Effects of heat-treatment of colostrum on the development of calves in the neonatal and pre-weaned periods

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Abstract

Calves are born agammaglobulinemic and must ingest vital immunoglobulins via colostrum for protection from infection during the neonatal period. Colostrum also contains many other biologically active factors, such as growth factors, immune cells and antimicrobial peptides, which are important for development, growth and health of the calf. Although essential to the calf, colostrum also represents one of the earliest sources of exposure to infectious pathogens. Pasteurization of milk and colostrum is becoming a common practice used to reduce vertical transmission of pathogens to young calves. Recently, there has been a growing interest in the effects of heating colostrum on its components and on the development of calves. The objective of this study was to investigate the effects of heat-treatment of colostrum on gut absorption and development in bull calves in the first 12 hours of life, and the effects of absorption, growth and health in pre-weaned heifer calves. Bull calves (n=23) and heifer calves (n=17) were fed 2L of either unheated or heat-treated colostrum within an hour after birth. In the first experiment, absorption of IgG and β-lactoglobulin (BLG), villus height and crypt depth of the ileum and ileal goblet cell number were measured in bull calves. Bulls that received heat-treated colostrum had 18% lower plasma IgG at 12 hours of age compared to those fed unheated colostrum. Bull calves receiving heat-treated colostrum also had lower plasma BLG concentrations beginning at 6 hours of life. By 9 hours, those that received heat-treated colostrum had 51% less plasma BLG compared to bulls fed unheated colostrum. The BLG concentration decreased more rapidly from circulation than IgG. No significant differences in villus height or crypt depth were detected with regards to colostrum treatment. Calves that received heat-treated colostrum had a significantly higher number of goblet cells compared to those receiving unheated colostrum. In the second experiment, heifer calves were also analyzed for absorption of IgG and BLG into the blood. Average daily gain, grain intake, and daily rectal temperatures were recorded for each heifer until

weaning. Despite being fed from the same pools of colostrum, heifer calves did not demonstrate the same absorption patterns for IgG and BLG as were seen with the bulls. Heifer calves receiving heat-treated colostrum had higher circulating IgG levels during the first 24 hours of life compared to those fed unheated colostrum. No significant differences in plasma BLG concentration, grain intake, or health scores were detected. Daily rectal temperatures also did not differ significantly during weeks 1, 2, 3, 4, and 6. However, during week 5, calves that had received heat-treated colostrum had significantly higher rectal temperatures compared to those fed unheated colostrum. Although no statistically significant differences were detected for average daily gain, during week 5, there was a tendency for heifers receiving heat-treated colostrum to have a lower rate of gain. Week 5 corresponds to when calves were dehorned. The results of the study indicate that heat-treatment of colostrum influences absorption, gut development and health of calves during the neonatal and pre-weaned periods of life.

Research is what I'm doing when I don't know what I'm doing.
-Wernher von Braun

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ADG	Average daily gain
AEA	Apparent efficiency absorption
ANOVA	Analysis of variance
BCA	Bicinchoninic acid
BLG	Beta-lactoglobulin
BMP	Bone morphogenic protein
BSA	Bovine serum albumin
BW	Body weight
CD	Cluster of differentiation
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
EIA	Enzyme immunoassy
ELISA	Enzyme linked immunoassay
FcRn	Neonatal Fc receptor
FPT	Failure of passive transfer
GALT	Gut-associated lymph tissue
GIT	Gastrointestinal tract
GLP-2	Glucagon-like peptide-2
HRP	Horseradish peroxidase
IFN-γ	Interferon-gamma
IGF	Insulin-like growth factor
Ig	Immunoglobulin
IL	Interleukin
LSM	Least squares means
MHC	Major histocompatibility complex
MR	Milk replacer

PAS	Periodic acid-Schiff
PBS	Phosphate buffered saline
PIgR	Polymeric immunoglobulin receptor
RIPA	Radio-immuno precipitation assay
RNA	Ribonucleic acid
SDS	Sodium dodecyl sulfate
SPM	Sphingomyelin
TBS	Tris buffered saline
TGF	Transforming growth factor
TMB	3,3',5,5'-Tetramethylbenzidine
TP	Total protein

Chapter 1. Literature Review

1.0 Introduction

Passive transfer of immunity is defined as the absorption of maternal antibodies by the neonate during the first day of life. Antibodies, or immunoglobulins, from the dam help protect the newborn animal from infection in early life (Enders and Carter, 2004). In the cow, the binucleate trophoblast cells of the placenta, which facilitate transport of nutrients to the fetus, fuse with the maternal epithelial layer, separating the fetal and maternal blood supplies and thereby preventing transfer of antibodies from maternal blood to the fetus *in utero*. Ruminant-type of placentation is called syndesmochorial. In contrast, humans have a hemochorial placenta, in which trophoblast cells of the blastocyst are in direct contact with maternal blood, allowing passive transfer of immunity *in utero* (Enders and Carter, 2004).

Due to the bovine placental physiology, described above, calves are born agammaglobulinemic and rely on antibodies in colostrum for passive transfer of immunity. Colostrum is the first milk produced after parturition. It is honey-colored, more viscous than mature milk, due to its composition, and it is comprised of high concentrations of proteins, fat, vitamins and minerals. This ensures that a nutrient-rich "package" is delivered to the newborn soon after birth, including a high concentration of immunoglobulins critical to the survival and health of the calf.

Early studies demonstrated the importance of colostrum for reducing calf mortality and morbidity. The work of Smith and Little (1922) shed light on the specific role of colostral antibodies in the neonatal calf. In their experiment, ten newborn calves received colostrum and twelve were deprived of colostrum. All animals receiving colostrum survived and nine of the deprived animals died. The researchers described the colostrum-deprived calves as becoming septic, as indicated by the calves' symptoms and

presence of *E. coli* bacteria throughout the body. Much research has followed this early work in order to further understand the molecules in colostrum and mechanisms responsible for protecting the neonate from pathogens.

It is classically understood that immunoglobulins are the critical components that provide this early protection. The level of protection is described as either successful or failed passive transfer. Calves that achieve at least 10 mg/ml serum IgG concentration by 24 hours of age are considered to have adequate passive transfer, whereas less than 10 mg/ml is considered failure of passive transfer (FPT). The rate of FPT remains at nearly 20% (NAMHS, 2007). As much as 39% of calf mortality occurs as a result of failed passive transfer (Tyler et al., 1999). Despite these statistics, great strides have been made in improving newborn calf and colostrum management on farms. Of the 80% of calves that had successful passive transfer, approximately 83% had greater than 15 mg/ml serum IgG, which is considered excellent (NAHMS, 2007).

Although immunoglobulins are essential, colostrum contains many other biologically active components, often in high concentration, that may contribute to the growth and development of the neonate. Examples of these compounds include hormones, growth factors, antimicrobial compounds and immune-related factors (Pakkanen and Aalto, 1997). These components are important for promoting and mediating normal development of the gut and immune system of the neonate and may influence the future growth and productivity of the animal. The functions of many bioactives are not well understood and more research is required to investigate the impact they have on the calf (Pakkanen and Aalto, 1997).

Pasteurization of milk and colostrum has become popular with producers, especially on large dairy farms, as a method for preventing vertical transmission of pathogens in young calves. However, research has shown that the temperatures recommended for pasteurization of milk cause significant reductions in IgG concentrations and undesirable thickening of the colostrum (Godden et al., 2003). Several studies have investigated the effects of heat-treatment on IgG concentration in colostrum and absorption in the newborn calf (Elizondo-Salazar and Heinrichs, 2009b; Johnson et al., 2007; McMartin et al., 2006). Few studies have looked at the effects of pasteurization on components of bovine colostrum other than IgG. Since most of these components are

found at higher concentrations in colostrum as compared to mature milk, their importance to the neonate should not be overlooked. Although colostrum quality is most often defined by the concentration of IgG, research should also consider other biologically active compounds that may be critical to growth, development and health of the neonate.

The objective of this study was to investigate the effects of pasteurization of colostrum on absorption and gut development in neonatal calves and absorption, growth and health in pre-weaned calves.

1.1 Importance of colostrum to the calf

The newborn calf is born into a world laden with challenges it must overcome in order to grow and develop into a healthy, productive adult. As a result of the bovine's syndesmochorial, cotyledonary placenta, in which the maternal and fetal blood supplies are separated (See Figure 1.1), passive transfer of antibodies in utero is completely prevented (Enders and Carter, 2004). The neonate is thus born without protection against pathogens it will immediately encounter in the environment and must obtain passive immunity in the form of ingested maternal antibodies in colostrum (Morein et al., 2007; Nousiainen et al., 1994). Colostrum is the first milk secreted by the mammary gland. It is available to the calf immediately post-partum, and may be produced for several days (Larson et al., 1977). Immunoglobulins, which are highest in concentration at first milking, are critical to protect the newborn from transmission of pathogenic infections in early life (Godden, 2008; Weaver et al., 2000). The critical importance of colostrum to calves was initially shown in a carefully controlled study by Smith and Little (1922), in which calves were either fed colostrum or had colostrum withheld. Of the calves that had colostrum withheld, most died.

Maternal protection persists, but gradually declines, over the first few weeks of life. Simultaneously, the calf's own immune system matures and begins producing antibodies sufficient to protect itself from infection (Nousiainen et al., 1994). The precise timing of this transitional period in the calf is not entirely known, but most research suggests that active immunity in the calf develops gradually and begins to approach

functional levels in the first 2-4 weeks after birth and then reaches adult antibody levels by approximately four months (Chase et al., 2008; Robison et al., 1988) (See Figure 1.2).

Colostrum also contains a high concentration of nutrients including protein (much of which consists of the immunoglobulins), lipids, carbohydrates, minerals and vitamins. Nearly all components of colostrum are present at higher concentrations compared to those measured in mature milk, with the main exception being lactose (Blum and Hammond, 2000; Foley and Otterby, 1978). As the foremost osmoregulator of milk volume, lactose synthesis is low initially to ensure delivery of a highly concentrated dose to the neonate. Lactose synthesis rapidly increases after the first few days post-partum, which leads to a dilution of the other components (Brew et al., 1968). Transfer and production of many components, such as immunoglobulins also decrease over time, contributing to the lower concentrations (Barrington et al., 2001).

Table 1.1 lists some examples of components of colostrum and milk with known concentrations. Compared to mature milk, colostrum also contains a seemingly infinite variety of compounds such as hormones, growth factors, immune cells, cytokines, other anti-microbial factors, small peptides, oligosaccharides, metabolites and enzymes (Blum and Hammon, 2000; Pakkanen and Aalto, 1997). These compounds are referred to as biologically active components, or bioactives, and they function to promote and modulate development of the gut, protection against pathogens, regulate absorption and influence development of the immune system. For example, lactoferrin is widely known to negatively affect the growth of some bacteria (Pakkanen and Aalto, 1997). While some of the bioactives detected in milk or colostrum have known functions and have been studied in depth, the roles of many others have yet to be elucidated.

To the calf, colostrum is essential for survival and lowering the risk of disease during early life. The combination of passive transfer and the bioactivity of colostrum contributes to ensuring a successful start and may influence future growth, milk production, health and longevity (Godden, 2008) of the mature cow. For producers, improving colostrum quality, not only with regards to IgG concentration but also in terms of bioactivity, will have direct positive impacts on survival and productivity of their dairy animals, as well as other livestock species.

1.2 Immunoglobulins of colostrum

In bovine colostrum, the immunoglobulin class present in highest concentration, and most important to the immediate health of the calf, is IgG, which consists of two subclasses: IgG_1 and IgG_2. Whereas these two subclasses are similar in concentration in the maternal circulation, IgG_1 predominates in colostrum, accounting for approximately 90% of the total IgG (Barrington et al., 2001; Larson et al., 1980). Transfer of IgG_1 increases close to parturition, allowing for a large accumulation in the mammary gland (Sasaki et al., 1976).

Immunoglobulin accumulation occurs as a result of a number of different mechanisms in cows. Brandon et al. (1971) observed that whereas IgG_2, IgA and IgM concentrations in maternal blood remain at the same concentration during colostrum formation, serum IgG_1 levels decline significantly, suggesting a specific transport mechanism that selects for this class of immunoglobulin.

Indeed, IgG_1 is selectively transferred from the maternal circulation into the mammary gland by the neonatal Fc receptor (FcRn), a receptor that has a variety of functions, but which is best known for its role in transporting colostral IgG across the gut wall in the neonatal rodents (Rodewald, 1973; Rodewald and Kraehenbuhl, 1984). The FcRn receptor was originally found to be expressed in the mammary gland in mice (Cianga et al., 1999), and was later found in the bovine mammary gland by Kacskovics et al. (2000). The FcRn receptor is comprised of two subunits: β2-microglobulin (the light chain) and an integral membrane polypeptide (the heavy chain), the latter of which bears a similarity to the major histocompatibility complex (MHC) class I molecules (Simister and Mostov, 1989). Research suggests that the Fc portion of IgG binds to the FcRn receptor on the apical surface of intestinal epithelial cells and the function of this receptor is to transport IgG from colostrum in the gut into circulation (Raghavan et al., 1993; Rodewald et al., 1976). Results from several studies show that binding of IgG to, and release from, the receptor are pH-dependent. Studies by Rodewald (1976) and Raghavan et al. (1993) show that IgG binds FcRn at a pH between 6.0 and 6.5 but dissociates at a pH around 7.4. This provides strong evidence that pH is important for binding of IgG in colostrum (pH 6.0), and its subsequent release into the blood (pH 7.4).

Results for the above studies suggest that colostrum with a lower than normal pH may promote an increase in absorption of IgG by the newborn calf. However, a study examining the effects of pH levels of colostrum showed no difference in blood IgG concentration in calves that were fed colostrum replacer containing a pH of 7.5, 7.0, 6.0, and 5.0 (Quigley et al., 2000). Although a narrow pH appears to be required for the transport of IgG from the gut lumen into circulation, homeostatic mechanism are likely returning colostral pH to normal levels in the gut prior to IgG reaching the site of absorption.

Although the pH-dependent FcRn-IgG relationship has been well recognized, the system remains complex. The FcRn receptor on the apical surface of intestinal cells is down-regulated 1000-fold after the neonatal period (Qiao et al., 2007), but the receptor continues to function in the adult animal and appears to be responsible for bidirectional transcytosis of IgG across epithelial layers (Horton and Vidarsson, 2013; Yoshida et al., 2004). The regulatory mechanism by which IgG is transported from circulation into the gut is not well understood.

The two other immunoglobulin classes that have significant importance to the calf are IgA and IgM. Their concentrations in colostrum are approximately 5% and 7%, respectively (Larson et al., 1980). Whereas IgG is sourced from circulation, IgA and IgM are produced locally in the mammary gland. Evidence suggests that B lymphocytes originating from other mucosal surfaces, such as the gastrointestinal tract (GIT), migrate to the mammary gland during colostrogenesis and differentiate into plasmacytes, capable of producing immunoglobulins (Devery et al., 1979; Salmon, 1999).

Colostral IgA and IgM are passively absorbed in the small intestine and are subsequently transported back into the mucosal lumen as polymeric structures. The polymeric immunoglobulin receptor (pIgR) is responsible for transcytosis of IgA and IgM across mucosal epithelial cells, where they are released into the mucosal lumen. Upon release, a fragment of the pIgR molecule, called the secretory component, is cleaved off bound to the immunoglobulin. This secretory component is anti-inflammatory and is important for normal immune function of IgA (Horton and Vidarsson, 2013).

1.3 Passive transfer of immunity in the calf

Due to the importance of maternal antibodies to the immediate health of the neonatal calf, it must be emphasized that studies of colostrum have predominantly focused on passive transfer of immunity with regard to immunoglobulins, specifically IgG_1. Research going back many decades has investigated strategies for achieving successful passive transfer. There are many things that contribute to successful passive transfer, but there are three main factors that producers and researchers consider: 1) colostrum quality 2) colostrum quantity and 3) timing of colostrum feeding (Godden, 2008). Inability to meet all or some of these criteria will result in failure of passive transfer.

Colostrum fed to calves should contain a minimum of 50 mg/mL IgG concentration, with the goal of achieving $\geq$ 5.2 g/dL serum total protein in calves 24-48 hours old (McGuirk and Collins, 2004). Serum total protein, typically assessed by refractometer, is highly correlated with serum IgG and can be used to approximate passive transfer. Colostrum quality is influenced by a number of factors including parity, breed of dam, nutritional status pre-partum, season, volume of colostrum produced, and length of the dry period (Godden, 2008; Weaver et al., 2000).

Volume of colostrum has also been shown to impact the passive transfer status in calves. Morin et al. (1997) fed calves 4 or 2 liters of high quality colostrum (~60 mg/ml) at birth. Both groups were fed 2 additional liters at 12 hours. Results reported that when high quality colostrum was fed to calves in larger volumes (4 versus 2 liters), there was higher serum concentration of IgG. However, this outcome appears to depend on the quality of the colostrum being administered. In a separate experiment, but part of the same study, calves were fed either 4 or 2 liters of low quality colostrum (23.9 mg/ml) at birth, followed by 2 additional liters 12 hours later. No differences were seen in serum IgG concentrations at 24 and 48 hours after birth. Calves in both these groups did not achieve adequate levels of serum IgG. An additional group in the low quality colostrum experiment received an extra 2 liters at 6 hours. These calves had significantly higher serum IgG concentrations compared to the other two low quality groups, and they achieved successful passive transfer. These results suggest that when calves are fed low

quality colostrum, multiple feedings may be advantageous, and that timing of feeding may be more important than volume.

Allowing the calf to ingest colostrum by natural suckling, even from dams with high colostral IgG concentrations (for adequate passive transfer, a calf would need to consume 2 liters of colostrum with an IgG concentration of 50 mg/ml), has been shown to result in higher rates of FPT (61%) as compared to feeding via bottle or esophageal feeding (19 and 10%, respectively) (Besser et al., 1991). This high rate of FPT has been attributed largely to the calf's inability to voluntarily consume a high enough volume of the dam's colostrum prior to the cessation of the ability of the gut to absorb macromolecules.

