyme, and β-LG (Hurley, 1993; Lampreave et al., 1990), as well as to DNA and heparin (Zou et al., 1992). The biochemical nature and functional significance of complexes between Lf and other macromolecules are unknown, but the may reduce Lf antimicrobial activity, or when bound to Ig, they may enhance antimicrobial activity (Wang and Hurley, 1998). However, human Lf and IgA act cooperatively with increased bacteriostatic activity (Stephens et al., 1980).

**Lactoferrin Fed to Dairy Calves**

A dairy calf is exposed to a variety of pathogens that result in illnesses such as respiratory disease and scours. The biological functions of Lf as discussed could have multiple benefits in fighting these diseases. However, the research involving feeding Lf to dairy calves has resulted in conflicting data.

The effect of supplemental Lf (0, 1, 10g/d) in a conventional milk replacer on preweaning growth, blood, and health characteristics was studied by Joslin et al. (2002).
Results indicate that calves receiving supplemental Lf had higher starter intakes, increased average daily gains, and increased heart girth measurements. It was also shown that calves fed 1 g/d of Lf had greater average daily gains then calves fed the 10 g/d treatment. The follow up study of Robblee et al. (2003) was designed to determine the optimum level of Lf to achieve the greatest response in health, growth and weaning of dairy calves. When supplementing non-medicated milk replacer with 0, 1, 2, or 3 g of Lf per day, results indicated 1g/d of Lf was the optimum level for good calf performance. Preweaning fecal scores and total days medicated showed a quadratic response with1g having the lowest score. There was also a linear effect in calves supplement with LF on the preweaning average daily gain and feed efficiency, however it did not maintain the same effect postweaning. These studies indicate that 1g/d of supplemental Lf would be beneficial to the dairy calf, possibly by aiding in the immune system of calves by destroying pathogens, which may result in illness. However, in a study conducted by Cowles et al. (2006), in which they compared the effect of 0 or 1g/d of Lf in both a conventional or intensified milk replacer, there was no increase in starter consumption, average daily gain, feed efficiency, or fecal scores between Lf treatments. The difference in results between studies could be caused by the difference in days medicated. In the study by Cowles et al. (2006), calves had at least 50% lower fecal scores and day’s medicated then calves used by Joslin et al. (2002). These findings indicate that supplemental Lf is only beneficial when calves are sick.

Not only would the antibacterial properties of Lf be beneficial to the dairy calf, but its ability to influence the development of intestinal epithelia would also be considered beneficial (Nicholas et al., 1987; Van Leeuwen et al., 2000). The theory
being if Lf increases intestinal development, then nutrient absorption will increase.

Schottstedt et al. (2005) investigated the effects of dietary bovine Lf and vitamin A alone or in combination on epithelia of the small intestine and colon and on the gut-associated lymphoid tissues, especially of Peyer's patches (PP). In this study, they found a variety of positive and negative results depending on which location of the GIT was measured. First, plasma Lf concentrations in all formula plus Lf fed calves remained low, indicating Lf was not absorbed. It was also found that crypt cell proliferation of the colon was negatively affected by Lf supplementation. However, the duodenum of calves fed the formula supplemented with Lf had a thicker tunica muscularis layer then calves fed colostrum, indicating that orally administered Lf might have an effect on both protein synthesis (Burrin et al., 1996) and on GIT muscle synthesis.
CHAPTER II

Serum IgG Concentration of Neonatal Calves Fed Colostrum Replacer Supplemented with Lactoferrin

Introduction

The neonatal calf is born hypogammaglobulinemic. Successful passive immunity is dependent upon adequate consumption and absorption of IgG soon after birth. Passive immunity is reliant on a variety of factors such as quality and quantity of colostrum fed (Morin et al., 1997), apparent efficiency of IgG absorption (AEA) (Morin et al., 1997), age of first feeding (Stott et al., 1979), stress (Hough et al., 1990; Johnston and Stewart, 1986; Stott, 1980), and method of feeding (Lateur-Rowet and Breukink, 1983). Successful passive transfer is defined as having a blood serum concentration of $\geq$ 10 mg of IgG/mL at 24 h after birth. Blood serum concentrations $< 10$ mg of IgG/mL at this time are related to calf morbidity and mortality (Besser and Gay, 1994), as well as long term depressed calf performance (Wittum and Perino, 1995). The national dairy heifer evaluation project found that 40% of all calves sampled between 24 and 48 h had an IgG concentration of $< 10$ mg/mL and over 25% $< 6.2$ mg/mL (Wells et al., 1996). There is a need for improvement of management procedures on the farm and development of products that will increase the number of calves that acquire passive immunity (Arthington et al., 2000).