Perhaps more important than quality and quantity of colostrum is timing of delivery. This factor has been subject to much debate over the years, but the general consensus is that the earlier the better in order to optimize absorption of IgG and other important components of colostrum. A process termed gut closure results in the inability of the gut to absorb macromolecules approximately 36 hours after birth. Several studies have suggested that the optimal time for feeding is in the first 4-6 hours after birth but passive transfer could still be achieved if colostrum was fed within the first 12-24 hours (Besser et al., 1985; Stott et al., 1979). Gut closure will be further discussed in a later section (see gut development).

The factors that influence the recommendations for quality, quantity and timing have been well studied for IgG, but they may be very different for other bioactive compounds. Little is known about similar criteria for these other components of colostrum. Although the importance of IgG to the neonatal calf is irrefutable, future recommendations for colostrum and calf management should also consider both the classic components of passive immunity (i.e. IgG) as well as the potential absorbance and bioactivity of other potentially essential factors.

1.4 Bioactive components in colostrum

Colostrum contains numerous compounds- perhaps as many as 2000 molecules or more- that are thought to have essential biologically-active functions (Guimont et al., 1997; Pakkanen and Aalto, 1997). These functions are proposed to include: promoting and facilitating development of the gut and immune system, mediating absorption of nutrients, stimulating enzymatic activity and providing early protection from disease. To be considered a bioactive compound, components of colostrum must be active or become active, have a functional role, and be associated with signs of deficiency in the colostrum-deprived animal. (Ellis et al., 1996).

Some components, such as immunoglobulins, have been studied extensively and their functions are understood. Many others are recognized as having a potential benefit but their importance to the neonate is not fully understood. Nearly all bioactive components secreted by the mammary gland occur at a significantly higher concentration in colostrum as compared to mature milk (Blum and Hammon, 2000). This fact lends itself to the idea that an important role of colostrum, via these bioactives, is to facilitate communication or signaling from dam to offspring in order to prepare the newborn for the environment into which it has been born. This process is referred to as lactocrine signaling (Bartol et al., 2013) and may be critical during the initial few weeks of life, mediating development and growth of the newborn, and may also be important for future health and productivity of the calf.

Immunoglobulins make up a large percentage of the protein fraction of colostrum, but other specific proteins may have biological functions in the newborn calf. Whey proteins are an important nutritional component of milk and colostrum, but may also have bioactive properties. The most abundant whey protein, β-lactoglobulin (BLG), is found in many species including the cow. It is not, however, present in the milk of humans or rodents. Two main genetic variants of the protein, A and B, are found in the cow, and it is present in bovine colostrum at approximately 14 mg/ml at the first milking (Levieux and Ollier, 1999). The function of BLG is not completely understood but it is known to have mitogenic characteristics (Capiaumont et al., 1994), and is of interest in terms of its function in the gut beyond providing a source of amino acids to the calf. BLG is a source

of biologically active peptides, arising from proteolytic cleavage of the mother protein. Although BLG is not found in human milk, the protein shares 83% homology with the human protein, glycodelin A, which is heavily involved in regulation of the immune system (Chatterton et al., 2013). Bioactive properties of BLG include angiotensin-I-converting enzyme inhibition, anti-hypertensive effects, antioxidant properties and antimicrobial activities or immunomodulating activities (Hernandez-Ledesma et al., 2008). Biziulevicius et al. (2006) investigated further studied BLG and its immuno-enhancing effects. Common food proteins, including casein, α-lactalbumin, β-lactoglobulin, ovalbumin and serum albumin were enzymatically hydrolyzed. The hydrolysates of these proteins were tested for their stimulatory effects on enzymatic self-destruction, called autolysis, in bacteria. The protein hydrolysates were also fed to mice, and the phagocytic capacity of macrophages from these mice was analyzed. All hydrolysates stimulated autolysis in all bacterial strains tested. Hydrolysates derived from milk proteins (casein, α-lactalbumin and β-lactoglobulin) produced increased activity compared to ovalbumin and serum albumin. All hydrolysates also enhanced phagocytic capacity of macrophages from the mice. These results suggest a bioactive role of BLG in enhancing the function of the immune system (Biziulevicius et al., 2006).

Other previous studies have also demonstrated immunoenhancing effects of BLG (Wong et al., 1998). An experiment, performed *in vitro*, evaluated the effect on mouse spleen cells when incubated with purified proteins from bovine milk. Both variants, A and B, of BLG were each added separately as well as together, and their effects were compared to that of bovine serum albumin (BSA). Variants A and B together had the greatest effect, stimulating proliferation of the splenic lymphocytes, as well as promoting production of IgM by cultured spleen cells. The authors suggest that the results of this study indicate a potential role as an immune-modulator and milk proteins, especially BLG, could have beneficial effects on immune function (Wong et al., 1998). Another study reported stimulatory effects of BLG-derived peptides on the secretion of interleukin-10 (IL-10), an anti-inflammatory cytokine, and a suppressive effect on pro-inflammatory interferon-gamma (IFN-γ) (Prioult et al., 2004). These results suggest a regulatory role for BLG on the immune system. Although the above reports provide

valuable information, they were performed with mice, and few studies have looked at the role of BLG and other milk peptides in the neonatal calf.

Beta-lactoglobulin is categorized as a lipocalin, meaning a protein that transports hydrophobic molecules, and it has been shown to enhance transport of retinol and some fatty acids, across the gut (Said et al., 1989). Another study was conducted to look at its role in maturation of the gut in piglets, which also require passive transfer of immunity via colostrum (Sutton and Alston-Mills, 2006). The authors suggested that BLG may be an important factor involved in IgG absorption and gut maturation due to its high concentrations detected in colostrum. Neonatal piglets were fed (immediately after birth) either bovine colostrum supplemented with bovine-derived BLG, bovine colostrum without BLG or were allowed to suckle from the sow. The researchers analyzed the gut morphology, specifically looking at villus height, and the activity of brush–border enzymes in the small intestine, specifically alkaline phosphatase, lactase and maltase, which are proposed markers of gut maturation. The authors suggested that the enzyme activity may be indicative of gut development, but did not find sufficient evidence to show that BLG contributes significantly to gut maturation. The results may have been partially confounded by the fact that the two treatment groups receiving bovine colostrum were fed *ad libitum* whereas the piglets suckling from the sow would most likely have had restricted intake.

Colostrum also contains many antimicrobial components that help protect the calf from pathogens entering the GIT. Several of these have been well studied including lactoferrin, lactoperoxidase and lysozymes. These substances provide bacteriostatic and/or bactericidal protection and are secreted into colostrum at higher concentrations than in mature milk (Molenaar et al., 1996; Pakkanen and Aalto, 1997). Lactoferrin is found in many different bodily secretions and has the ability to inhibit some types of bacteria, such as *E. coli*., by binding iron required for their optimal growth (Nuijens et al., 1996). Lactoferrin may have many other important roles, including promoting normal neutrophil function (Lakritz et al., 2000), which may have important implications for the newborn animal. Lactoperoxidase and lysozyme both have potent antibacterial properties and are able to enzymatically lyse a variety of both gram positive and negative bacteria (Kussendrager and van Hooijdonk, 2000; Pellegrini et al., 1992).

Colostrum also contains significant levels of maternal leukocytes, compared to normal mature milk. Colostral leukocytes are able to pass from ingested colostrum into the newborn's lymphatic circulation within a few hours after birth, as shown in a study with newborn piglets, which had been administered radiolabelled immune cells into the stomach (Tuboly et al., 1988). Maternal, colostrum-derived immune cells have been found in blood of the neonate in other studies as well, including in piglets (Williams, 1993) and in neonatal lambs (Sheldrake and Husband, 1985). It is probable that these cells provide some protection against pathogens, however development and priming of the young animal's own immune system also appears to be a major function. Donovan et al. (2007) and Reber et al. (2008) fed newborn calves either whole colostrum or cell-free colostrum. The results from these studies showed that calves fed whole colostrum had enhanced development and response of the immune system compared to calves that received cell-free colostrum.

A plethora of hormones and growth factors are transferred in large quantities from bovine colostrum to the neonatal calf. A few interesting examples are insulin-like growth factor (IGF), growth hormone and prolactin, although there are many others that may have important effects in the calf. These components, which follow the general pattern of being concentrated in colostrum, are important stimulators of cell differentiation and growth (Pakkanen and Aalto, 1997). Bioactive components may modify the development and absorptive capacity of the GIT, and they may have long-term effects on growth, development, health and productivity of the animal (Blum and Hammon, 2000). With a colostral concentration of more than 150 times that of mature milk, it can be postulated that IGF has an essential bioactive function in the calf. Results from research studying the effects of IGF have been variable. Buhler et al. (1998) administered IGF-1 to calves and saw no effect on histological measurements gut development, An earlier study had suggested that dietary IGF-1 increases growth of the small intestine in calves within the first week of life (Baumrucker et al., 1994). Effects of IGF-1 on GIT growth have also been demonstrated in other species such as piglets (Drozdowski and Thomson, 2009; Xu et al., 1994). Growth hormone from colostrum interacts with IGF by causing an increase in circulating IGF-1 in the calf and may have an effect on development of the GIT (Hammon and Blum, 1997).

Prolactin is typically thought of as a hormone of lactation, but high levels in colostrum and the presence of prolactin receptors in the neonatal intestine suggest other biological functions (Ellis et al., 1996; Nagano et al., 1995). The function of prolactin on neonatal calves has not been extensively studied, although evidence from other species and older cattle demonstrates a role in immune function. A study by Grove et al. (1991) showed that prolactin is involved in lymphocyte proliferation. In this study, rat pups demonstrated altered lymphocyte populations and activity as a result of the administration of prolactin-deficient milk. This suggests that the high concentrations in colostrum have a biologically functional value to the neonate.

Some nutritive components of colostrum also have bioactive functions. Vitamin A and Vitamin E have both been the subject of research into their roles in the neonate. These fat-soluble vitamins are present in bovine colostrum at higher concentrations than in mature milk (Foley and Otterby, 1978). Vitamin A comes in many different forms including retinol, retinal, retinoic acid and retinyl esters. There are also several vitamin A precursors, the most important being β-carotene (Debier and Larondelle, 2005). In general, vitamin A is known for being essential for a broad range of functions in the body including vision, immune function, reproduction, growth and maintenance of epithelial surfaces but each of the forms and precursors have specific biological roles. For example, retinoic acid seems to be important for regulating the activity of protein kinase C, which regulates cellular processes (Blomhoff and Blomhoff, 2006), whereas retinol appears to be more involved in function of immune cells (Buck et al., 1990). Vitamin E has multiple functions as well, but is essential for normal immune system functions, especially with regards to its antioxidant activity (Debier and Larondelle, 2005).

In calves, research has been conducted looking at the specific roles and interactions between vitamin A and other components of colostrum such as vitamin E, lactoferrin and β-lactoglobulin. Early work indicated that high levels of vitamin A fed to calves might be beneficial, but that it may also have a negative interaction with vitamin E. A study by Eicher et al. (1994), in which dairy calves were fed milk replacers supplemented with various concentrations of vitamin A and E, found that there was a positive effect of vitamin A on immune function without negatively impacting vitamin E plasma concentrations. Other studies have shown contrary results, suggesting that high

levels of vitamin A decreased vitamin E bioavailability, resulting in reduced plasma concentrations (Nonnecke et al., 1999). In a more recent report by Schottstedt et al. (2005) results indicated that supplementation not only had a positive effect on small intestinal development, but also that interactions between vitamin A and lactoferrin supplemented in milk replacer, induced crypt cell proliferation in the ileum and colon, and may have influenced growth of Peyer's Patches in the ileum in calves. Said et al. (1989) showed a significant effect of BLG in bovine milk on retinol uptake in rat intestine. This effect has also been seen in other species including piglets and calves (Sutton and Alston-Mills, 2006).

Another component of colostrum, and one that may be overlooked due to its unconventional nature is the population of microbes. Although some species of bacteria are pathogenic, many beneficial microbes exist and contribute to the development of the gut and to the health of the young animal (Lara-Villoslada et al., 2007). There is substantial evidence that commensal bacterial establish themselves in the gut soon after birth (Ducluzeau, 1983; Mackie et al., 1999) and are likely to have positive influences on development of the gut, especially the mucosal immune system. The characteristics and functions of the gut microbiota have gained more interest recently, especially with regards to digestive health and disease treatment in humans. Colonization of the gut begins with exposure in the birth canal and environment during birth. A major source of commensal bacteria immediately after birth is through ingestion of colostrum or milk. Studies in humans have shown that breast milk contains a plethora of microbes that colonize the infant's gut, such as *Lactobacilli* (Wagner et al., 2008). Commensals play a crucial role in many physiological processes including normal development of the mucosal immune system and providing a physical barrier to pathogenic bacteria by out-competing them for nutrients and attachment sites. It has been shown that disruption of the bacterial population not only leads to abnormal development, but may also contribute or be responsible for certain diseases (Sekirov et al., 2010). Hypotheses implicating aberrant microbial populations with disease states in humans include those which lead to an increased risk of allergies and development of intestinal inflammatory diseases such as Crohn's (Salminen et al., 2006). Although there is no known link, Crohn's disease is thought to have similar characteristics as Johne's disease in cattle, caused by

Mycobacterim avium subspecies *paratuberculosis*. The exact pathology is unknown, but a disruption of commensal bacterial is thought to be involved in both diseases (Shanahan and O'Mahony, 2005).

Many factors, including bacteria, can influence goblet cell secretion of mucin, a gel-forming glycoprotein that may be released into the gut lumen in response to a number of bioactive factors, including compounds that are found in colostrum (Deplancke and Gaskins, 2001; Drozdowski and Thomson, 2009). Bacteria in the gut have been found to influence the number of goblet cells and the composition of mucins secreted, as seen in studies in germ-free rodents (Kandori et al., 1996). In addition to the effect of the microbes, goblet cell numbers and mucin composition are diet-dependent (Ganessunker et al., 1999; McCracken et al., 1995). Quantifying mucin production in the gut has proven to be difficult, but some studies have been successful in accurately measuring mucin secretion and goblet cells. In a study by Sakamoto et al. (2000), analysis of goblet cells using periodic acid-Schiff (PAS) and alcian blue staining determined that the two stains were comparable and could be used to quantify the mucus gel. These researchers also used lectin staining with fluorescein isothiocyanate-labeled *Ulex europaeus* agglutinin I followed by image analysis to quantify mucin and other morphological parameters in the intestine. The number of goblet cells and the composition of secreted mucin may be indicators of development and adaptation to enteral nutrition in the gut of the neonatal animal (Ganessunker et al., 1999).

Another role of commensal microbes is to aid in establishing immune tolerance in the gut, a mechanism that has developed to prevent the immune system from reacting to commensals and instead directing it towards pathogenic organisms (Kelly et al., 2005). The effects and benefits of microbes in colostrum and milk may be largely underestimated and more research is required to understand their roles in metabolic and immunomodulatory mechanisms, especially outside of human health research.

Other bioactives, such as cytokines, oligosaccharides, other small peptides, specific fatty acids and nucleotides in colostrum may play important roles in the neonate (Pakkanen and Aalto, 1997), and the precise effects or mechanisms of many of these components are not fully understood. Hundreds of compounds have been recognized as being influential in the physiological mechanisms of the neonate, especially in rodents

and humans. However, more research is needed to understand the functional mechanisms of such compounds in the neonatal calf. As the gut is one of the first places of exposure to bioactive compounds of colostrum, it is important to recognize that neonatal gut development is easily influenced by enteric factors.

1.5 Gut development of the neonatal calf

The major site of absorption of colostrum in the calf is the small intestine, which is comprised of three anatomically and physiologically distinct sections (Ross et al., 2009). The first portion is the duodenum, which is the main site of chemical digestion of nutrients in the mature gut. The duodenum ends at the ligament of Treitz. The second section, the jejunum, and the most distal section, the ileum, are both involved in absorption of nutrients and play a critical role in mucosal immune function. The layers of the gut, shown in Figure 1.3 include the outer layer of serosa, two layers of muscularis externa (the outer longitudinal and the inner circular), the submucosa, a layer of mucosal muscle, and the mucosa. Large, fold-like structures called plicae circulares (Figure 1.3A) aid in increasing the surface area of the small intestine. The mucosal surface of the gut is lined with finger-like projections called villi, which are composed of simple columnar epithelial cells (Figure 1.3B and D). The lamina propria, a layer of thin connective tissue, lies just under the epithelial layer, which lines the villi (Figure 1.3B). Between the villi, invaginations, known as the crypts of Lieberkuhn (Figure 1.3D), are the source of intestinal stem cells. Villi of the duodenum are short and blunt, whereas the villi of the jejunum are long, slender and are more finger-like in appearance. The ileum contains villi that have a tongue-like shape (Ross et al., 2009). In the calf these differences in villi shape are more subtle (Pearson et al., 1978). Although the sections of the gut are distinct, changes in morphology and function are gradual throughout the small intestine (Ross et al., 2009). The small intestine, especially the distal portions, is characterized by gut-associated lymphoid tissue (GALT), which plays a key role in detecting antigens and neutralizing pathogens (Miura et al., 2011). The GALT includes important structures of

the intestinal immune system such as Peyer's patches (Figure 1.3C) and mesenteric lymph nodes.

The epithelial cells of intestinal villi are covered by microvilli that create a "brush border" on the enterocytes, helping to increase surface area and thus absorptive capacity of the gut. In the neonate, the small intestine grows extremely quickly and allometrically to the rest of the body. This effect is more pronounced in piglets than calves (Zabielski et al., 2008). The neonatal piglet's small intestine increases in weight by 70% in the first day. The gut grows at this precipitous rate in early life due to rapid proliferation of the mucosal cell populations, elongation of the villi, lengthening of the GIT and the accumulation of colostral proteins (Xu, 1996).