The recognition of high rates of failure of passive transfer and the risk of transferring disease to calves through maternal colostrum has led to areas of research,
evaluating alternative sources of maternal colostrum. Maternal colostrum is the best source of Ig and nutrients for the calf. However, good quality colostrum is not always available. Alternative sources of providing Ig and nutrients to the calf have been developed such as colostrum replacer (CR) and colostrum supplements. These products are derived from a variety of sources such as lacteal secretions, blood serum, and eggs. Colostrum replacer is defined as containing > 100 g IgG/dose and providing other nutrients required by the calf (Quigley et al., 2002a). A colostrum supplement contains < 100 g IgG/dose and is not formulated to replace maternal colostrum but rather be fed with it (Quigley et al., 2002a). Supplements do not provide the necessary energy, amino acids, or vitamins and minerals necessary for the calf’s development. Although CR contain the required amount of 100g of IgG when fed at recommended levels, no milk or colostrum based replacer product has routinely shown results of serum IgG concentrations ≥ 10mg/mL in dairy calves (Garry et al., 1996; Mee et al., 1996; Hopkins and Quigley, 1997; Arthington et al., 2000). The reason for poor IgG absorption of colostrum replacers and supplements has yet to be determined. Colostrum replacer not only needs to replicate maternal colostrum in its energy, protein, fat, mineral and vitamin content, but also other bioactive proteins such as lactoferrin (Lf). Lactoferrin is an iron binding glycoprotein present in colostrum and milk. The concentration of Lf in bovine colostrum is 1 to 2 mg/mL, compared to bovine milk which only has a concentration of 0.01 to 1 mg/mL (Molenaar et al., 1996). Lactoferrin has been shown to be important for intestinal development and development of the immune system in species such as mice and humans (Shah, 2000). It has three functions, which may be beneficial to the development of a neonatal calf: inhibiting bacterial growth (Teraguchi et al., 1994), increasing intestinal
cell growth (Zhang et al., 2001), and stimulating glucose absorption (Teraguchi et al., 1998). The ability of Lf to inhibit bacterial growth may protect the intestine of the newborn calf from infection, while increasing intestinal cell growth may increase both IgG and nutrient absorption by the intestine (Robblee et al., 2003). White (2005) saw an effect of Lf on average apparent efficiency of absorption (AEA), when calves were fed maternal colostrum with 1g/d of supplemental Lf. The objective of this experiment was to determine the effect of various levels of Lf supplementation to colostrum replacer fed as 1 or 2 doses on apparent efficiency of IgG absorption, serum IgG concentrations, and intestinal development in neonatal Holstein bull calves.

Materials and Methods

Calves, Feeding, and Treatments

This experiment was reviewed and approved by the University of New Hampshire Institutional Animal Care and Use Committee (Approval # 051102).

A total of 80 Holstein bull calves born between March 2006 and November 2006 at two different dairies were used. Calves were assigned randomly at birth by location to a 2 x 4 factorial arrangement of treatments in a randomized complete block design. Calves were blocked in groups of 8 at birth by location. Randomization of treatments occurred within blocks. Forty-eight bull calves assigned to the experiment were born at the University of New Hampshire, Fairchild Teaching and Research Center (initial BW of 43.8 ± 4.9 kg). Thirty-two calves were from a local New Hampshire dairy farm. All calves were removed from their dam and not allowed to suckle. Calves born at the university were weighed at birth and placed in a naturally ventilated, enclosed calf room in individual pens. Pens were bedded with kiln-dried sawdust. Calves born at the
commercial dairy were not weighed at birth, as a scale was unavailable. Calves from the commercial dairy were placed in a naturally ventilated maternity pen and bedded with wheat straw. The treatment groups consisted of 1 dose\(^1\) of CR versus 2 doses of CR with or with/out supplemental Lf (Immucell, Portland ME). The levels of supplemental Lf were 0, 0.5, 1, or 2 g/d. All calves received 1 dose\(^1\) of CR with or without supplemental Lf within 90 min of birth. The source of Lf was bovine milk (Immucell Corp., Portland, ME); iron saturation was 13.2g/100g. The second feeding was at 12 h in which the calves received either a second dose\(^1\) of CR or 2 L of non-medicated milk replacer (MR) (20%CP:20%fat) with each containing the varying levels of supplemental Lf depending on which treatment the calf was assigned. Calves continued to be fed MR with or without supplemental Lf in 12 h increments throughout study. Calves received 1.2% of its initial BW, in MR powder. The MR powder was divided into two equal portions, and each portion was reconstituted in 2 L of warm water immediately before feeding. At the sixth feeding in which calves were 60 ± 2 h of age, a xylose challenge was conducted (university calves only) to determine xylose absorption by the small intestine. Calves from the commercial dairy were removed from study at 48 h, while calves born at the university were removed by 72 h.

**Feed Analysis**

The DM of the CR and MR was determined by drying samples in the same forced-air convection oven at 60°C for 24 h. Samples from each bag of CR and MR were saved and stored at -20 °C. When the experiment was completed, samples were composited for nutrient analysis. Colostrum replacer and MR were analyzed for CP (AOAC, 1979), fatty acid (AOAC, 1995), and Ca, P, Mg, and Fe (AOAC, 1990), and Lf

---

\(^1\) 1 dose is equal to 470g of CR reconstituted in 1L of warm water
after reconstituting the MR to 15% DM (RID; Cardiotech). Chemical analysis of CR and MR are shown in Table 1.

**Blood Collections for Immunoglobulin G and Serum Protein**

Blood samples were collected via jugular venipuncture before the first feeding of colostrum replacer (within 90 min of birth, referred to as 0 h) and at 6, 12, 18, 24, 48 h after birth. Samples were collected in 5-mL tubes. Samples were allowed to clot at room temperature for at least 1 h and then centrifuged at 3300-x g at 25°C for 20 min. Serum samples were stored at -20°C until analyzed for IgG by radial immunoassay (WCVM, Saskatoon Canada) and total serum proteins with a digital refractometer (SPER Scientific, model 300027 from VWR).

**Blood Collections for Xylose Challenge**

The xylose challenge was only performed on calves born at the UNH Fairchild Dairy Teaching & Research Center. D-xylose (0.5g/kg BW) was mixed with the MR and fed at the sixth feeding (60 ± 2 h of age). D-xylose is used as an indirect marker for glucose absorption across the small intestine. Calves were fasted throughout the sampling period but allowed access to ad libitum water. Blood samples were taken before D-xylose treatment (0 h) and at 2, 4, 6, 8, and 12 h after feeding. Blood samples were collected into evacuated tubes via jugular venipuncture, containing tripotassium EDTA, to measure D-xylose and glucose concentrations in the plasma. Glucose levels were measured in the same blood samples to compare xylose and glucose concentrations. Blood samples were immediately centrifuged at 3300 x g at 5°C for 20 min to harvest plasma. Two aliquots of plasma were stored in 5-ml polypropylene tubes at -20°C and later analyzed for D-xylose and glucose concentrations. D-xylose was measured as
described by Merritt and Duely (1983). Plasma glucose concentrations were determined using a glucose oxidase base kit (Wako, Richmond VA).

**Measurements:**

Apparent efficiency of IgG absorption (AEA) at 0, 6, 12, 18, 24 h of age was estimated using the equation: (plasma IgG [g/L] x body weight [kg] x 0.09/IgG intake) x 100% (Quigley et al., 1998a). The IgG concentration, area under the curve (AUC), and mean IgG were analyzed. Total serum protein concentrations were also analyzed. The blood samples collected on d 3 at 0, 2, 4, 6, 8, and 12 h were analyzed for the concentration mean and AUC for both D-xylose and glucose.