Proliferation of the epithelial cells occurs by the activation of intestinal stem cells, located in the crypts, to produce progenitor cells. There are four different epithelial lineages that form from the immature stem cells, with the predominant type being enterocytes that make up approximately 90% of the mucosal epithelial cells. Smaller percentages of mucin-producing goblet cells, enteroendocrine cells and Paneth cells also arise. The latter two cells types secrete hormones and antimicrobial peptides, respectively. As these cells differentiate, they migrate towards the apical surface of the villi, with the exception of Paneth cells, which remain in the bottom of the crypts (Rao and Wang, 2011; Stockinger et al., 2011). Mucin-producing goblet cells (Figure 1.3D) are important for providing a physical and chemical barrier in the gut that protects not only against abrasion, but also against pathogenic bacteria. The proliferation rate of goblet cells and the composition of the mucins they produce may be significantly altered by diet and the presence of commensal bacteria (Deplancke and Gaskins, 2001).

Three major signaling pathways control regulation of intestinal cell differentiation and self-renewal: the Wnt signaling pathway, the bone morphogenic protein (BMP) pathways and the Notch pathway. Maintenance of progenitor cell differentiation and migration of new cells is mediated by the Wnt signaling pathway. This pathway is essential for maintaining normal intestinal stem cell differentiation (Pinto et al., 2003), targeting many regulatory genes, such as those responsible for normal positioning of Paneth cells in the bottom of the crypts. Paneth cells secrete antimicrobial peptides such as defensins and are an important aspect of mucosal immunity in the intestine. Target

genes that regulate Paneth cell development include SOX9, EPHB2 and EPHB3 (Bevins and Salzman, 2011). The Wnt pathway also interacts with members of the BMP pathway, key regulatory proteins that are members of the TGF-β super family. BMP signaling has been shown to inhibit proliferation of epithelial cells by suppressing Wnt-β-catenin-signaling. This function was elucidated by a study that used mice with a conditional knock-out of BMP receptor 1A, which is required for ligand binding of BMP and mediation of cell fate (He et al., 2004). Results from this study revealed an occurrence of hyperproliferation, an increased number of crypts and the development of intestinal polyps in the knock-out mice compared to their wild type counterparts. Lastly, the Notch pathway appears to play an essential role in determining the fate of progenitor cells and maintaining a balance between cell lineages (Richmond and Breault, 2010; Stanger et al., 2005). The pathways mentioned above are key regulators of gut development, but many other signaling pathways are also involved. Examples of other important regulatory pathways of intestinal development include Ephrin, JAK/STAT1, PTEN, AKT, and PI3K pathways (Rao and Wang, 2011). The combined roles of all pathways contribute to normal development of a functional gut.

Recently it has been hypothesized that microRNAs (miRNAs) contribute significantly to regulation normal development and maintenance of the intestinal epithelium. These small RNA molecules are involved in regulating gene expression through gene silencing. Synthesis occurs by cleavage of a precursor miRNA by an enzyme called Dicer, thereby producing a functional miRNA. McKenna et al. (2010) showed that in mice that were deficient in the intestinal epithelium-specific Dicer1 enzyme, normal functions, such as goblet cell production and maintenance of the intestinal barrier, were inhibited. Results provide strong evidence that miRNAs are essential for regulating development and maintenance of intestinal cells. Research has also suggested that miRNAs may be important for mediating communication between the intestinal and immune systems (Biton et al., 2011). Although found throughout the body, the miRNA transcriptome is not completely known. A recent study detected many miRNAs secreted in milk and these are likely involved in immune-modulation and mediating gut development through gene silencing (Kosaka et al., 2010; Liang et al., 2014).

Besides miRNAs, other bioactive components of colostrum may have an effect on mediating and stimulating factors within the gut responsible for growth and development. Growth factors, such as EGF, TGF-α and TGF-β, may play a role in intestinal growth of the neonate. For example, TGF-β derived from colostrum is thought to influence gut integrity in the suckling rat (Playford et al., 2000). Some lipids found in colostrum may also contribute to gut development. One example is sphingomyelin (SPM), a phospholipid that has been found in, and implicated as a bioactive of, bovine milk (Graves et al., 2007). Motouri et al. (2003) fed SPM to artificially reared, pre-weaned rat pups to study its effects on intestinal development. Rats receiving SPM had significantly lower lactase activity in their small intestines compared to the control animals. Rats fed SPM were also found to have a lower number of vacuolated enterocytes. These results strongly suggest a bioactive role for colostrum or milk-derived SPM in mediating gut mucosal maturation.

One factor that is of particular interest is glucagon-like peptide-2 (GLP-2), a pleiotropic peptide hormone that belongs to the glucagon superfamily. Production of GLP-2 occurs by cleavage of the proglucagon molecule by a post-translational process called prohormone convertase-1/3-mediated cleavage. The processed GLP-2 is secreted as a 33-amino acid peptide by enteroendocrine L-cells of the intestine, but is also secreted in the pancreas and some regions of the brain. Research has shown that secretion of GLP-2 can be stimulated by certain components of milk, especially milk proteins. Izumi et al. (2009) fed suckling rats either the lactose fraction, the cream fraction or the protein fraction from bovine milk. Serum GLP-2 concentration was measured by an enzyme immunoassay (EIA). Compared to rats receiving the lactose and cream fractions, the milk protein fraction resulted in a marked increase in GLP-2 secretion. In the same study, a different set of rats that were fed isolated whey proteins, obtained following milk processing, had significantly higher circulating GLP-2, whereas soy protein and ovalbumin did not have this effect.

GLP-2 has a wide range of functions but is best known for its stimulatory effects on small intestinal growth, mucosal growth and increasing nutrient absorptive capacity of the intestine (Burrin et al., 2003; Dubé and Brubaker, 2007). In neonatal piglets, administration of GLP-2 in parenteral-fed animals had a stimulatory effect on growth of

the small intestine and resulted in increased absorptive capacity (Sangild et al., 2006). Some evidence has suggested similar effects in ruminating calves (Taylor-Edwards et al., 2011), however studies with neonatal calves are limited. In other studies, GLP-2 appeared to play an important role in glucose and hexose transport in the intestines. The peptide also promoted proliferation and repair of mucosal epithelial cells (Drucker, 2002). The mechanism by which GLP-2 functions is still not fully understood but evidence has suggested that it is at least partially responsible for stimulating secretion of intestinal IGF-I. A recent study demonstrated that GLP-2 promoted proliferation of epithelial cells by activating β-catenin signaling in intestinal crypts of mice. This process requires IGF-I as a mediator (Dube et al., 2008). The mechanisms involved with GLP-2 are of great interest as the peptide is thought to have health promoting and therapeutic effects, especially with regards to gastrointestinal disorders. However, more research is required to fully understand its multitude of functions and its potential interactions.

It has long been demonstrated that during the first few hours of life, macromolecules are mostly absorbed passively via non-selective pinocytosis. However evidence for receptor-meditated transport of some molecules also exists (Bush and Staley, 1980; Danielsen et al., 2011; Moog, 1979). Early studies (Kraehenbuhl and Campiche, 1969; Ockleford and Whyte, 1980) suggested the existence of a tubular vesicle mechanism in intestinal enterocytes in the neonate to facilitate transport of colostral macromolecules into circulation. The mechanism involves the transport of molecules through apical tubules, or invaginations, at the base of the microvilli, that "bead" and form small vesicles. These vesicles congregate into larger vacuoles of colostral contents, which subsequently are dispensed at the basal cell surface so that they can be absorbed into circulation (Kraehenbuhl and Campiche, 1969; Ockleford and Whyte, 1980).

The early work that investigated the ultrastructure of neonatal enterocytes looked mostly at rodents or rabbits. Some studies (Jochims et al., 1994; Kaup et al., 1996; Staley et al 1971) have determined that vacuoles are also present in both piglets and calves in the periods corresponding to colostral uptake. A study by Staley et al. (1971) demonstrated the presence of this vesicular-vacuolar system in calves by using electron micrographs to image sections taken through intestinal cells. Results showed that,

whereas the major site of absorption in rats appears to be in proximal-mid portions of the small intestine (Rodewald, 1973), in calves the ileal cells were most active in macromolecule uptake. The researchers also showed that vacuoles were smaller in jejunal enterocytes compared to large supranuclear vacuoles found in ileal cells.

Another study by Kaup et al. (1996) found similar characteristics in the intestinal ultrastructure of neonatal calves. This study investigated small intestinal morphology in calves prior to, and after, receiving colostrum and found that the vacuoles in ileal cells were much larger than in the jejunum. Results also demonstrated more pre-colostral vacuoles in enterocytes of the distal small intestine. More vacuoles also appeared on the tips of the villi compared to the bases, which indicates a progression in maturation of the enterocytes. After colostrum administration, absorptive vacuoles containing colostrum contents were clearly visible using light and electron microscopy to visualize intestinal villi. The authors of this study suggested that colostral absorption occurs mainly in the caudal portion of the small intestine, although they were able to see absorptive vacuoles in all sections. In piglets, researchers suggested that the disappearance of vacuolated enterocytes serve as an indicator of the maturation of intestinal cells (Skrzypek et al., 2007).

A unique aspect of gut development during the immediate post-natal period is the ability to absorb macromolecules, followed shortly by a cessation of permeability of the gut, a process that was originally termed “gut closure” by Lecce and Morgan (1962). Early researchers (Broughton and Lecce, 1970; Smeaton and Simpson-Morgan, 1985) suggested hypotheses to explain the mechanism of gut closure. One idea maintained that compounds could be absorbed via pinocytosis during a short window of time (species-dependent) post-partum. The suggested mechanism involved the formation of “pinocytotic channels”, but that this activity ceased as a result of a limited capacity by the enterocytes to form these channels (Broughton and Lecce, 1970). This study merely speculated on the proposed mechanism, however. Another hypothesis investigated by Smeaton and Simpson-Morgan (1985) suggested that the absorptive capacity of enterocytes reduced over time due to maturation of the cells. Results of this study indicated that mature epithelium gradually replace fetal enterocytes in the intestines of neonatal lambs, which reduces the absorptive capacity for macromolecules. Both of the

ideas described above have been the subject of debate and the exact mechanism/s are not entirely understood.

Although results have been largely inconclusive, evidence supports the idea that multiple mechanisms exist. One study performed on human infants suggested that different routes of passage might be explained by differences in the molecular weights of compounds. The authors suggested that some molecules may be taken up via pinocytosis, while others may traverse the gut by way of paracellular pathways (Weaver et al., 1984). Tight junctions in the gut, made of a protein complex, form between adjacent cells and create a barrier that prevents molecules from passing between the intestinal epithelial cells (Assimakopoulos et al., 2011). Tightening of "leaky" tight junctions has been proposed as a mechanism of gut closure, whereby the intestinal cells and junctions gradually mature (Louis and Lin, 2009). Some studies, primarily performed in humans or model species, have shown that tight junctions are regulated by a number of factors including nutrients and bioactives of milk, such as cytokines and immune cells (Nusrat et al., 2000). Few, if any, results have been conclusive in determining the role of tight junctions and gut permeability of neonatal calves or other animals requiring passive transfer via colostrum.

Timing of closure in the calf has been under much scrutiny. Efforts have been made to determine the optimal time to feed colostrum to maximize absorption before closure occurs. Most studies in calves have focused on timing of immunoglobulin absorption, specifically IgG. Some early studies suggested that different classes of maternal antibodies were absorbed with different efficiencies (Penhale et al., 1973) but evidence for this has not been consistent. In general, the ability to absorb macromolecules by the gut is lost spontaneously as seen in calves that were deprived of colostrum. Closure in these calves occurred gradually after 12 hours after birth and they were no longer able to absorb colostrum at 24-36 hours (Stott et al., 1979). This effect has also been seen in other species.

In many other species, it has been shown that intake of nutrients or colostrum has promoted closure of the gut. A study in piglets by Lecce et al. (1964) demonstrated that neonatal piglets that were deprived of colostrum after birth had delayed gut closure, compared to piglets fed colostrum. This "diet-induced closure" has also been

demonstrated in calves. As described above in Stott et al. (1979), delay of colostrum feeding prolonged the period in which the gut remained permeable until spontaneous closure occurred. Feeding colostrum earlier than 12 hours stimulated closure 2-3 hours earlier than in its absence. However, once colostrum-deprived calves were fed, closure occurred at a more rapid rate than those fed earlier than 12 hours. Delaying colostrum consumption reduced the amount of time the calf ultimately had to absorb colostrum by 13 hours. Although the gut does appear to remain permeable in delayed-feeding animals, it was evident that these calves had a higher risk of mortality.

Another factor that has been proposed to affect timing or capacity for colostrum absorption is stress. Calves and many neonate species with higher circulating corticosteroids had a reduced ability to absorb macromolecules from colostrum. This effect was seen in calves from cows that had been given corticosteroids during gestation, suggesting that stress of the dam may play a role in colostrum uptake in the newborn (Husband et al., 1973; Patt, 1977). Results regarding the effects of stress of the dam on absorption of colostrum in calves have not been consistent, however. Some studies found no significant effect of stress on IgG uptake, whereas others provided evidence that environmental conditions of the dam may have influenced absorption in the calf (Johnston and Oxender, 1979; Stott, 1980). In a study by Nardone et al. (1997), cows that were exposed to high air temperatures not only had lower colostrum concentrations of IgG, but also had a negative effect on colostral IgA, total protein, lactose and fatty acids. Interestingly, heat stress did not have a significant effect on IgM or lactoglobulin concentrations. These results suggest that stress on the dam results in lower quality colostrum, which may have negative consequences in the calf. Whether stress on the dam leads to early gut closure remains to be determined.

Following the rapid development of the small intestine, the neonatal calf must transition from essentially being a monogastric to becoming a fully functional ruminant by the time it is weaned. Proper rumen development depends on volatile fatty acid production during the pre-weaned period. Initially, calves solely consume a liquid milk diet, but will soon begin eating small quantities of solid feed. Research has shown that milk, combined with a coarsely texturized starter grain results in butyrate and propionate, volatile fatty acids that favor papillae development and elongation. These papillae

increase surface area in the rumen and are critical to a healthy, functional rumen. Physical stimulation in transition calves also contributes to an increase in rumen growth and muscle development (Drackley, 2008; Lesmeister and Heinrichs, 2005). Little is known about how bioactivity of colostrum may influence rumen development.

1.6 Heat-treatment of colostrum

Colostrum's role is to deliver a package of nutrients, immunoglobulins, growth factors and many other bioactive compounds. Due to improper colostrum collection and handing techniques, contaminated feeding equipment or transmission of pathogens directly from the mammary gland, colostrum is potentially the initial source of infection for calves (Stewart et al., 2005). Common pathogens that pose a risk to the newborn calf include *Escherichia coli*, *Salmonella spp*, *Mycoplasma spp* and *Mycobacterium avium ssp. Paratuberculosis* (the cause of Johne's). Bacteria from colostrum and milk can cause scours and septicemia, reduced growth in the pre-weaned period and can have long-term effects on health and productivity. Some evidence also suggests that bacteria may influence absorption of immunoglobulins. A study by James et al. (1981) reported that live bacteria reduced the uptake of immunoglobulins in experiments performed using excised gut segments. In a more recent study (Elizondo-Salazar and Heinrichs, 2009a), calves that were fed high levels of bacteria in colostrum did not differ in the absorption of IgG compared with calves fed low levels, suggesting that bacteria do not influence uptake. More research is needed to clarify the effects of bacteria on macromolecule absorption by the gut, and to understand the associated mechanisms.

In order to reduce the bacterial load ingested by the calf, on-farm pasteurization of milk and colostrum prior to feeding has been adopted and is now a common management practice. Pasteurization is the process of heating to a temperature high enough to adequately reduce the bacterial load to a level that poses little risk of infection. Waste milk (milk that is unfit for human consumption, used for feeding calves) is often pasteurized at 63°C or higher (Godden et al., 2003). Although pasteurization at this temperature successfully reduced the pathogen load, early research found that these

temperatures also resulted in a reduction in IgG in colostrum. A 12% decrease was found by Meylan et al. (1996) when colostrum was held at 63°C for 30 minutes. Godden et al. (2003) found that there was on average a 26.2% loss in IgG using the same temperature and time. This study also reported that larger volumes of colostrum and higher quality colostrum had greater reductions in IgG. As well as these effects on IgG, there was an increase in viscosity. Two subsequent research studies discovered that using a lower temperature could alleviate the problems with pasteurization of colostrum. Colostrum heated to 60°C for as long as 120 minutes resulted in negligible reductions in IgG concentration. However, colostrum that had a concentration of > 75 mg/ml IgG was shown to have a higher loss than lower quality colostrum, decreasing by almost 10%. The authors suggested that this loss is biologically insignificant, as a 10% reduction would still be a sufficient concentration for successful passive transfer (Godden et al., 2006; McMartin et al., 2006).

The effects of heat-treatment of colostrum on the calf are of great interest. Research has looked at whether pasteurization influences absorption of immunoglobulins, growth, nutritional status and health of the young calf. In a study by Johnson et al. (2007) calves were fed 3.8 liters of either raw colostrum or colostrum after pasteurization at 60°C for 60 minutes. Blood samples were collected from calves prior to feeding of colostrum and at 24 hours of life, for analysis of serum total protein and, specifically, for IgG, IgA and IgM. Other immune and nutritional parameters, such as vitamins and leukocytes, were also measured. IgG concentration of colostrum was not significantly affected by heat-treatment. Calves that were fed heat-treated versus raw colostrum had significantly higher (22 versus 18 mg/mL) serum IgG concentration at 24 hours of age. These results suggest that although IgG concentration in the colostrum was not affected by heat-treatment, the bioactivity of the colostrum was altered in such a way that enhanced uptake of IgG by the gut. Serum total protein was also higher in calves receiving heat-treated colostrum (63 mg/mL) compared to those that were fed raw colostrum (59 mg/mL). Concentrations of IgA and IgM did not differ by that time. Apparent efficiency of absorption (AEA), which is an equation used to estimate the amount of IgG actually consumed by the calf, was also higher in calves that received heat-treated colostrum.

A similar study (Elizondo-Salazar and Heinrichs, 2009b) reported results that were consistent with this earlier study. In their study, researchers fed colostrum that was either unheated or pasteurized at 60°C for 30 minutes. Blood was collected prior to colostrum feeding to establish an initial concentration. Blood was also collected at 4, 8, 12, 16, 20, 24 and 48 hours of life, and then once per week until weaning. Calves that received pasteurized colostrum had higher serum IgG concentration up to the fifth week of life and higher AEA of IgG through the first 48 hours. This study also investigated the effects on body weight, feed intake, growth, health and lymphocyte counts during the entire pre-weaned period but found no significant differences between treatment groups.