**Statistical Analysis:**

A randomized complete block design with a 2 x 4 factorial arrangement of treatments was used, and calves were randomly assigned to treatments in blocks of eight based on birth order and location. Analysis of varience was conducted using the MIXED procedure of SAS (SAS Institute, 2001). The mixed model effect used was:

\[
Y_{ijk} = \mu + b_i + L_j + R_k + LR_{jk} + KC_{ijk} + E_{ijk}
\]

Where:

- \( Y \) = the dependent variable,
- \( \mu \) = the overall mean,
- \( b_i \) = the random effect of block \( i \) (\( i = 1, \ldots, 10 \)),
- \( L_j \) = the fixed effect of the \( j \)th lactoferrin level (\( j = 0, 0.5, 1, 2 \)),
- \( R_k \) = the fixed effect of \( k \)th colostrum replacer feeding (\( k = 1, 2 \)),
- \( LR_{jk} \) = the fixed effect of the interaction between the \( j \)th lactoferrin level and the \( k \)th colostrum replacer feeding,
- \( K \) = the regression coefficient of the covariate \( C \)
- \( C_{ijk} \) = is the covariate variable for the \( l \)th calf, of the \( i \)th block, of the \( j \)th lactoferrin level, \( k \)th colostrum replacer feeding (\( l = 1, \ldots, 8 \)), and
- \( E_{ijk} \) is the random residual \( \sim N(0, \sigma^2_e) \).

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
Significance was determined at a $P \leq 0.05$. Data were tested for linear, quadratic, and cubic response to level of Lf supplementation. Degrees of freedom were determined using the Satterwaite option of the MIXED procedure of SAS (SAS® Institute, 2001).

**Results**

Chemical analysis of CR and MR are presented in Table 1. The Lf content of CR was 0.78g/dose and the lactoferrin content of MR was 0.45 mg/g of DM.

Calves fed two doses of CR had higher mean serum IgG concentrations ($P < 0.001$) and total serum protein ($P < 0.01$) compared to calves fed one dose of CR (Table 2). Calves fed a second dose of CR at 12 h had higher plasma IgG and total serum protein levels at 18, 24, and 48h, resulting in a significant feed x time interaction ($P < 0.05$) (Figure 1 and 2). On average, calves attained blood serum IgG concentration ≥ 10 mg/mL resulting in successful passive transfer by 24 h, except for calves fed 1 dose of CR + 1 or 2 g/d of supplemental Lf. The proportion of calves that attained passive IgG transfer per treatment is shown in Table 2. There were no effects of Lf supplementation on serum IgG concentrations and total serum protein concentrations. Area under the curve of IgG (Table 2) was greater for the calves fed 2 doses CR compared to the calves fed 1 dose CR ($P<0.0001$). Lactoferrin supplementation had no effect on the results for AUC of IgG.

There was a decrease ($P < 0.0001$) in AEA of IgG when 2 doses of CR were fed versus 1 dose of CR (Table 2). Apparent efficiency of absorption of IgG decreased quadratically ($P < 0.05$) with increasing levels of Lf (Table 2).

Two hours after xylose intake, plasma xylose concentrations were higher in calves fed two doses of CR ($P < 0.01$), although at 8 and 12 h after xylose intake ($P<0.05$) plasma xylose concentrations were higher in the calves fed one dose of CR (Figure 3).
However, the overall mean for plasma xylose concentrations of all the time points' were not different between calves fed one dose verses calves fed two doses of CR (Table 2). Plasma xylose concentration and AUC for xylose decreased quadratically ($P < 0.01$) with increasing Lf supplementation (Table 2). There was a trend ($P < 0.1$) for plasma glucose with calves fed two doses of CR to have higher concentrations of plasma glucose then calves fed 1 dose of CR. There was no effect of Lf on glucose concentrations (Table 2).

**Discussion**

Serum IgG concentration can be affected by dystocia (Donovan et al., 1986) and by birth weight, with larger calves having lower IgG concentrations on average because of they have higher plasma volumes (Quigley et al., 1998). In this experiment, there were no differences among treatments in calf birth weights or calving scores.

In this study, on average calves attained blood serum IgG concentration $\geq 10$ mg/mL resulting in successful passive transfer by 24 h, except for calves fed 1 dose of CR + 1 or 2 g/d of supplemental Lf. This is different from other studies in which it was observed that lacteal secretion based CR did not consistently provide successful passive transfer (Zaremba et al., 1993; Garry et al., 1996; Mee et al., 1996; Hopkin and Quigley, 1997; Arthington et al., 2000). However, the number of calves which attained passive transfer was higher when fed 2 doses of CR vs 1 dose CR ($P = 0.03$). The AEA of IgG was lower in calves fed 2 doses of CR, perhaps the result of more IgG supplied then the calves were able to absorb.

The purpose of adding Lf to the CR was to determine if the quality of CR could be improved by increasing IgG absorption. A previous study conducted in our laboratory (White, 2005) showed an effect of Lf on average AEA over time, when bull
calves were fed maternal colostrum with 1g/d of supplemental Lf. However, at 24 h only there was no effect of Lf on AEA of IgG. The present study showed no effect of Lf on serum IgG concentration but there was a suppressing quadratic effect ($P<0.05$) on AEA of IgG with increasing levels of Lf. White (2005) added 1 g/d of Lf to maternal colostrum, which has an average of about 2 mg/mL of Lf (Molenaar et al., 1996). The calves received 2 feedings (2L/feeding) of maternal colostrum bringing the total Lf consumed to be about 9 mg/mL. In the present study, Lf was added to CR which only had an Lf concentration of 0.78g/dose of CR so at the highest supplementation of 2g these calves were still receiving less Lf then the calves used by White (2005). In addition, maternal colostrum may have other nutrients such as vitamins and other bioactive proteins that may aid in IgG absorption and intestinal development that may not be present in the CR.

The decrease in AEA of IgG when Lf was supplemented to CR may have been caused by Lf ability to bind to Ig such as IgG and IgA prior to absorption (Wang and Hurley, 1998). This would increase the overall size of the molecule, thus possibly decreasing it ability to be absorbed. Other components found in maternal colostrum may compensate for this effect, resulting in Lf not hindering IgG absorption as shown by White (2005). The complexes between Lf and other proteins may either enhance or reduce its biological functions (Wang and Hurley, 1998). In the case of Lf binding to IgA, there is a synergistic effect on bacteriostatic activity (Stephans et al., 1980) but the Lf-casein complex decreases the antibacterial activity of Lf (Ellison and Giehl, 1991; Hurley, 1993).
There was a decreasing quadratic effect ($P<0.01$) with increasing Lf supplementation on xylose absorption and AUC with calves fed 0.5 and 1 g of Lf having lower plasma xylose concentration than calves fed 0 and 2 g of Lf. The xylose absorption test is used as an indirect measurement of glucose absorption, intestinal epithelial size and function (Hammon and Blum, 1997; Kuhne et al., 2000; Rauprich et al., 2000). The results from this study indicate that Lf is impairing xylose absorption and possibly intestinal development when supplemented at 0.5 and 1 g per day for the first 3 days of life. Cowles et al. (2006) also performed a xylose challenge on calves fed MR supplemented with 1 g/d of Lf and found no effect of Lf. However, calves were 10 days old at the time of the xylose challenge and only fed one level of Lf at 1g/d added to either a nonmedicated conventional MR (20% CP: 20% fat) or a nonmedicated intensified MR (28% CP:20% fat). These authors reported no effect of feeding the 1g/d of Lf on xylose absorption while in the present study both 0.5 and 1g/d of Lf had a negative impact on xylose absorption. The maximum Lf fed per feeding was 1.78 mg/mL in the present study compared to Cowles et al. (2006) in which the maximum Lf fed per feeding was 1.05 mg/mL.

This is contradictory to other studies in which Lf was observed to increase intestinal development (Zhang et al., 2001; Humphrey et al., 2002). However, Zhang et al. (2001) fed milk from transgenic dams to neonatal mice for 10 d. The milk from these transgenic dams had a Lf concentration of 12 mg/mL. Zhang et al. (2001) fed Lf at a much higher concentration and for a longer period of time than in the current study. Humphrey et al. (2002) observed greater villous height, better nutrient absorption, and greater feed efficiency in chicks when Lf was fed in combination with lysozyme. There
was no effect on intestinal development when Lf was fed alone. These studies would indicate that if the neonatal calf responded in the same way as neonatal mice and chicks than Lf would have to be fed at a much higher concentration or in conjunction with another component of maternal colostrum such as lysozyme.