Both studies cited above used colostrum with a minimum concentration of 50 g/L IgG. Colostrum was pooled and mixed thoroughly before processing, and no differences were found in colostral IgG concentration. Results from both studies demonstrated higher serum IgG concentrations in calves that had been fed heat-treated colostrum. The reason for these differences is unknown but the authors suggested several explanations. It may be that with unheated colostrum, IgG may bind bacteria either before or during ingestion, and before absorption occurs in the gut following ingestion, rendering the IgG unavailable for absorption. High bacteria levels in the unheated colostrum may also bind to receptors, interfering with uptake of IgG by the intestinal epithelial cells (James et al., 1981). However, Elizondo-Salazar and Heinrichs (2009a) fed calves colostrum containing low or high bacterial loads and found no difference in IgG absorption. Another explanation is that pasteurization denatures some proteins, which subsequently affects specific aspects of the gut physiology, such as brush-border enzymes including alkaline phosphatase lactase and maltase, which are thought to promote gut closure and therefore increase the time or ability of the gut to absorb immunoglobulins. These results may have important implications for calf health and growth. A recent study by (Godden et al., 2012) using > 500 calves per treatment, from six different dairy farms, showed that calves receiving pasteurized colostrum were at significantly lower risk for illness compared to calves that were fed unheated colostrum.

Besides the effects on absorption of IgG, other bioactives may also be affected by heat-treatment and influence absorption in the calf. In the study by Johnson et al. (2007) serum concentrations of several other components including IgA, IgM, vitamin A,

vitamin E, cholesterol and β-carotene were analyzed. However, no significant treatment effect was observed in these components between calves receiving heated versus unheated colostrum. Another study found that pasteurization of colostrum resulted in reduced serum lactoferrin levels and had a negative effect on neutrophil function (Lakritz et al., 2000). Although these effects were significant, the colostrum used in this study was pasteurized at 76°C for 15 minutes, a temperature well above what is recommended for colostrum. Therefore, the effects on lactoferrin and neutrophil activity require validation at lower temperatures. Pasteurization of colostrum and milk at temperatures higher than 60°C has been shown to affect other components of bovine milk and colostrum especially proteins such as β-lactoglobulin (Guimont et al., 1997) but the effects have yet to be determined at temperatures suitable for preventing denaturation of IgG. Research on humans has shown significant effects on many bioactive components of milk and colostrum (Ewaschuk et al., 2011). The major findings from a number of studies focusing on the effects of heat-treatment on bioactive compounds in colostrum or milk from various species are presented in Table 1.2. Many bioactives are affected by heat-treated, although the degree to which they are affected often depends on the temperature used for pasteurization (Trujillo et al., 2007; Henderson et al., 1998). Other colostral components that can be affected by pasteurization include amylase (Henderson et al., 1998), IGF-1 (Goelz et al., 2009), free fatty acids (Lepri et al., 1997) and lysozyme (Czank et al., 2009), to name a few examples. The influence of heat-treatment on bioactives and how they affect the neonatal calf requires more investigation.

1.7 Summary

An indicator of good management of colostrum and its application to newborn calves is the concentration of IgG in the calf's blood after ingestion of colostum. Therefore research objectives often focus heavily on passive transfer of immunity, with the knowledge that IgG is critical for reducing early mortality and morbidity rates. However, other bioactive factors in colostrum should not be overlooked as they may have profound impacts on growth, development, health, productivity and longevity of the animal. Some research has recognized the importance of some of these bioactive

compounds. However, there are perhaps hundreds of molecules for which we have very little understanding, especially in the neonatal calf.

As part of an improved strategy for managing disease transmission, on-farm pasteurization of colostrum is becoming more popular. Researchers have investigated the effects of heat-treatment on colostrum and milk and found that not only are there compounds that are affected by the pasteurization but also those changes are reflected in modulation of the physiology of the animal. In the calf, most research has targeted immunoglobulins, but a gap exists with regards to other bioactive compounds and the mechanisms by which they may influence the newborn calf.

1.8 Objectives

The overall objective of this study was to investigate the effects of heat-treatment on the bioactivity of colostrum. Specifically, there were two main objectives to the experiment. 1) To investigate the effects of heat-treatment of colostrum on gut absorption of colostral protein (specifically IgG and BLG), gut development in bull calves as measured by villus height, crypt depth and goblet cell numbers in the first 12 hours of life, and 2) To examine the effects of heat-treated colostrum on gut absorption, growth and health in pre-weaned heifer calves.

Table 1.1: Composition of colostrum and mature milk.

Component	Colostrum	Milk
Total Solids (%)	23.9	12.9
Lactose (%)	2.7	5
Fat (%)	6.7	4
Total Protein (%)	14	3.1
Casein (%)	4.8	2.5
Albumin (%)	6	0.5
B-lactoglobulin (g/L)	14.3	4.2
Immunoglobulins (%)	6	0.09
IgG_1 (g/L)	69.5	0.35
IgG_2 (g/L)	1.85	0.055
IgA (g/L)	4.9	0.045
IgM (g/L)	4.7	0.05
IGF-I (µg/L)	310	< 2
Insulin (µg/L)	65	< 2
Glucagon (µg/L)	0.16	0.01
Growth Hormone (µg/L)	1.4	< 2
Prolactin (µg/L)	280	15
Total Ash (%)	1.11	0.74
Vitamin A (µg/100 mL)	295	34
Vitamin E (µg/g fat)	84	15
Lactoferrin (g/L)	1.84	N.D.
TNF-α (µg/L)	5	< 2

Data from Blum and Hammon, 2000; Foley and Otterby, 1978

Table 1.2: Effects of pasteurization on bioactives in colostrum and milk

Component	Species	Heating Conditions[1]	Effect[2]	Reference
Amylase	Human	62.5°C for 30m	15% Reduction	Henderson et al., 1998
B-lactoglobulin	Bovine	Approx. 60°C for 60m	~15% Reduction	Chen et al., 2005
B-lactoglobulin	Bovine	63°C for 30m	Aggregation	Roth-Walter et al., 2008
Copper	Human	62.5°C for 30m	4.48% Reduction	da Costa et al., 2003
Free fatty acids	Human	62.5°C for 30m	80% Increase	Lepri et al., 1997
IgA	Human	70°C for 30m	33.3% reduction	Evans et al., 1978
IgA (Secretory)	Human	62.5°C for 30m	27.1-31.5% Reduction	Czank et al., 2009
IGF-1	Human	63°C for 30m	39.4% reduction	Goelz et al., 2009
IgG	Caprine	56°C for 60m and 63°C for 30m	14-15% reduction	Trujillo et al., 2007
IgG	Bovine	60°C for 30m	2.63% Reduction	Elizondo-Salazar and Heinrichs, 2009
IgG	Bovine	63°C for 60m	24.2% reduction	Teixeira et al., 2013
IgG	Human	65°C for 30m	77.2% reduction	Evans et al., 1978
IgM	Human	62.5°C for 30m	Abolished	Koenig et al., 2005
Iron	Human	62.5°C for 30m	12.75% Reduction	da Costa et al., 2003
Lactoferrin	Bovine	63°C for 60m	Reduced	Teixeira et al., 2013
Lactoferrin	Human	65°C for 30m	85% reduction	Evans et al., 1978
Lactoferrin	Human	62.5°C for 30m	75.2-81.7% Reduction	Czank et al., 2009
Linolenate	Human	62.5°C for 30m	22% Reduction	Wardell et at., 1981
Lysozyme	Human	62.5°C for 30m	47.5-67.2% Reduction	Czank et al., 2009
sCD14	Human	62.5°C for 30m	88% Reduction	Cossey et al., 2009
Total Protein	Bovine	60°C for 30m	9.8% Increase	Elizondo-Salazar and Heinrichs, 2009
Vitamin E	Bovine	60°C for 30m	42.3% Reduction	Elizondo-Salazar and Heinrichs, 2009
Zinc	Human	62.5°C for 30m	2.88% Reduction	da Costa et al., 2003

Table adapted from Ewaschuk et al., 2011
[1]Temperature and duration at which milk or colostrum was held
[2]Effect on component in colostrum or milk

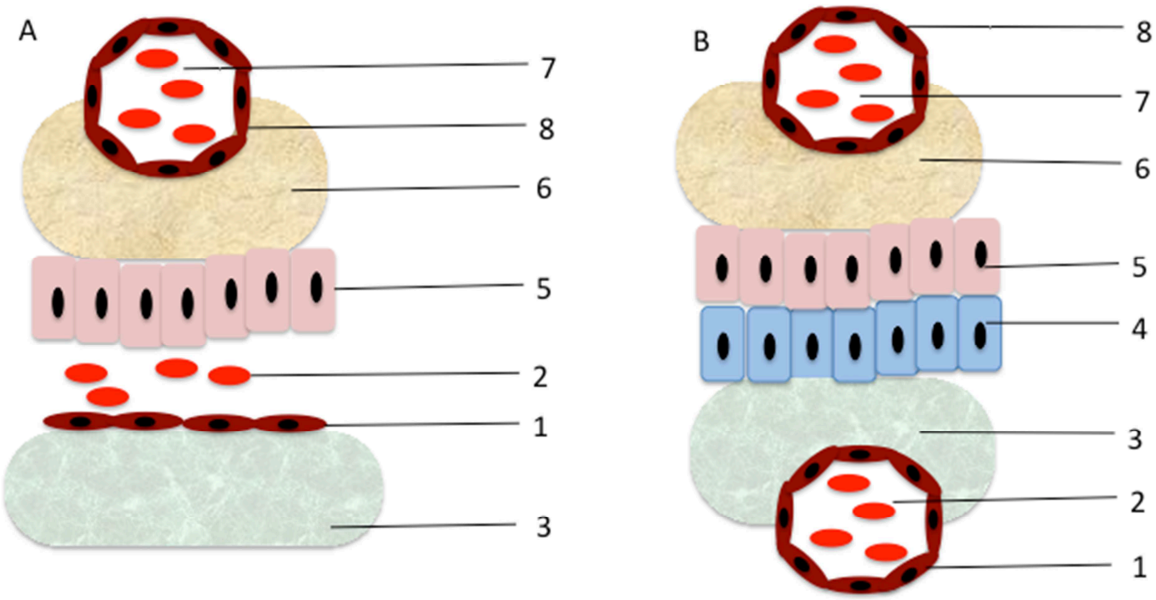

Figure 1.1: Layers separating maternal and fetal blood supplies in the human, an example of hemochorial placentation (A) and cow, an example of syndesmochorial placentation (B). 1) Maternal endothelium 2) Maternal blood 3) Maternal connective tissue 4) Maternal endometrial epithelium 5) Fetal chorionic epithelium 6) Fetal connective tissue 7) Fetal blood 8) Fetal endothelium. Image adapted from Gilbert (2012).

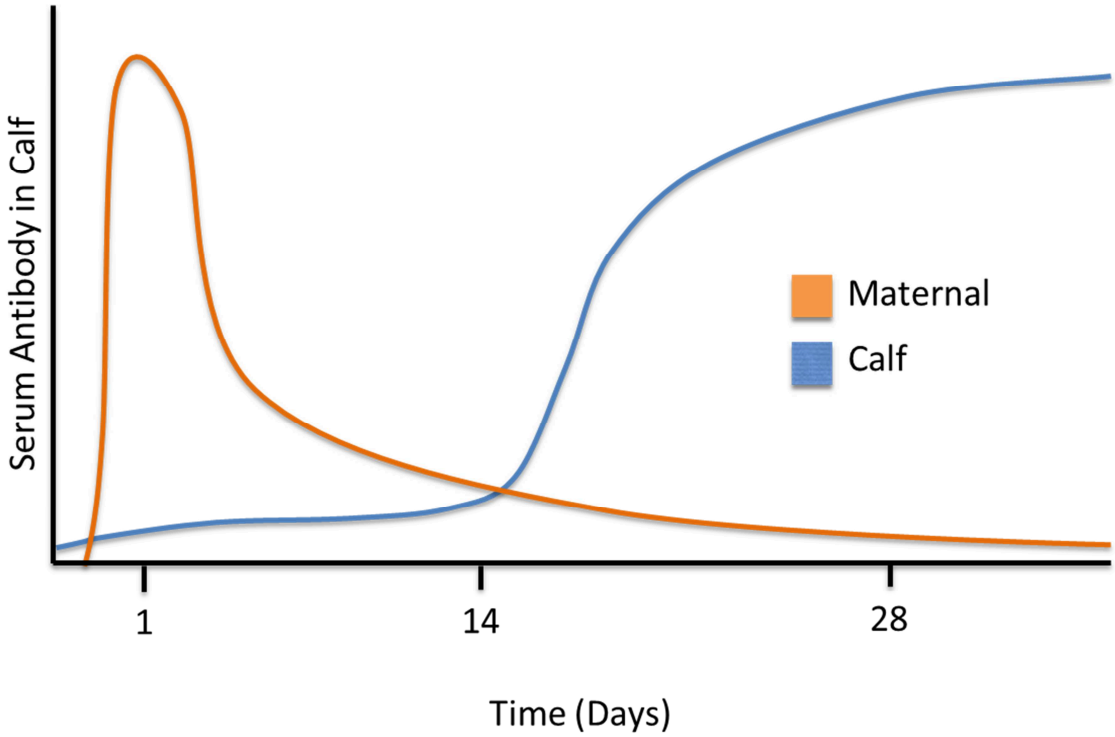

Figure 1.2: Serum antibody levels in the calf during the first 4 weeks of life (Chase et al., 2008; Robison et al., 1988). Orange indicates colostral antibodies absorbed by the calf. Blue indicates endogenous antibody production.

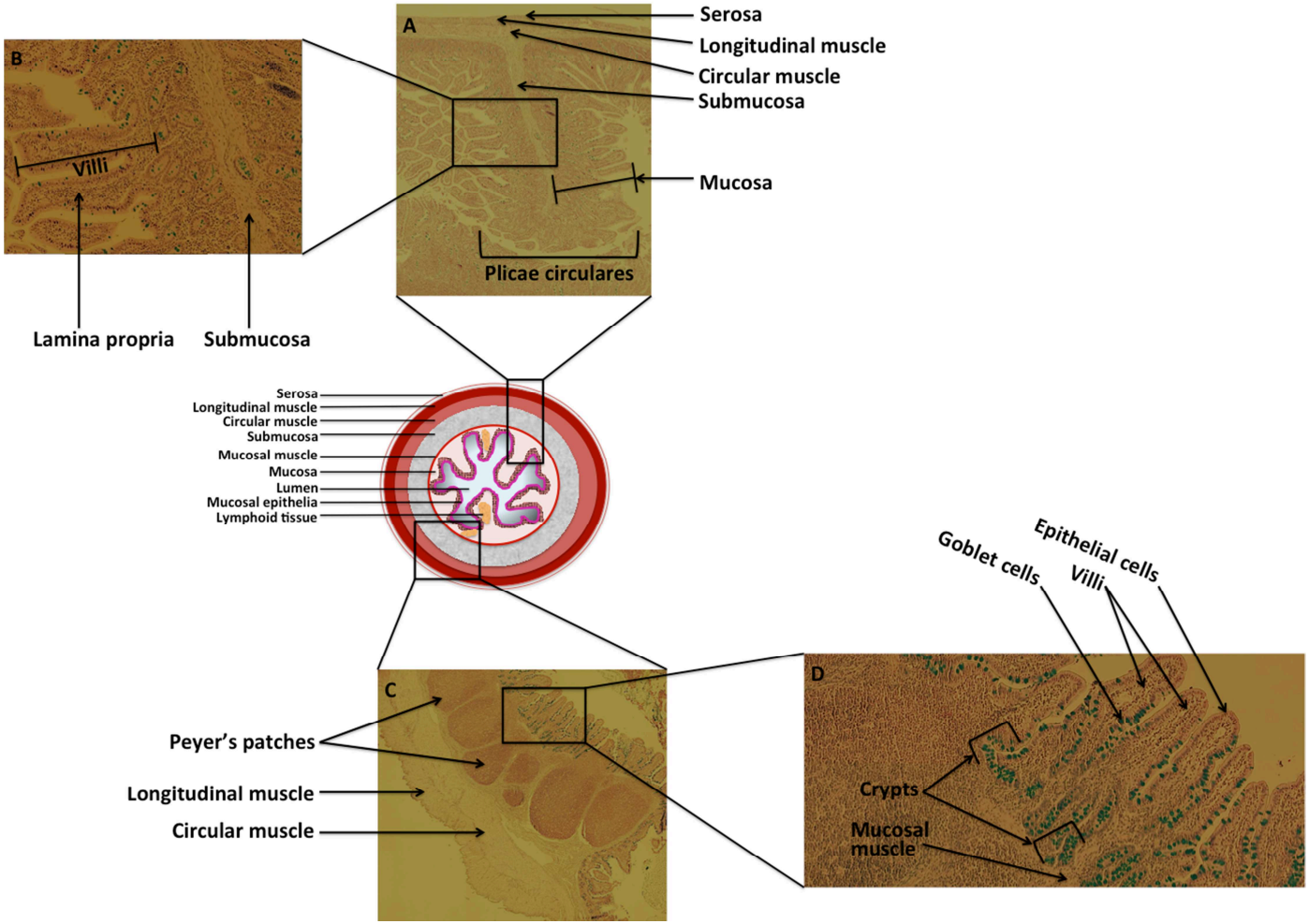

Figure 1.3: Major tissue layers and functional structures of the small intestine. Slides stained with Alcian Blue and Nuclear Fast Red.

1.9 References:

Assimakopoulos, S. F., I. Papageorgiou, and A. Charonis. 2011. Enterocytes' tight junctions: From molecules to diseases. World journal of gastrointestinal pathophysiology 2: 123-137.

Barrington, G. M., T. B. McFadden, M. T. Huyler, and T. E. Besser. 2001. Regulation of colostrogenesis in cattle. Livestock Production Science 70: 95-104.

Bartol F.F., A.A. Wiley, D. J.Miller, A.J. Silva, K.E. Roberts, M.L. Davolt, J.C. Chen, A.L. Frankshun, M.E. Camp, K.M. Rahman, J.L. Vallet, C.A. Bagnell. 2013. Lactation Biology Symposium: lactocrine signaling and developmental programming. J Anim Sci. 2013 Feb;91:696-705

Baumrucker, C. R., D. L. Hadsell, and J. W. Blum. 1994. Effects of dietary insulin-like growth factor I on growth and insulin-like growth factor receptors in neonatal calf intestine. Journal of animal science 72: 428-433.

Besser, T. E., A. E. Garmedia, T. C. McGuire, and C. C. Gay. 1985. Effect of colostral immunoglobulin G1 and immunoglobulin M concentrations on immunoglobulin absorption in calves. Journal of dairy science 68: 2033-2037.

Besser, T. E., C. C. Gay, and L. Pritchett. 1991. Comparison of three methods of feeding colostrum to dairy calves. Journal of the American Veterinary Medical Association 198: 419-422.

Bevins, C. L., and N. H. Salzman. 2011. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. Nature reviews. Microbiology 9: 356-368.

Biton, M. A. Levin, M. Slyper, I. Alkalay, E. Horwitz, H. Mor, S. Kredo-Russo, T. Avnit-Sagi, G. Cojocaru, F. Zreik, Z. Bentwich, M.N. Poy, D. Artis, M.D. Walker , E. Hornstein, E. Pikarsky, Y. Ben-Neriah. 2011. Epithelial microRNAs regulate gut mucosal immunity via epithelium-T cell crosstalk. Nature immunology 12: 239-246.

Biziulevicius, G. A., O. V. Kislukhina, J. Kazlauskaite, and V. Zukaite. 2006. Food-protein enzymatic hydrolysates possess both antimicrobial and

immunostimulatory activities: a "cause and effect" theory of bifunctionality. FEMS immunology and medical microbiology 46: 131-138.

Blomhoff, R., and H. K. Blomhoff. 2006. Overview of retinoid metabolism and function. Journal of neurobiology 66: 606-630.

Blum, J. W., and H. Hammon. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livestock Production Science 66: 151-159.

Brandon, M. R., D. L. Watson, and A. K. Lascelles. 1971. The mechanism of transfer of immunoglobulin into mammary secretion of cows. Aust J Exp Biol Med 49: 613-623.

Brew, K., T. C. Vanaman, and R. L. Hill. 1968. The role of alpha-lactalbumin and the A protein in lactose synthetase: a unique mechanism for the control of a biological reaction. Proceedings of the National Academy of Sciences of the United States of America 59: 491-497.

Broughton, C. W., and J. G. Lecce. 1970. Electron-microscopic studies of the jejunal epithelium from neonatal pigs fed different diets. The Journal of nutrition 100: 445-449.

Buck, J. F. Derguini, E. Levi, K. Nakanishi, U. Hammerling. 1990. Retinol is essential for growth of activated human B cells. The Journal of experimental medicine. 171: 1613-1624.

Buhler, C., H. Hammon, G. L. Rossi, and J. W. Blum. 1998. Small intestinal morphology in eight-day-old calves fed colostrum for different durations or only milk replacer and treated with long-R3-insulin-like growth factor I and growth hormone. Journal of animal science 76: 758-765.

Burrin, D. G., B. Stoll, and X. Guan. 2003. Glucagon-like peptide 2 function in domestic animals. Domestic animal endocrinology 24: 103-122.

Bush, L. J., and T. E. Staley. 1980. Absorption of colostral immunoglobulins in newborn calves. Journal of dairy science 63: 672-680.

Capiaumont, J. et al. 1994. Whey and Beta-Lactoglobulin .2. Milk by-Products Which Can Replace Fetal Calf Serum in Mouse Hybridoma Cell-Culture. Lait 74: 127-137.

Chase, C. C., D. J. Hurley, and A. J. Reber. 2008. Neonatal immune development in the calf and its impact on vaccine response. The Veterinary clinics of North America. Food animal practice 24: 87-104.

Chatterton, D. E., D. N. Nguyen, S. B. Bering, and P. T. Sangild. 2013. Anti-inflammatory mechanisms of bioactive milk proteins in the intestine of newborns. The international journal of biochemistry & cell biology 45: 1730-1747.

Chen WL, M.T. Hwang, C.Y. Liau, J.C. Ho, K.C. Hong, S.J.T. Maoemail. 2005. β-Lactoglobulin is a Thermal Marker in Processed Milk as Studied by Electrophoresis and Circular Dichroic Spectra. J Dairy Sci 88:1618

Cianga, P., C. Medesan, J. A. Richardson, V. Ghetie, and E. S. Ward. 1999. Identification and function of neonatal Fc receptor in mammary gland of lactating mice. European journal of immunology 29: 2515-2523.

Cossey, V., Jeurissen, A., Bossuyt, X., and Schuermans, A. 2009. Effect of pasteurisation on the mannose-binding lectin activity and the concentration of soluble CD14 in human milk. J. Hosp. Infect.73: 96–97.

Czank, C., Prime, D., Hartmann, B., Simmer, K., and Hartmann, P.E. 2009. Retention of the immunological proteins of pasteurized human milk in relation to pasteurizer design and practice. Pediatr. Res. 66(4): 374–379.

da Costa, R.S., do Carmo, M.G., Saunders, C., de Jesus, E.F., Lopes, R.T., and Simabuco, S.M. 2003. Characterization of iron, copper and zinc levels in the colostrum of mothers of term and pre-term infants before and after pasteurization. Int. J. Food Sci. Nutr. 54:111–117.

Danielsen, M., L. J. Pedersen, and E. Bendixen. 2011. An in vivo characterization of colostrum protein uptake in porcine gut during early lactation. Journal of proteomics 74: 101-109.

Debier, C., and Y. Larondelle. 2005. Vitamins A and E: metabolism, roles and transfer to offspring. The British journal of nutrition 93: 153-174.

Deplancke, B., and H. R. Gaskins. 2001. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. Am J Clin Nutr 73: 1131S-1141S.

Devery, J. E., C. L. Davis, and B. L. Larson. 1979. Endogenous production of immunoglobulin IgG1 in newborn calves. Journal of dairy science 62: 1814-1818.

Donovan, D. C., A.J. Reber, J.D. Gabbard, M. Aceves-Avila, K.L. Galland, K.A. Holbert, L.O. Ely, D.J. Hurley. 2007. Effect of maternal cells transferred with colostrum on cellular responses to pathogen antigens in neonatal calves. American journal of veterinary research 68: 778-782.

Drackley, J. K. 2008. Calf nutrition from birth to breeding. The Veterinary clinics of North America. Food animal practice 24: 55-86.

Drozdowski, L., and A. B. Thomson. 2009. Intestinal hormones and growth factors: effects on the small intestine. World journal of gastroenterology : WJG 15: 385-406.

Drucker, D. J. 2002. Gut adaptation and the glucagon-like peptides. Gut 50: 428-435.

Dubé, P. E., and P. L. Brubaker. 2007. Frontiers in glucagon-like peptide-2: multiple actions, multiple mediators. American Journal of Physiology - Endocrinology And Metabolism 293: E460-E465.

Dube, P. E., K. J. Rowland, and P. L. Brubaker. 2008. Glucagon-like peptide-2 activates beta-catenin signaling in the mouse intestinal crypt: role of insulin-like growth factor-I. Endocrinology 149: 291-301.

Ducluzeau, R. 1983. Implantation and development of the gut flora in the newborn animal. Annales de recherches veterinaires. Annals of veterinary research 14: 354-359.

Eicher, S. D., J. L. Morrill, F. Blecha, C. G. Chitko-mckown, N. V. Anderson, and J. J. Higgins. 1994. Leukocyte functions of young dairy calves fed milk replacers supplemented with vitamins A and E. Journal of dairy science 77: 1399-1407.

Elizondo-Salazar, J. A., and A. J. Heinrichs. 2009a. Feeding heat-treated colostrum or unheated colostrum with two different bacterial concentrations to neonatal dairy calves. Journal of dairy science 92: 4565-4571.

Elizondo-Salazar, J. A., and A. J. Heinrichs. 2009b. Feeding heat-treated colostrum to neonatal dairy heifers: Effects on growth characteristics and blood parameters. Journal of dairy science 92: 3265-3273.

Ellis, L. A., A. M. Mastro, and M. F. Picciano. 1996. Milk-borne prolactin and neonatal development. Journal of mammary gland biology and neoplasia 1: 259-269.

Enders, A. C., and A. M. Carter. 2004. What can comparative studies of placental structure tell us?--A review. Placenta 25 Suppl A: S3-9.

Evans, T.J., Ryley, H.C., Neale, L.M., Dodge, J.A., and Lewarne, V. M. 1978. Effect of storage and heat on antimicrobial proteins in human milk. Arch. Dis. Child. 53: 239–241.

Ewaschuk, J. B., S. Unger, S. Harvey, D. L. O'Connor, and C. J. Field. 2011. Effect of pasteurization on immune components of milk: implications for feeding preterm infants. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme 36: 175-182.

Foley, J. A., and D. E. Otterby. 1978. Availability, Storage, Treatment, Composition, and Feeding Value of Surplus Colostrum: A Review1,2. Journal of dairy science 61: 1033-1060.

Ganessunker, D., H. R. Gaskins, F. A. Zuckermann, and S. M. Donovan. 1999. Total parenteral nutrition alters molecular and cellular indices of intestinal inflammation in neonatal piglets. JPEN. Journal of parenteral and enteral nutrition 23: 337-344.

Gilbert, S. F. 2014. Developmental biology. Tenth edition. ed. Sinauer Associates, Sunderland, MA.

Godden, S. 2008. Colostrum management for dairy calves. The Veterinary clinics of North America. Food animal practice 24: 19-39.

Godden, S., S. McMartin, J. Feirtag, J. Stabel, R. Bey, S. Goyal, L. Metzger, J. Fetrow, S. Wells, and H. Chester-Jones. 2006. Heat-Treatment of Bovine Colostrum. II: Effects of Heating Duration on Pathogen Viability and Immunoglobulin G. Journal of dairy science 89: 3476-3483.

Godden, S. M. S. Smith, J. M. Feirtag, L. R. Green, S. J. Wells, and J. P. Fetrow. 2003. Effect of on-farm commercial batch pasteurization of colostrum on colostrum and serum immunoglobulin concentrations in dairy calves. Journal of dairy science 86: 1503-1512.

Godden, S. M., D. J. Smolenski, M. Donahue, J. M. Oakes, R. Bey, S. Wells, S. Sreevatsan, J. Stabel, and J. Fetrow 2012. Heat-treated colostrum and reduced morbidity in preweaned dairy calves: results of a randomized trial and

examination of mechanisms of effectiveness. Journal of dairy science 95: 4029-4040.

Goelz, R., Hihn, E., Hamprecht, K., Dietz, K., Jahn, G., Poets, C., and Elmlinger, M. 2009. Effects of different CMV-heat-inactivationmethods on growth factors in human breast milk. Pediatr. Res. 65: 458–461.

Graves, E. L., A. D. Beaulieu, and J. K. Drackley. 2007. Factors affecting the concentration of sphingomyelin in bovine milk. Journal of dairy science 90: 706-715.

Grove, D. S., B. Bour, B. Kacsoh, and A. M. Mastro. 1991. Effect of neonatal milk-prolactin deprivation on the ontogeny of the immune system of the rat. Endocrine regulations 25: 111-119.

Guimont, C., E. Marchall, J. M. Girardet, G. Linden, and H. Otani. 1997. Biologically active factors in bovine milk and dairy byproducts: Influence on cell culture. Critical Reviews in Food Science and Nutrition 37: 393-410.

Hammon, H., and J. W. Blum. 1997. The somatotropic axis in neonatal calves can be modulated by nutrition, growth hormone, and Long-R3-IGF-I. The American journal of physiology 273: E130-138.

He, X. C., J. Zhang, W.G. Tong, O. Tawfik, J. Ross, D.H. Scoville, Q. Tian, X. Zeng, X. He, L.M. Wiedemann, Y. Mishina, L. Li. 2004. BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-beta-catenin signaling. Nature genetics 36: 1117-1121.

Henderson, T. R., T. N. Fay, and M. Hamosh. 1998. Effect of pasteurization on long chain polyunsaturated fatty acid levels and enzyme activities of human milk. The Journal of pediatrics 132: 876-878.

Hernandez-Ledesma, B., I. Recio, and L. Amigo. 2008. Beta-lactoglobulin as source of bioactive peptides. Amino acids 35: 257-265.

Horton, R. E., and G. Vidarsson. 2013. Antibodies and their receptors: different potential roles in mucosal defense. Frontiers in immunology 4: 200.

Husband, A. J., M. R. Brandon, and A. K. Lascelles. 1973. The effect of corticosteroid on absorption and endogenous production of immunoglobulins in calves. The Australian journal of experimental biology and medical science 51: 707-710.

Izumi, H., S. Ishizuka , A. Inafune, T. Hira, K. Ozawa, T. Shimizu, M. Takase, H. Hara. 2009. α-Lactalbumin Hydrolysate Stimulates Glucagon-Like Peptide-2 Secretion and Small Intestinal Growth in Suckling Rats. The Journal of nutrition 139: 1322-1327.

James, R. E., C. E. Polan, and K. A. Cummins. 1981. Influence of administered indigenous microorganisms on uptake of [iodine-125] gamma-globulin in vivo by intestinal segments of neonatal calves. Journal of dairy science 64: 52-61.

Jochims, K.. F.-J. Kaup, W. Drommer. andM. Pickel, 1994: Colostral IgG transfer in newborn calves: an immunoelectron microscopic investigation of IgG absorption across the intestinal barrier. Res.Vet. Sci. 57, 75-80.

Johnson, J. L., S. M. Godden, T. Molitor, T. Ames, and D. Hagman. 2007. Effects of Feeding Heat-Treated Colostrum on Passive Transfer of Immune and Nutritional Parameters in Neonatal Dairy Calves. Journal of dairy science 90: 5189-5198.

Johnston, N. E., and W. D. Oxender. 1979. Effect of altered serum glucocorticoid concentrations on the ability of the newborn calf to absorb colostral immunoglobulin. American journal of veterinary research 40: 32-34.

Kacskovics, I., Z. Wu, N. E. Simister, L. V. Frenyó, and L. Hammarström. 2000. Cloning and characterization of the bovine MHC class I-like Fc receptor. Journal of Immunology 164: 1889-1897.

Kandori, H., K. Hirayama, M. Takeda, and K. Doi. 1996. Histochemical, lectin-histochemical and morphometrical characteristics of intestinal goblet cells of germfree and conventional mice. Experimental animals / Japanese Association for Laboratory Animal Science 45: 155-160.

Kaup, F. J., W. Drommer, K. Jochims, and M. Pickel. 1996. Ultrastructure of pre- and postcolostral enterocytes of the newborn calf. Anatomia, histologia, embryologia 25: 249-255.

Kelly, D., S. Conway, and R. Aminov. 2005. Commensal gut bacteria: mechanisms of immune modulation. Trends in Immunology 26: 326-333.

Koenig, A., de Albuquerque Diniz, E.M., Barbosa, S.F., and Vaz, F. A. 2005. Immunologic factors in human milk: the effects of gestational age and pasteurization. J. Hum. Lact. 21: 439–443.

Kosaka, N., H. Izumi, K. Sekine, and T. Ochiya. 2010. microRNA as a new immune-regulatory agent in breast milk. Silence 1:7.

Kraehenbuhl, J. P., and M. A. Campiche. 1969. Early stages of intestinal absorption of specific antibiodies in the newborn. An ultrastructural, cytochemical, and immunological study in the pig, rat, and rabbit. J Cell Biol 42: 345-365.

Kussendrager, K. D., and A. C. van Hooijdonk. 2000. Lactoperoxidase: physico-chemical properties, occurrence, mechanism of action and applications. The British journal of nutrition 84 Suppl 1: S19-25.

Lakritz, J., J.W. Tyler, D.E. Hostetler, A.E. Marsh, D.M Weaver, J.M. Holle, B.J. Steevens, J.L. Denbigh. 2000. Effects of pasteurization of colostrum on subsequent serum lactoferrin concentration and neutrophil superoxide production in calves. American journal of veterinary research 61: 1021-1025.

Lara-Villoslada, F., M. Olivares, S. Sierra, J.M. Rodríguez, J. Boza, J. Xaus J. 2007. Beneficial effects of probiotic bacteria isolated from breast milk. The British journal of nutrition. 98 Suppl 1: S96-100.

Larson, B. L., H. L. Heary Jr, and J. E. Devery. 1980. Immunoglobulin Production and Transport by the Mammary Gland. Journal of dairy science 63: 665-671.

Larson, L. L., F.G. Owen, J.L. Albright, R.D. Appleman, R.C. Lamb, L.D. Muller. 1977. Guidelines Toward More Uniformity in Measuring and Reporting Calf Experimental Data1. Journal of dairy science 60: 989-991.

Lecce, J. G., and D. O. Morgan. 1962. Effect of Dietary Regimen on Cessation of Intestinal Absorption of Large Molecules (Closure) in the Neonatal Pig and Lamb. The Journal of nutrition 78: 263-268.

Lecce, J. G., D. O. Morgan, and G. Matrone. 1964. Effect of Feeding Colostral and Milk Components on the Cessation of Intestinal Absorption of Large Molecules (Closure) in Neonatal Pigs. The Journal of nutrition 84: 43-48.

Lepri, L., Del Bubba, M., Maggini, R., Donzelli, G.P., and Galvan, P. 1997. Effect of pasteurization and storage on some components of pooled human milk. J. Chromatogr. B Biomed. Sci. Appl. 704:1–10.

Lesmeister, K. E., and A. J. Heinrichs. 2005. Effects of adding extra molasses to a texturized calf starter on rumen development, growth characteristics, and blood parameters in neonatal dairy calves. Journal of dairy science 88: 411-418.

Levieux, D., and A. Ollier. 1999. Bovine immunoglobulin G, β-lactoglobulin, α-lactalbumin and serum albumin in colostrum and milk during the early post partum period. Journal of Dairy Research 66: 421-430.