**Conclusion**

Calves can attain successful passive transfer when fed 1 dose of a lacteal based CR. However, feeding calves 2 doses of CR is recommended to provide a margin of safety due to factors affecting absorption. The AEA of IgG in CR is not improved by Lf supplementation at the levels fed. Lactoferrin does not increase xylose absorption in the calf at the levels fed; suggesting intestinal development is not improved by Lf supplementation. More research needs to be done to determine the components in maternal bovine colostrum that affect intestinal development and IgG absorption.
Table 1. Nutrient analysis on a DM basis of Colostrum Replacer and Milk Replacer

<table>
<thead>
<tr>
<th>Item</th>
<th>CR</th>
<th>MR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>93.5</td>
<td>89.5</td>
</tr>
<tr>
<td>CP, %</td>
<td>54.9</td>
<td>20.6</td>
</tr>
<tr>
<td>Fat (acid hydrolysis),%</td>
<td>22.6</td>
<td>18.9</td>
</tr>
<tr>
<td>Ca, %</td>
<td>1.1</td>
<td>0.97</td>
</tr>
<tr>
<td>P, %</td>
<td>0.99</td>
<td>0.64</td>
</tr>
<tr>
<td>Mg, %</td>
<td>0.16</td>
<td>0.15</td>
</tr>
<tr>
<td>Fe, ppm</td>
<td></td>
<td>102</td>
</tr>
<tr>
<td>Lactoferrin (g/dose(^3))</td>
<td>0.78</td>
<td>0.48</td>
</tr>
</tbody>
</table>

\(^1\) Land O Lakes colostrum replacer, Saskatoon, SK, Canada
\(^2\) Blue Seal Feeds non-medicated milk replacer, Londonderry, NH
\(^3\) 1 dose is equal to 470 g with $\geq 100$ g of bovine IgG
Table 2: Overall means of IgG, serum protein, xylose and glucose. Apparent efficiency of absorption of IgG and area under the curve for IgG, xylose, and glucose.

<table>
<thead>
<tr>
<th>Item</th>
<th>1 CR</th>
<th>2 CR</th>
<th>Lactoferrin</th>
<th>SE</th>
<th>CR Effect</th>
<th>Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPT, %</td>
<td>0.8</td>
<td>0.79</td>
<td>0.7</td>
<td>0.4</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Serum, IgG g/L</td>
<td>9.7</td>
<td>9.0</td>
<td>8.8</td>
<td>8.1</td>
<td>11.2</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>10.7</td>
<td>10.3</td>
<td>9.7</td>
<td>8.7</td>
<td>14.5</td>
<td>13.1</td>
</tr>
<tr>
<td>Serum Protein (g/L)</td>
<td>4.9</td>
<td>4.8</td>
<td>4.9</td>
<td>4.8</td>
<td>5.1</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>4.9</td>
<td>4.7</td>
<td>5.0</td>
<td>4.6</td>
<td>5.4</td>
<td>5.4</td>
</tr>
<tr>
<td>AUC IgG (g/L.h)</td>
<td>2160.1</td>
<td>1875.9</td>
<td>1827.4</td>
<td>1442.9</td>
<td>3306.2</td>
<td>2449.9</td>
</tr>
<tr>
<td>AEA, %</td>
<td>44.0</td>
<td>41.0</td>
<td>34.0</td>
<td>39.0</td>
<td>33.0</td>
<td>26.0</td>
</tr>
<tr>
<td>Xylose (mmol/L)</td>
<td>2.1</td>
<td>1.8</td>
<td>1.9</td>
<td>2.0</td>
<td>2.0</td>
<td>1.8</td>
</tr>
<tr>
<td>AUC Xylose (mmol/dL.h)</td>
<td>31.3</td>
<td>24.3</td>
<td>26.9</td>
<td>26.7</td>
<td>28.3</td>
<td>22.3</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>103.9</td>
<td>100</td>
<td>95.8</td>
<td>96.9</td>
<td>103.5</td>
<td>106.6</td>
</tr>
<tr>
<td>AUC Glucose (mg/dL.h)</td>
<td>66989.0</td>
<td>62349.0</td>
<td>57835.0</td>
<td>59067.0</td>
<td>66586.0</td>
<td>70293.0</td>
</tr>
</tbody>
</table>

1 Calves were fed either 1 dose of CR or 2 doses of CR with either 0, 0.5, 1, or 2 g/d of lactoferrin in the first two feedings.
2 CR = colostrum replacer
3 Contrast: linear and quadratic effects of lactoferrin.
4 Main effect of colostrum replacer.
5 SPT = Proportion of calves that attained successful passive IgG transfer > 10 g/L
6 P<0.05.
7 Data collected from all 80 calves collected from both dairies.
8 Data collected from 48 calves from University of New Hampshire dairy.
Figure 1: Serum IgG concentrations (mg/mL) of calves fed either 1 or 2 doses of CR with varying levels of lactoferrin over the first 48h of life. 1L0 = 1 dose CR + 0g of lactoferrin, 1L0.5 = 1 dose CR + 0.5g of lactoferrin, 1L1 = 1 dose CR + 1g of lactoferrin, 1L2 = 1 dose CR + 2g of lactoferrin, 2L0 = 2 dose CR + 0g of lactoferrin, 2L0.5 = 2 dose CR + 0.5g of lactoferrin, 2L1 = 2dose CR + 1g of lactoferrin, 2L2 = 2 dose CR + 2g of lactoferrin. The largest SEM was 0.87 and occurred in calves fed 1L2 for all time points. * (P < 0.05) Calves fed two doses of CR had greater plasma IgG.
Figure 2: Serum Protein concentration (mg/mL) of calves fed either 1 or 2 doses of CR with varying levels of lactoferrin over the first 48h of life. 1L0 = 1 dose CR + 0g of lactoferrin, 1L0.5 = 1 dose CR + 0.5g of lactoferrin, 1L1 = 1 dose CR + 1g of lactoferrin, 1L2 = 1 dose CR + 2g of lactoferrin, 2L0 = 2 dose CR + 0g of lactoferrin, 2L0.5 = 2 dose CR + 0.5g of lactoferrin, 2L1 = 2 dose CR + 1g of lactoferrin, 2L2 = 2 dose CR + 2g of lactoferrin. The largest SEM was 0.13 and occurred in calves fed 1L2 for all time points.
* (P < 0.05) Calves fed two doses of CR had greater serum protein.
Figure 3: Plasma xylose concentration (mg/mL) of calves fed either 1 or 2 doses of CR with varying levels of lactoferrin over the first 48h of life. 1L0 = 1 dose CR + 0g of lactoferrin, 1L0.5 = 1 dose CR + 0.5g of lactoferrin, 1L1 = 1 dose CR + 1g of lactoferrin, 1L2 = 1 dose CR + 2g of lactoferrin, 2L0 = 2 dose CR + 0g of lactoferrin, 2L0.5 = 2 dose CR + 0.5g of lactoferrin, 2L1 = 2 dose CR + 1g of lactoferrin, 2L2 = 2 dose CR + 2g of lactoferrin. The largest SEM was 0.15 and occurred in calves fed 1L1 for all time points.

* (P < 0.01) Calves fed two doses of CR had higher plasma xylose concentrations and a quadratic response to lactoferrin with 0.5g/d and 1g/d being less than 0 and 2 g/d.

*(P < 0.05) Calves fed one dose of CR had higher xylose concentrations.
Figure 4: Plasma glucose concentration (mg/mL) of calves fed either 1 or 2 doses of CR with varying levels of lactoferrin over the first 48h of life. 1L0 = 1 dose CR + 0g of lactoferrin, 1L0.5 = 1 dose CR + 0.5g of lactoferrin, 1L1 = 1 dose CR + 1g of lactoferrin, 1L2 = 1 dose CR + 2g of lactoferrin, 2L0 = 2 dose CR + 0g of lactoferrin, 2L0.5 = 2 dose CR + 0.5g of lactoferrin, 2L1 = 2 dose CR + 1g of lactoferrin, 2L2 = 2 dose CR + 2g of lactoferrin. The largest SEM was 5.12 occurring at all time points.

* (P < 0.05) Calves fed two doses of CR had greater glucose concentrations.
REFERENCES


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


APPENDIX

Institutional Animal Care and Use Committee Approval
December 22, 2005

Erickson, Peter
The Dairy Nutrition Research Center
30 O'Kane Road
Durham, NH 03824

IACUC #: 051102
Approval Date: 12/22/2005
Review Level: B

Project: The Supplementation of Colostrum Replacer with Lactoferrin

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category B on Page 4 of the Application for Review of Vertebrate Animal Use in Research or Instruction - the study involves either no pain or potentially involves momentary, slight pain, discomfort or stress. The IACUC made the following comment on this protocol:

1. In Section VII, 1, e (animal procedures, blood sampling), the Committee changed "13" to "12."

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 study. Requests for extension must be filed prior to the expiration of the original approval.

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

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

For the IACUC,

[Signature]
Jessica A. Bolker, Ph.D.
Chair

cc: File

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

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.