Liang G, Malmuthuge N, McFadden TB, Bao H, Griebel PJ, et al. 2014. Potential Regulatory Role of microRNAs in the Development of Bovine Gastrointestinal Tract during Early Life. PLoS ONE 9(3): e92592. doi:10.1371/journal.pone. 0092592

Louis, N. A., and P. W. Lin. 2009. The intestinal immune barrier. NeoReviews 10: e180-e190.

Mackie, R. I., A. Sghir, and H. R. Gaskins. 1999. Developmental microbial ecology of the neonatal gastrointestinal tract. The American journal of clinical nutrition 69: 1035s-1045s.

McCracken, B. A., H. R. Gaskins, P. J. Ruwe-Kaiser, K. C. Klasing, and D. E. Jewell. 1995. Diet-dependent and diet-independent metabolic responses underlie growth stasis of pigs at weaning. The Journal of nutrition 125: 2838-2845.

McGuirk, S. M., and M. Collins. 2004. Managing the production, storage, and delivery of colostrum. The Veterinary clinics of North America. Food animal practice 20: 593-603.

McKenna, L. B., J. Schug, A. Vourekas, J.B. McKenna, N.C. Bramswig, J.R. Friedman, K.H. Kaestner. 2010. MicroRNAs control intestinal epithelial differentiation, architecture, and barrier function. Gastroenterology 139: 1654-1664, 1664 e1651.

McMartin, S., S. Godden, L. Metzger, J. Feirtag, R. Bey, J. Stabel, S. Goyal, J. Fetrow, S. Wells, and H. Chester-Jones. 2006. Heat Treatment of Bovine Colostrum. I: Effects of Temperature on Viscosity and Immunoglobulin G Level. Journal of dairy science 89: 2110-2118.

Meylan, M, D.M. Rings, W.P. Shulaw, J.J. Kowalski, S. Bech-Nielsen, G.F. Hoffsis. 1996. Survival of Mycobacterium paratuberculosis and preservation of

immunoglobulin G in bovine colostrum under experimental conditions simulating pasteurization. American journal of veterinary research 57: 1580-1585.

Miura, S., R. Hokari, and S. Komoto. 2011. Intestinal immune system / Soichiro Miura, Ryota Hokari and Shunsuke Komoto. San Rafael : Morgan & Claypool, c2011.

Molenaar, A. J., Y. M. Kuys, S. R. Davis, R. J. Wilkins, P. E. Mead, And J. W. Tweed. 1996. Elevation of lactoferrin gene expression in developing, ductal, resting, and regressing parenchymal epithelium of the ruminant mammary gland. Journal of dairy science 79: 1198-1208.

Moog, F. 1979. Endocrine Influences on the Functional Differentiation of the Small Intestine. Journal of animal science 49: 239-249.

Morein, B., G. Blomqvist, and K. Hu. 2007. Immune responsiveness in the neonatal period. Journal of comparative pathology 137: S27-S31.

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

Motouri, M., H. Matsuyama, J. Yamamura, M. Tanaka, S. Aoe, T. Iwanaga, H. Kawakami. 2003. Milk sphingomyelin accelerates enzymatic and morphological maturation of the intestine in artificially reared rats. Journal of pediatric gastroenterology and nutrition 36: 241-247.

Nagano, M., E. Chastre, A. Choquet, J. Bara, C. Gespach, P.A. Kelly. 1995. Expression of prolactin and growth hormone receptor genes and their isoforms in the gastrointestinal tract. The American journal of physiology 268: G431-442.

Nardone, A., N. Lacetera, U. Bernabucci, and B. Ronchi. 1997. Composition of colostrum from dairy heifers exposed to high air temperatures during late pregnancy and the early postpartum period. Journal of dairy science 80: 838-844.

National Animal Health Monitoring System. Dairy 2007. Heifer Calf Health and Management Practices on U.S. Dairy Operations. USDA:APHIS Veterinary Services, Ft. Collins, CO.

Nonnecke, B. J., R. L. Horst, W. R. Waters, P. Dubeski, and J. A. Harp. 1999. Modulation of fat-soluble vitamin concentrations and blood mononuclear

leukocyte populations in milk replacer-fed calves by dietary vitamin A and beta-carotene. Journal of dairy science 82: 2632-2641.

Nousiainen, J., H. Korhonen, E. L. Syvaoja, S. Savolainen, H. Saloniemi, and H. Halonen 1994. The effect of colostral, immunoglobulin supplement on the passive immunity, growth and health of neonatal calves. Agric. Sci. Finland 3: 421-428.

Nuijens, J. H., P. H. van Berkel, and F. L. Schanbacher. 1996. Structure and biological actions of lactoferrin. Journal of mammary gland biology and neoplasia 1: 285-295.

Nusrat, A., J. R. Turner, and J. L. Madara. 2000. Molecular physiology and pathophysiology of tight junctions. IV. Regulation of tight junctions by extracellular stimuli: nutrients, cytokines, and immune cells. American journal of physiology. Gastrointestinal and liver physiology 279: G851-857.

Ockleford, C. D., and A. Whyte. 1980. Coated vesicles. Cambridge University Press, Cambridge ; New York.

Pakkanen, R., and J. Aalto. 1997. Growth factors and antimicrobial factors of bovine colostrum. International dairy journal / published in association with the International Dairy Federation 7: 285-297.

Patt, J. A. 1977. Factors affecting the duration of intestinal permeability to macromolecules in newborn animals. Biological Reviews 52: 411-429.

Pearson, G. R., M. S. McNulty, and E. F. Logan. 1978. Pathological changes in the small intestine of neonatal calves with enteric colibacillosis. Veterinary pathology 15: 92-101.

Pellegrini, A., U. Thomas, R. von Fellenberg, and P. Wild. 1992. Bactericidal activities of lysozyme and aprotinin against gram-negative and gram-positive bacteria related to their basic character. The Journal of applied bacteriology 72: 180-187.

Penhale, W. J., E. F. Logan, I. E. Selman, E. Fisher, and A. D. McEwan. 1973. Observations on the absorption of colostral immunoglobulins by the neonatal calf and their significance in colibacillosis. Annales de recherches veterinaires. Annals of veterinary research 4: 223-233.

Pinto, D., A. Gregorieff, H. Begthel, and H. Clevers. 2003. Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. Genes & development 17: 1709-1713.

Playford, R. J., C. E. Macdonald, and W. S. Johnson. 2000. Colostrum and milk-derived peptide growth factors for the treatment of gastrointestinal disorders. The American Journal of Clinical Nutrition 72: 5-14.

Prioult, G., S. Pecquet, and I. Fliss. 2004. Stimulation of interleukin-10 production by acidic beta-lactoglobulin-derived peptides hydrolyzed with Lactobacillus paracasei NCC2461 peptidases. Clinical and diagnostic laboratory immunology 11: 266-271.

Qiao, S. W., W. I. Lencer, and R. S. Blumberg. 2007. How the controller is controlled - neonatal Fc receptor expression and immunoglobulin G homeostasis. Immunology 120: 145-147.

Quigley, J. D., 3rd, P. French, and R. E. James. 2000. Short communication: effect of pH on absorption of immunoglobulin G in neonatal calves. Journal of dairy science 83: 1853-1855.

Raghavan, M., L. N. Gastinel, and P. J. Bjorkman. 1993. The class I major histocompatibility complex related Fc receptor shows pH-dependent stability differences correlating with immunoglobulin binding and release.

Rao, N. J., and J.-Y. Wang. 2011. Regulation of gastrointestinal mucosal growth. In: Colloquium Series on Integrated Systems Physiology: From Molecule to Function. p 1-114.

Reber, A. J., D.C. Donovan, J. Gabbard, K. Galland, M. Aceves-Avila, K.A. Holbert, L. Marshall, D.J. Hurley. 2008. Transfer of maternal colostral leukocytes promotes development of the neonatal immune system Part II. Effects on neonatal lymphocytes. Veterinary immunology and immunopathology 123: 305-313.

Richmond, C. A., and D. T. Breault. 2010. Regulation of gene expression in the intestinal epithelium. Progress in molecular biology and translational science 96: 207-229.

Robison, J. D., G. H. Stott, and S. K. DeNise. 1988. Effects of passive immunity on growth and survival in the dairy heifer. Journal of dairy science 71: 1283-1287.

Rodewald, R. 1973. Intestinal transport of antibodies in the newborn rat. J Cell Biol 58: 189-211.

Rodewald, R. 1976. pH-dependent binding of immunoglobulins to intestinal cells of the neonatal rat. J Cell Biol 71: 666-669.

Rodewald, R., and J. P. Kraehenbuhl. 1984. Receptor-mediated transport of IgG. J Cell Biol 99: 159s-164s.

Ross, M. H., W. Pawlina, and T. A. Barnash. 2009. Atlas of descriptive histology. Sinauer Associates, Sunderland, Mass.

Roth-Walter F., M.C. Berin, P. Arnaboldi, C.R. Escalante, S. Dahan, J. Rauch, E. Jensen-Jarolim, L. Mayer. 2008. Pasteurization of milk proteins promotes allergic sensitization by enhancing uptake through Peyer's patches. Allergy. 63:882-90.

Said, H. M., D. E. Ong, and J. L. Shingleton. 1989. Intestinal uptake of retinol: enhancement by bovine milk beta-lactoglobulin. The American Journal of Clinical Nutrition 49: 690-694.

Sakamoto, K., H. Hirose, A. Onizuka, M. Hayashi, N. Futamura, Y. Kawamura, T. Ezaki. 2000. Quantitative study of changes in intestinal morphology and mucus gel on total parenteral nutrition in rats. The Journal of surgical research 94: 99-106.

Salminen, S., Y. Benno, and W. de Vos. 2006. Intestinal colonisation, microbiota and future probiotics? Asia Pacific journal of clinical nutrition 15: 558-562.

Salmon, H. 1999. The mammary gland and neonate mucosal immunity. Veterinary immunology and immunopathology 72: 143-155.

Sangild, P. T., K.A. Tappenden, C. Malo, Y.M. Petersen, J. Elnif, A.L. Bartholome, R.K. Buddington. 2006. Glucagon-like peptide 2 stimulates intestinal nutrient absorption in parenterally fed newborn pigs. Journal of pediatric gastroenterology and nutrition 43: 160-167.

Sasaki, M., C. L. Davis, and B. L. Larson. 1976. Production and turnover of IgG1 and IgG2 immunoglobulins in the bovine around parturition. Journal of dairy science 59: 2046-2055.

Schottstedt, T., C. Muri, C. Morel, C. Philipona, H. M. Hammon, and J. W. Blum. 2005. Effects of feeding vitamin A and lactoferrin on epithelium of lymphoid tissues of intestine of neonatal calves. Journal of dairy science 88: 1050-1061.

Sekirov, I., S. L. Russell, L. C. Antunes, and B. B. Finlay. 2010. Gut microbiota in health and disease. Physiol Rev 90: 859-904.

Shanahan, F., and J. O'Mahony. 2005. The mycobacteria story in Crohn's disease. The American journal of gastroenterology 100: 1537-1538.

Sheldrake, R. F., and A. J. Husband. 1985. Intestinal uptake of intact maternal lymphocytes by neonatal rats and lambs. Research in veterinary science 39: 10-15.

Simister, N. E., and K. E. Mostov. 1989. An Fc receptor structurally related to MHC class I antigens. Nature 337: 184-187.

Skrzypek, T., J.L. Valverde Piedra, H. Skrzypek, W. Kazimierczak, M. Biernat, R. Zabielski. 2007. Gradual disappearance of vacuolated enterocytes in the small intestine of neonatal piglets. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society 58 Suppl 3: 87-95.

Smeaton, T. C., and M. W. Simpson-Morgan. 1985. Epithelial cell renewal and antibody transfer in the intestine of the foetal and neonatal lamb. The Australian journal of experimental biology and medical science 63 (Pt 1): 41-51.

Smith, T., and R. B. Little. 1922. The significance of colostrum to the new-born calf. The Journal of experimental medicine 36: 181-198.

Staley, T. E., E. W. Jones, and L. J. Bush. 1971. Maternal transport of immunoglobulins to the calf. Journal of dairy science 54: 1323.

Stanger, B. Z., R. Datar, L. C. Murtaugh, and D. A. Melton. 2005. Direct regulation of intestinal fate by Notch. Proc Natl Acad Sci U S A 102: 12443-12448.

Stewart, S. et al. 2005. Preventing bacterial contamination and proliferation during the harvest, storage, and feeding of fresh bovine colostrum. Journal of dairy science 88: 2571-2578.

Stockinger, S., M. W. Hornef, and C. Chassin. 2011. Establishment of intestinal homeostasis during the neonatal period. Cellular and molecular life sciences : CMLS 68: 3699-3712.

Stott, G. H. 1980. Immunoglobulin absorption in calf neonates with special considerations of stress. Journal of dairy science 63: 681-688.

Stott, G. H., D. B. Marx, B. E. Menefee, and G. T. Nightengale. 1979. Colostral immunoglobulin transfer in calves I. Period of absorption. Journal of dairy science 62: 1632-1638.

Sutton, L. F., and B. Alston-Mills. 2006. β-lactoglobulin as a potential modulator of intestinal activity and morphology in neonatal piglets. The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology 288A: 601-608.

Taylor-Edwards, C. C., D. G. Burrin, J. J. Holst, K. R. McLeod, and D. L. Harmon. 2011. Glucagon-like peptide-2 (GLP-2) increases small intestinal blood flow and mucosal growth in ruminating calves. Journal of dairy science 94: 888-898.

Teixeira, A.G., M.L. Bicalho, V.S. Machado, G. Oikonomou, C. Kacar, C. Foditsch, R. Young, W.A. Knauer, D.V. Nydam, R.C. Bicalho. 2013. Heat and ultraviolet light treatment of colostrum and hospital milk: effects on colostrum and hospital milk characteristics and calf health and growth parameters. Vet J. 2013 Aug;197:175 81.

Trujillo, A.J., N. Castro, J. M. Quevedo, A. Arguello, J. Capote, and B. Guamis. 2007. Effect of Heat and High-Pressure Treatments on Microbiological Quality and Immunoglobulin G Stability of Caprine Colostrum. J. Dairy Sci. 90:833–839.

Tuboly, S., S. Bernath, R. Glavits, and I. Medveczky. 1988. Intestinal absorption of colostral lymphoid cells in newborn piglets. Veterinary immunology and immunopathology 20: 75-85.

Tyler, J. W., D. D. Hancock, J. G. Thorne, C. C. Gay, and J. M. Gay. 1999. Partitioning the mortality risk associated with inadequate passive transfer of colostral immunoglobulins in dairy calves. Journal of veterinary internal medicine / American College of Veterinary Internal Medicine 13: 335-337.

Wagner, C. L., S. N. Taylor, and D. Johnson. 2008. Host factors in amniotic fluid and breast milk that contribute to gut maturation. Clinical reviews in allergy & immunology 34: 191-204.

Wardell, J.M., Hill, C.M., and D'Souza, S.W. 1981. Effect of pasteurization and of freezing and thawing human milk on its triglyceride content. Acta Paediatr. Scand. 70: 467–471.

Weaver, D. M., J. W. Tyler, D. C. VanMetre, D. E. Hostetler, and G. M. Barrington. 2000. Passive transfer of colostral immunoglobulins in calves. Journal of veterinary internal medicine / American College of Veterinary Internal Medicine 14: 569-577.

Weaver, L. T., M. F. Laker, and R. Nelson. 1984. Intestinal permeability in the newborn. Archives of disease in childhood 59: 236-241.

Williams, P. P. 1993. Immunomodulating effects of intestinal absorbed maternal colostral leukocytes by neonatal pigs. Canadian journal of veterinary research = Revue canadienne de recherche veterinaire 57: 1-8.

Wong, K. F., N. Middleton, M. Montgomery, M. Dey, and R. I. Carr. 1998. Immunostimulation of murine spleen cells by materials associated with bovine milk protein fractions. Journal of dairy science 81: 1825-1832.

Xu, R. 1996. Development of newborn GI tract and its relation to colostrum/milk uptake a review. Reprod. Fertil. Dev. 8: 35-48.

Xu, R. J., D. J. Mellor, M. J. Birtles, B. H. Breier, and P. D. Gluckman. 1994. Effects of oral IGF-I or IGF-II on digestive organ growth in newborn piglets. Biology of the neonate 66: 280-287.

Yoshida, M., S.M. Claypool, J.S. Wagner, E. Mizoguchi, A. Mizoguchi, D.C. Roopenian, W.I. Lencer, R.S. Blumberg. 2004. Human neonatal Fc receptor mediates transport of IgG into luminal secretions for delivery of antigens to mucosal dendritic cells. Immunity 20: 769-783.

Zabielski, R., M. M. Godlewski, and P. Guilloteau. 2008. Control of development of gastrointestinal system in neonates. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society 59 Suppl 1: 35-54.

Chapter 2. Effects of heat-treatment of colostrum on absorption and gut development in neonatal bull calves during the first 12 hours of life

2.0 Introduction

In ruminants, the syndesmochorial placenta separates fetal and maternal blood supplies (Enders and Carter, 2004), preventing the direct transfer of immunoglobulins *in utero.* As a result, colostrum is the sole source of passive transfer of immunity for the neonatal calf. Transfer of immunoglobulins, specifically IgG, via colostrum significantly improves mortality and morbidity rates in calves, and therefore research on newborn calf management has placed an emphasis on methods for improving passive transfer. Calves are considered to have successful passive transfer if they are found to have a minimum of 10 mg/ml serum IgG concentration at approximately 24 hours after birth. Values less than this indicate failure of passive transfer (FPT) (Godden, 2008). Research has shown that FPT can account for as much as 40% of calf mortality during the pre-weaned period, in modern production systems (Tyler et al., 1999).

Ensuring successful passive transfer depends on three major factors: Quality, quantity and timing. Although all three of these variables can greatly influence the passive transfer status in calves, the time at which colostrum is delivered is often considered the most important. Immediately after the calf is born, the neonatal gut is in a naïve state and is able to passively absorb macromolecules. A process, termed "gut closure" by Lecce and Morgan (1962), is completed by approximately 36 hours of life and results in the inability to absorb large molecules. Because of this narrow window, it is generally recommended that calves should receive colostrum as soon as possible after birth.

Studies investigating passive transfer have determined optimal conditions for administering colostrum to newborn calves but, for the most part, this research has focused on IgG. Although IgG is considered critical to the survivability and health of the calf, colostrum also delivers high concentrations of many other compounds, broadly known as bioactives. These compounds are found at high concentrations in colostrum compared to milk and are thought to be important factors for the normal development of the calf. However, little is known about the properties and functions of such molecules, especially in the neonatal calf. Bioactives include hormones, growth factors, immune cells, cytokines, other anti-microbial factors, small peptides, metabolites and enzymes. Perhaps thousands of bioactive compounds exist in colostrum, many of which have not been characterized (Pakkanen and Aalto, 1997).

Some colostral bioactives are known to have important functional roles in the neonatal calf, mediating development of the gut, promoting growth and influencing the immune system (Pakkanen and Aalto, 1997). Lactoferrin, for example, is present at high concentrations in colostrum, and has been shown to have bacteriostatic effects within the gut by binding iron, an important component for growth of some bacteria types, such as *E. coli* (Molenaar et al., 1996). Lactoferrin also promotes normal neutrophil function (Lakritz et al., 2000). Maternal cells delivered to the calf in colostrum play an influential role in development of the neonatal immune system (Donovan et al., 2007; Reber et al., 2008). Some metabolites of vitamin A, in colostrum, may be important for regulating cellular processes and function of immune cells (Blomhoff and Blomhoff, 2006).

The most abundant whey protein in cow's milk, β-lactoglobulin (BLG), has been implicated as a potential factor involved in immunomodulation and gut development. A study by Wong et al. (1998) suggested that BLG has a beneficial effect on the stimulation of the immune system, which is consistent with results from Prioult et al. (2004) that show stimulatory effects of BLG on secretion of interleukin-10 (IL-10), an anti-inflammatory cytokine. Studies investigating the effects of BLG on gut development in production animals have been inconclusive (Sutton and Alston-Mills, 2006). However, BLG is known to interact with a number of other molecules, including growth factors, retinol and fatty acids (Chatterton et al., 2013; Madureira et al., 2007), some of which may influence developmental aspects of the gut.

Bioactives in colostrum may also affect (positively or negatively) proliferation of intestinal cells, such as goblet cells (Deplancke and Gaskins, 2001; Ganessunker et al., 1999) or mucosal growth (Blum and Hammon, 2000). These components may also influence production or secretion of molecules in the gut, thus affecting development or function. Glucagon-like peptide-2 (GLP-2) is a small peptide secreted by enteroendocrine L-cells in the small intestine and plays a crucial role in development of the gut during the neonatal period. GLP-2 has a strong stimulatory effect on small intestinal growth and also in increasing its absorptive capacity (Dubé and Brubaker, 2007; Sangild et al., 2006). A study by Izumi et al. (2009) has shown that secretion of GLP-2 is stimulated by proteinaceous components of milk in rats that were fed either the lactose, cream or protein fractions of milk. Purified soy protein or ovalbumin did not elicit this same response. High concentrations of GLP-2 are secreted in the gut in neonatal animals (Burrin et al., 2001). The effects of exogenously administered GLP-2 have been studied in weaned calves (Taylor-Edwards et al., 2011) but few studies have examined the effects of GLP-2 in neonates.

Pasteurization of milk and colostrum is becoming a popular calf management practice on dairy farms as a method of controlling transmission of infectious pathogens, such as that which causes Johne's disease. Previous experiments have found that the temperatures used for heat-treating milk are unsuitable for colostrum, since temperatures greater than 60°C result in an undesirable increase in viscosity and a 26% loss of IgG (Godden et al., 2003). Studies conducted by Johnson et al. (2007) and Elizondo-Salazar and Heinrichs (2009), in which colostrum was pasteurized at 60°C, demonstrated that calves fed heat-treated colostrum had 18% higher serum concentrations of IgG and higher apparent efficiency of absorption (AEA) compared to calves that received unheated colostrum. In these studies there were no significant differences detected in colostral IgG concentrations, suggesting that heat-treatment influenced the absorption of IgG in the calf's gut. These studies also analyzed a limited number of other bioactive components such as IgA, IgM, vitamin A, vitamin E, cholesterol and β-carotene, but did not detect any significant differences in the calves' serum.

Significant effects of pasteurization on IgG have been reported, but little attention has been given to the effects on other bioactives and their role in the neonatal calf. Most

bioactive compounds are found at colostrum in higher concentrations compared to mature milk, suggesting an important role in the newborn. The aim of the current experiment was to use the neonatal calf in order to study the effects of pasteurization on the bioactivity of colostrum. Specifically, serum IgG and BLG were analyzed to investigate the absorption of two common proteins found in colostrum. Furthermore, morphology of the small intestine was analyzed in order to investigate development of the gut as measured by villus height, crypt depth and goblet cell numbers.

Because previous research (Elizondo-Salazar and Heinrichs, 2009; Johnson et al., 2007) found that calves receiving heat-treated colostrum had higher serum IgG concentrations compared to those fed unheated colostrum, it was hypothesized that heat-treatment of colostrum in the present study leads to higher plasma levels of IgG. It was also hypothesized that BLG, a heat-labile protein, would be negatively affected by heat, resulting in lower plasma BLG concentrations in neonatal calves. It was also speculated that heat-treatment of colostrum would affect gut development by promoting growth of small intestinal villi and crypts, and decreasing the proliferation of ileal goblet cells, as a result of a lower bacterial count.

2.1 Materials and Methods

2.1.1 Colostrum Management

First-milking colostrum was collected from primi- and multi-parous cows and its specific gravity was measured at room temperature using a hydrometer. Colostrum with an IgG concentration of 50 mg/ml or greater was aliquoted into 1-liter volumes in plastic freezer bags, which were immediately laid flat on wire racks and frozen in -20°C. Two batches, consisting of ~56 liters each, were collected. Batch 1 was collected from 6 cows from May to mid-June and Batch 2 was collected from 10 cows from mid-June to late-July. Once 56 liters were collected, colostrum was placed in a 4°C cold room and thawed slowly for 24 hours. After thawing, colostrum was mixed thoroughly by manually

whisking for several minutes. Half of the colostrum (28L) was subsequently aliquoted into 1-liter volumes and refrozen in plastic freezer bags. The other half of the colostrum (28L) was pasteurized for 60 minutes at 60°C using an on-farm pasteurizer (Dairy Tech Inc, Greeley, CO, USA). The temperature was carefully monitored and did not fluctuate more than 1.5 degrees for the entire 60 minutes. At the end of the pasteurization cycle, colostrum was rapidly cooled, aliquoted into 1-liter volumes and refrozen in plastic freezer bags. Subsamples from both unheated and heat-treated colostrum were frozen and stored for later analysis, at which point they were thawed, aliquoted into smaller tubes, and re-frozen. Two batches of colostrum were used for calves, and a third batch was used for statistical analysis only.

2.1.2 Bull Calf Management and Sample Collection

Protocols for this study were approved by the University of Alberta Animal Care and Use Committee for Livestock and conducted in accordance with the Canadian Council of Animal Care (Ottawa, ON, Canada). Twenty-eight bull calves were enrolled in the study as they were born and assigned in a semi-random fashion to receive either heat-treated or unheated colostrum. Near parturition, cows were placed in individual maternity pens and monitored closely with video cameras. At birth, cows were allowed to lick their calf dry. Calves were removed from the cow within 30 minutes of calving and always before suckling could occur. Calves were moved to a clean pen bedded with fresh wood shavings and their navels were dipped with 7% (v/v) iodine. Each calf was fed 2 liters of either heat-treated or unheated colostrum via an esophageal tube feeder within 1 hour after birth. Prior to colostrum feeding, approximately 8.5 ml of blood was collected in a heparinized tube, which was centrifuged at 3000 x g at 4°C for 20 minutes. Plasma was aliquoted into five 1.5 ml microcentrifuge tubes and frozen at -80°C for later analysis. Blood samples were also collected at 3, 6, 9 and 12 hours after birth. Calves were kept in individual pens until either 6 (n=11) or 12 (n=12) hours of life, at which point the calves were taken to a surgical suite for tissue sample collection. Of the 6-hour bulls, 6 bulls received heat-treated colostrum and 5 received unheated colostrum. Of the 12-hour bulls, 6 bulls received heat-treated colostrum and 6 bulls received unheated

colostrum. Five extra bulls also received heat-treated (n=3) or unheated (n=2) colostrum. Blood samples from these calves were analyzed with the other 12-hour bulls, but they were not euthanized for tissue collection.

Bull calves were euthanized at either 6 or 12 hours after birth by penetrative captive bolt followed by exsanguination. The abdominal cavity was opened and clamps were placed on the esophagus and rectum, and the entire GIT was removed. Figure 2.1 depicts the methods used for dissection. The small intestine was dissected out in the following order: ileum, distal jejunum, duodenum and proximal jejunum. Ileum was measured 10 cm caudally from the ileocecal junction and the section was removed. Only the ileum was used for the present study. Prior to removal, all sections were clamped shut with hemostats at both the proximal and distal ends to prevent leakage of gut contents.

The tissue was processed by removing a 0.5 cm section from each end (distal and proximal ileum), and these were then placed in cassettes for histological analysis. The tissue was fixed in 10% (v/v) formalin for 24 hours at which point they were rinsed with phosphate buffered saline (PBS) (pH 7.4), placed in 70% (v/v) ethanol and stored for later analysis. The remaining section was laterally opened and laid flat, mucosal side facing up. Using the edge of a glass microscope slide, the mucosal surface was scraped off and placed in small plastic screw top tubes, which were snap frozen in liquid nitrogen.

2.1.3 Colostrum and Plasma Analysis of IgG and BLG Concentrations

Colostrum aliquots were analyzed for total IgG and β-lactoglobulin concentrations by enzyme-linked immunoassay (ELISA) (Bethyl Laboratories, Montgomery, TX, USA). Affinity purified coating antibodies for bovine IgG or bovine BLG were diluted in coating buffer (0.05 M Sodium Carbonate-Bicarbonate, pH 9.6) and 100 µl was added to each well of a 96-well plate. The plate was incubated at room temperature for one hour, and wells were washed five times, using an automated plate washer with Tris-buffered saline (TBS) (50 mM Tris, 0.14 M NaCl, 0.05% (v/v) Tween 20, pH 8.0). This was used as the blocking, washing and sample buffer. After washing, 200 µl of blocking buffer was added to each well and the plate was incubated at room temperature for 30 minutes or overnight at 4°C. Samples were thawed on ice. Plasma

samples were centrifuged at 3000 x g at 4°C for 5 minutes. All samples were diluted based on the predicted concentration of the target analyte so as to fall within the range of the standards. Standard dilutions from 7.8 to 500 ng/ml for IgG and 1.95 to 125 ng/ml for BLG were prepared. The 0 (buffer only) standard was used as the blank and its absorbance measurement was subtracted from all the other standards and unknowns. Following vigorous mixing, 100 µl of each standard and sample was added to assigned wells and incubated at room temperature for one hour. The plate was then washed five times, 100 µl of horseradish peroxidase (HRP) conjugated bovine detection antibody and the plate was incubated at room temperature for an hour. The concentration of the IgG- and BLG-specific HRP antibodies was optimized (1:100,000 and 1:30,000 for IgG and BLG, respectively) to result in an optical density of 1.8-2.2 O.D. for the lowest dilution of standard. The plate was again washed five times, followed by the addition of 100 µl of the enzyme substrate, 3,3',5,5'-Tetramethylbenzidine (TMB) to each well. The plate was incubated at room temperature for 15 minutes in the dark. The enzymatic reaction develops as a blue color. After 15 minutes, 100 µl of stop solution (0.18 M H_2SO_4) was added, stopping the reaction and causing the solution to change from blue to yellow. Absorbance was measured on a plate reader (Molecular Devices LLC, Sunnyvale, CA, USA) at 450nm.

Apparent efficiency of absorption (AEA) was calculated with the following equation: ((Birth weight x Estimated blood volume x serum IgG)/Total IgG fed)x100 (Elizondo-Salazar and Heinrichs, 2009).

2.1.4 Colostrum and Plasma Total Protein

Total protein (TP) quantification of plasma was determined using a bicinchoninic acid (BCA) assay (Pierce Biotechnology, Rockford, IL, USA). The assay was performed using the microplate procedure according to manufacturer's directions. Briefly, standards were prepared using bovine serum albumin (BSA) in water to achieve final concentrations of 2000, 1500, 1000, 750, 500, 250, 125, 25 and 0 µg/ml. Plasma samples were diluted 1:100. The working reagent was prepared from Reagent A and B, supplied with the kit, at a 50:1 ratio. Twenty-five microliters of each standard and sample was

pipetted in duplicates into a 96-well microtiter plate and 200 µl of working reagent was added to each well using a multichannel pipette. The plate was incubated at 37°C for 30 minutes and absorbance was read at 562nm using a microplate reader (Molecular Devices LLC, Sunnyvale, CA, USA). The 0 standard was used as the blank and its absorbance measurement was subtracted from all the other standards and unknowns.

2.1.5 Colostrum Bacteria

Colostrum samples from each batch were analyzed for total bacterial plate count and for the levels of individual bacteria types in unheated and heat-treated colostrum. See Appendix I.

2.1.6 Gut Morphology Measurements

Gut samples destined for histology were stored in 70% (v/v) ethanol until processing. Ileum samples from 12 and 6-hour bull calves were embedded in a paraffin block. Sections, 4-5 µm thick, were cut, placed on glass slides and stained with hematoxylin and eosin. Villus height and crypt depth were analyzed using Axiovision software (Zeiss, Oberkochen, Germany), with measurements made by a single observer. Specific criteria were implemented for making consistent measurements. Figure 2.2 shows an example of a villi and a crypt measured by the following criteria: 1) Villi must have normal, recognizable leaf-, finger-, or tongue-like shape. 2) Villus body and tip must be present. 3) A visible crypt-villus junction must be present. Villus-crypt junctions are defined as forming a mouth of a pouch-like structure 4) Villus height will be measured starting at the tip and ending at the villus-crypt junction, and always down the center of the lamina propria. 5) Villi tips must have a recognizable lining of epithelial cells. 6) Crypts must be identifiable invaginations or pouch-like structures. 7) Crypt depth will be measured from the villus-crypt junction to the base of the crypt, including basement membrane of crypt cells, just above the layer of muscularis. To control for as much error as possible, at least 10 well-oriented villi and crypts were measured from sections of the ileum. Often many more than 10 were measured. Two sections of the

ileum- one from the distal end and one from the proximal end- were used in order to have a more representative sample. The mean height and depth were calculated. (Ross et al., 2009).

2.1.7 Goblet Cell Staining and Quantification

Slides were de-paraffinized in two changes of Xylene for 10 min each. The tissue was then rehydrated using the following sequence: 100% ethanol (2x10 minutes), 95% ethanol (1x10 minutes), 75% ethanol (1x10 minutes), 50% ethanol (1x10 minutes) and distilled water (dH2O) (1x10 minutes). Slides were then stained for 25 min in Alcian Blue (1% w/v Alcian blue in 3% v/v Acetic Acid, pH 2.5). Following a rinse in dH2O the slides were washed 2 times in dH2O for 5 minutes. They were then stained for 7 minutes in Nuclear Fast Red (0.1 % w/v Nuclear Fast Red in 5% (w/v) Aluminum Sulfate, boiled for 5 min and then filtered to remove solids) and again rinsed and washed in dH2O, 2 times for 5 minutes. Slides were dehydrated using the following sequence: 95% ethanol (1X5 minutes), 100% ethanol (2X5 minutes). Cover slips (#1.5) with Mowiol (a solid non-xylene mounting media) were then placed over the tissue.

Goblet cells were counted on at least 10 well-oriented villi in each sample, and the average number of goblet cells per animal was calculated. Only 12-hour bulls were used for goblet cell quantification. Goblet cells in the crypts were too numerous to accurately count.

2.1.8 Statistical analysis

Colostral concentrations of IgG, TP and BLG of each subsample were used to calculate the mean sample concentration in heat-treated and unheated colostrum for each batch (the experimental unit). Differences between colostrum measurements were analyzed by paired, one-tailed paired t-test. Blood observations were analyzed by two-way ANOVA (treatment by time) with repeated measures of time using the MIXED procedure of SAS 9.3 (SAS Institute Inc. 2012). Calves from both treatment groups

received colostrum (within each batch) from the same original pool; therefore batch was considered a random effect. The statistical model used for this analysis was:

$$Y_{ijk} = \mu + C_i + T_j + (CT)_{ij} + Batch_l + e_{ijk}$$

Where Y_{ijk} = dependent variables, μ = overall mean, C_i = fixed effect of colostrum treatment i, where i = heat-treated or unheated, T_j = repeated measure of time j, $(CT)_{ij}$ = effect of treatment by time interaction, $Batch_l$ = random effect of batch l, and e_{ijk} = residual. Birth weight and minutes to first feeding were offered as covariates. However neither of these characteristics was significant and they were subsequently removed from the final model. Intestinal morphology observations were analyzed by one-way ANOVA using the MIXED procedure of SAS. The treatment LS means for villi height, crypt depth and goblet cell numbers were compared for 6- and 12-hour bull calves (goblet cells were only analyzed at12 hours). Batch was considered a random effect.

2.2 Results

2.2.1 Colostral total IgG, β-lactoglobulin concentrations total protein, and bacterial counts

In this study, colostrum was heat-treated and analyzed for the effects of heat-treatment. Colostral IgG, BLG and total protein concentrations and colostral bacterial counts in heated colostrum were compared to unheated colostrum. Effects of heat-treatment are presented in Table 2.1. There was no statistical difference ($p>0.05$) in the mean colostral IgG concentration, however there was a tendency for the concentration to be lower in heat-treated colostrum. The IgG concentration in unheated and heat-treated colostrum was 74.49 mg/ml and 69.05 mg/ml, respectively. Heat-treatment of colostrum significantly decreased colostral BLG concentration by 9.5%. The BLG concentration in unheated colostrum was 11.2±1.6 mg/ml, whereas the concentration in heat-treated colostrum was 10.14±1.4 mg/ml. Total protein did not differ significantly with regards to colostrum treatment. The standard plate count, Figure 2.6, shows colostral bacteria levels before and after heat-treatment for each of the three batches. Numerically there was a

large decrease in total colostral bacteria after heat-treatment, but analysis revealed that statistically this decrease was not significant. As seen in the graph, batch 1 was low in total bacteria counts even before heat-treatment. Figure 2.7 shows the counts for individual bacterium in each of the three batches before and after colostrum treatment. The bacteria profiles differed between the batches, but in general batch 1 was low in most types. *Streptococcus* spp. (Figure 2.7I) was the most predominant bacteria type (before heat-treatment) followed by *Enterobacter* (D), *Escherichia coli* (C), *Pseudomonas* (G), *Acinetobacter* (A), *Staphylococcus* spp. (H), *Enterococcus* (E) and *Bacillus (B)*. A small number of *Micrococcus* (F) were present only in heat-treated colostrum and was presumed to be due to contamination after heat-treatment. As with the standard plate count, there was no significant difference between treatments.

2.2.2 Plasma IgG, BLG and total protein concentrations in 6-hour bull calves

The results for plasma IgG, TP and BLG concentrations are shown in Table 2.2. There was no difference found in plasma IgG or TP concentrations between calves receiving heat-treated or unheated colostrum at the 0 hour time point (pre-colostrum sample). Plasma BLG in the pre-colostrum sample was not detectable, and its concentration was presumed to be zero. The plasma IgG, BLG and TP concentrations at 3 and 6 hours were not statistically different between treatments in the 6-hour bull calves. The AEA for both IgG and BLG was not significantly different between treatment groups (see Table 2.3). Although there was no difference in treatment, IgG, TP and BLG all increased in plasma concentrations from 3 to 6 hours of life.

2.2.3 Plasma IgG, BLG and total protein concentrations in 12-hour bull calves

As with the 6-hour bulls, plasma concentrations of IgG and TP were the same for both treatment groups, and BLG was not detectable, prior to receiving colostrum. For IgG (see Figure 2.8A), no significant differences due to heat-treatment were found at 3 or 6 hours. At 9 hours, there was a tendency for IgG concentration to be lower in calves receiving heat-treated (12.37 ± 1.4 mg/ml) versus unheated (14.72 ± 1.4 mg/ml; $p=0.06$) colostrum. The IgG concentration in calves at 12 hours was significantly lower in calves

that were fed heat-treated (12.89 ± 1.4 mg/ml) versus unheated (15.83 ± 1.4 mg/ml; p=0.02) colostrum.

Plasma TP concentrations (shown in Figure 2.8B) were not statistically different between the two treatment groups at 3 or 12 hours after birth. The blood TP concentrations in calves fed unheated colostrum was higher compared to calves fed heat-treated colostrum at 6 hours (62.01 ± 1.6 versus 57.29 ±1.5 mg/ml; p=0.04) and 9 hours (68.16 ± 1.6 versus 62.35 ± 1.6 mg/ml; p=0.01).

For BLG (see Figure 2.8C), plasma concentrations were not significantly different at 3 hours after birth, however concentrations were significantly higher in calves that receiving unheated compared to heat-treated colostrum at 6 hours (324.34 ± 52 versus 239.90 ± 51.0 μg/ml; p=0.04) and 9 hours (258.23 ± 52.0 versus 126.32 ± 51.9 μg/ml; p=0.0023). By 12 hours there was no difference between the two treatment groups. Table 2.4 shows plasma concentrations of IgG, TP and BLG in bull calves in the first 12 hours of life.

The AEA (Table 2.5) for both IgG and BLG was not significantly different between treatment groups, except at 9 hours where the AEA for BLG was significantly lower in calves receiving heat-treated (2.3 ± .70 %) versus unheated (4.0 ± .67 %; p=0.02) colostrum.

The plasma IgG and TP concentrations increased from 3 to six hours. The concentrations began to level off after 6 hours, but remained above 10 mg/ml IgG and 60 mg/ml TP through 12 hours after birth, at which point they were euthanized for tissue collection. Interestingly, plasma BLG concentrations peaked by 6 hours, but after 6 hours the concentration decreased rapidly to levels comparable to what was observed at 3 hours.

2.2.4 Effect of Heat-Treatment on Intestinal Development of Ileum

Intestinal mucosal growth in the neonatal calf reflects the development of the gut from the fetal state to full functionality. Figure 2.3A shows the distinct layers of the ileum, including the mucosa, mucosal muscle (muscularis mucosae), submucosa, and the two layers of muscularis externa. The characteristic tongue-shaped villi, as seen in figure 2.3B, were clearly visible, and crypts formed pocket-shaped structures. Figure 2.3B also

shows common morphological structures such as the crypts, lamina propria, surface epithelial cells and aggregated lymph tissue (Peyer's patches). In order to determine the effect of heat-treated colostrum on development of the gut, villus height and crypt depth were measured in the ileum of both 6-hour and 12-hour bull calves. Morphometric results are shown in Table 2.6. Analysis revealed that 6- and 12-hour bulls that received heat-treated colostrum had numerically longer villi but the results were not statistically significant. There were no differences in crypt depth at either time point.

Villus height and crypt depth in 6 and 12-hour bulls were compared to measurements taken in calves that had been euthanized at birth in a previous experiment. Figure 2.4 shows an image from a 0-hour calf (A) compared to a 6-hour calf from the present study (B). The villi in the 0-hour samples were shorter, and the crypts appeared shallow. There were also a high number of goblet cells surrounding the villi. Table 2.7 shows the mean villus heights and crypt depths in the calves from the previous experiment compared to those in the present study. Average villus height in 0-hour bulls was approximately 486 um and did not grow significantly from 0 to 6 hours. From 6 to 12 hours villi grew significantly (566.84±29.6 to 677.85±25.4 μm; p=0.006). Crypts were about 113 um in 0-hour bulls and did not grow significantly from birth to 6 hours, but did significantly increase in depth from 6 to 12 hours (101.63 ± 4.3 to 182.2±6.2 μm; p=<.0001).

2.2.5 Effect of heat-treatment on goblet cell counts

The average goblet cell numbers in 12-hour bull calves are described in Table 2.6. Calves receiving heat-treated colostrum had significantly higher numbers of goblet cells on ileal villi as compared to calves fed unheated colostrum (63.42±3.3 versus 48.76±3.3, respectively; p=0.02). Figure 2.5 shows an example of a villus stained with Alcian Blue and Nuclear Fast Red. Goblet cells, which appear as bright blue dots, seemed to be clustered at the base of villi and were sparse further towards the tips.

2.3 Discussion

Pasteurization of colostrum is a common method used on dairy farms to reduce vertical transmission of pathogens to newborn calves (Godden, 2008). Early studies on the effects of heat-treating colostrum used processing methods common to pasteurization of mature milk. However, temperatures used for milk are not suitable for colostrum due to the negative effect on IgG concentrations. Godden et al. (2003) reported that pasteurization at 63ºC for 30 minutes in batches of 57 liters, reduced the IgG concentration by 23.6%, and increased its viscosity. They also found that larger batches (95 liters) of colostrum were more severely affected by heating; IgG concentration was reduced by 58.5%. Under laboratory conditions and using small volumes of colostrum (50 ml), another study demonstrated that colostrum could be heat-treated at 60ºC for up to 120 minutes without significantly decreasing IgG concentration or increasing viscosity (McMartin et al., 2006). These processing methods were also sufficient to significantly reduce bacterial load in the colostrum to undetectable levels (Godden et al., 2006).

In the present study, colostrum was pasteurized at 60ºC for 60 minutes, in batches of ~28 liters. The IgG concentration was not significantly affected by heat-treatment however there was a tendency for the concentration to be lower (by 7.5%) in heat-treated colostrum than unheated colostrum. In the study by Godden et al. (2003) the researchers found that colostrum with an IgG concentration > 75 mg/ml was more negatively affected by heat-treatment then colostrum with lower concentrations. In the colostrum that was fed to calves in the present study, the average IgG concentration prior to pasteurization was approximately 80 mg/ml, which may at least partially explain the decrease post-heating. However, this small decrease did result in an average IgG concentration of approximately 73.3 mg/ml, which is still well above the recommended dose for ensuring passive transfer of immunity in the newborn calf.

Although the effect of heat on IgG concentration in colostrum is vital to the survival and health of the newborn calf, other biologically important components may also be affected (Guimont et al., 1997; Pakkanen and Aalto, 1997). β-Lactoglobulin is the predominant whey protein in milk, and is found at concentrations four times higher in bovine colostrum compared to mature milk (Perez et al., 1990), with a range of 7.9 to 30

mg/ml in the first milking (Levieux and Ollier, 1999). In the current study, the average BLG concentration in colostrum (prior to heat-treatment) was 12.6 mg/ml. Upon pasteurization, the concentration dropped by about 9.5%. Previous work has shown that BLG is heat-labile when exposed to the conditions common to processing milk for human consumption, which uses temperatures of above 70°C for at least 15 seconds (Guimont et al., 1997), but little is known about the effects at lower temperatures. Some research has shown that during pasteurization, BLG has the ability to bind to other proteins, such as casein and membrane proteins of fat globules (Guimont et al., 1997). Heat-induced changes, such as the formation of disulfide bonds between BLG and casein, have been demonstrated (Hae and Swaisgood, 1990). It has been suggested that the temperatures required for denaturation of BLG, and the formation of protein aggregates, are greater than 65ºC (Parris et al., 1991). Results from the current study demonstrate that even when colostrum is pasteurized at 60ºC (considered to be acceptable for IgG) significant decreases in BLG concentration were observed, suggesting that the protein undergoes denaturation or binds to other molecules in colostrum.

The profile of bacteria found in colostrum suggests that there is a large diversity in the microbial population of colostrum collected in a commercial dairy system Elizondo-Salazar and Heinrichs, 2009; Johnson et al., 2007). Numerically, there was a decrease in total colostral bacteria. It was expected that heat-treatment would significantly decrease the number of bacteria in the colostrum. However, analysis revealed that statistically this decrease was not significant. The reason for this appears to be due to a combination of contamination of the heat-treated colostrum, as well as a significant batch effect. The first batch of colostrum collected was inherently low in bacteria before heat-treatment, and thus contributed to the large variation between colostrum samples. A larger sample size may have helped to reduce some of the variation and increase the power. Some bacteria appeared to have survived the heat-treatment, an effect that may lead to different microbial profiles being fed to calves. Since colostrum is one of the first substances to be ingested by the neonate (after amniotic fluid and environmental materials in the calving areas) different microbial populations may have an influence on the calf's gut microbiota (Wagner et al., 2008). Due to these results, inferences about the effect of bacteria on gut immune development in the present study

should be made with caution. These results also emphasize the point that even under controlled circumstances, bacterial contamination does occur and care should be taken when processing colostrum.

In this study, bull calves were fed either heat-treated or unheated colostrum, and plasma IgG and BLG levels were measured. In the first 6 hours of life, no differences were detected in plasma IgG concentrations between treatment groups. At 9 hours there was a tendency for the plasma IgG concentration to be lower in calves that received heat-treated colostrum, and at 12 hours this effect was statistically significant. These results are contrary to previous research (Elizondo-Salazar and Heinrichs, 2009; Johnson et al., 2007), which showed that calves that received heat-treated colostrum had significantly higher circulating IgG levels. One explanation for this discrepancy may be due to the use of bull calves in the present study and the use of heifer calves in the previous work. There is some evidence that bull calves absorb IgG less efficiently compared to heifers (Quigley, 2002) compared to heifer calves. In the present study, there was no difference in birth weight between the males and females. The reason for the difference in plasma IgG concentration as it relates to heat-treated colostrum is unknown. Some research suggests that there may be interactions between various compounds (e.g. growth factors, immunoglobulins) in colostrum and hormones such as gonadotropin-releasing hormone, luteinizing releasing hormone and glucocorticoids (Shing et al., 2009). Changes in heat-treated colostrum may lead to different interactions among a different panel of hormones in bull calves, than that seen in heifer calves, and thus promoted the increased uptake of IgG at 9 and 12 hours after birth. However, there is little evidence to support this hypothesis in neonates. In addition, the present study did not measure hormone levels in calves. For more on gender-related differences, see chapter 3.

In addition to IgG, BLG is thought to carry out biologically active functions in the neonate, including effects on the immune system, gut development and absorption of hydrophobic molecules (Chatterton et al., 2013; Prioult et al., 2004). Although no difference was observed at 3 hours, plasma concentrations of BLG were significantly lower in calves that had been fed heat-treated colostrum, at 6 and 9 hours. The difference quickly disappeared by 12 hours. The AEA of BLG was the same for both treatment groups at all time points except at 9 hours, when it was significantly lower in the heat-

treated group. At 9 hours, calves from the heat-treated group had 2.3% AEA, whereas calves in the unheated colostrum group remained at around 4%. Since AEA is a calculated measurement of the percentage of ingested component (BLG in this case) that is actually absorbed by the calf, these results suggest that even though heat-treated colostrum had a 9% lower BLG concentration, absorption was equally as efficient in both treatment groups for the first 6 hours. The reason for the difference demonstrated at 9 hours is not completely understood. β-Lactoglobulin is known to bind with casein, especially as a result of pasteurization (Parris et al., 1991). Studies have shown that the curd, which is formed in the abomasum of the calf, mainly consists of the casein fraction of milk (Longenbach and Heinrichs, 1998). Curd formation results in the casein fraction remaining longer in the abomasum, while the whey fraction passes quickly into the small intestine. One explanation for the difference in AEA at 9 hours is that by this time, much of the remaining BLG was bound to casein and therefore was less efficiently absorbed. If this were the case, it would suggest that the BLG in unheated colostrum remained in the whey fraction and was thus more available for absorption. β-Lactoglobulin bound to casein may also have impaired its detection by ELISA. Furthermore, the more time spent in the abomasum will increase gastric digestion of the proteins, potentially damaging antibody binding sites. Other molecules are also known to bind with BLG, and many of these could also interfere or influence the rate of absorption.

Along with the absorptive efficiency, BLG also appears to have a much shorter biological half-life compared to colostral IgG, which continues to circulate in pre-weaned calves for several weeks after ingestion of colostrum (Chase et al., 2008). In comparison, the results of the present study shows that BLG peaks in plasma concentration by 6 hours of life, and then rapidly decrease until it is close to zero by 24 hours after birth. This suggests that components of colostrum other than IgG have different biological functions and may have different mechanisms for uptake from the gut.

In order to assess the effects of heat-treatment of colostrum on neonatal gut development, two measurements were made: mucosal growth and number of goblet cells. Three bull calves from a separate experiment were used as a baseline for the 6 and 12-hour bulls in the current study. These three were euthanized for tissue sample collection immediately after birth. Although no significant growth was seen from birth to 6 hours,

analysis showed that villi and crypts increased in length and depth, respectively, from 6 hours to 12 hours, and that the effect was highly significant. This is in agreement with previous research that suggested that there is rapid mucosal growth in the first several hours after birth (Xu, 1996).

There were no differences of villus height and crypt depth observed at either time point with regards to treatment groups. It is possible that due to the small number of animals used for histological analysis that the effect was not detected, and with a higher sample size, the increase in power would show a significant difference. But in the present study, a treatment effect was not observed. It is also possible that the time of tissue collection was too early in life to see a significant effect.

Previous research in piglets has studied goblet cell numbers, especially around the time of weaning, and has suggested that goblet cells decrease in number at the time of weaning, thus decreasing the production of mucin in the gut (Dunsford et al., 1991; McCracken et al., 1995). Goblet cell number and mucin production may be stimulated by components in feedstuffs or bacteria (Deplancke and Gaskins, 2001). In the present study, calves that were 12-hours old had numerous goblets cells in the ileum. Observations revealed that there were large clusters of goblet cells at the base of the villi and in the crypt region (see Figure 2.5). Fewer goblet cells were seen on the villus tips, especially on longer villi. An average of 14 more goblet cells per villi was observed in calves that had received heat-treated colostrum. The explanation for these results is unknown, however previous work has shown that mucin and goblet cell production are stimulated by components in milk or other foodstuffs (McCracken et al., 1995). Many colostral bioactives are known to interact with factors in the GIT, contributing to an altered environment in the gut. Heat-treatment of colostrum may affect bioactives and influence this environment. For example, many proteins and peptides found in milk and colostrum, such as β-lactoglobulin among others, may be susceptible to heat damage (Guimont et al., 1997). Ingestion of these damaged components may induce or impair functions such as goblet cell and mucin production. Future research should focus on the roles of these bioactives in altering the developing gut and immune system.

2.4 Summary and Conclusions

Results from the current study revealed that heat-treatment of colostrum at 60°C for 60 minutes does not significantly affect colostral IgG concentration, but does significantly decrease BLG concentration. Plasma IgG concentrations were lower in calves receiving heat-treated colostrum, but the AEA did not differ. Plasma BLG concentrations were only significantly different at 9 hours, and the AEA of BLG was lower in calves that were fed heat-treated colostrum at this time. Mucosal growth was not affected by treatment, however the number of goblet cells differed significantly. This experiment indicates that absorption of colostral protein can be altered by consumption of heat-treated colostrum. Villus height and crypt depth do not appear to be affected significantly by heat-treated colostrum, but does increase goblet cells numbers.

Table 2.1: Colostral IgG, total protein and β-lactoglobulin levels

	Treatment Group		
Components	Unheated	Heat-Treated	P-value
lgG, mg/mL	74.49±7.9	69.05±8.6	0.06
TP[1], mg/mL	186.3±14.	177±10.8	0.21
BLG[2], mg/mL	11.2±1.6	10.14±1.4	0.01

$P < 0.05$
[1]Total Protein
[2]β-Lactoglobulin

Table 2.2: Plasma IgG (mg/mL), TP (mg/mL) and BLG (µg/mL) concentrations in the first 6 hours of life in bull calves receiving unheated or heat-treated colostrum

	IgG			TP			BLG		
Age (Hr)	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value
0	0.02±0.74	0.02±0.7	0.84	49.0±1.9	46.96±1.8	0.43	ND	ND	ND
3	0.05±0.74	0.04±0.7	0.84	48.87±1.9	49.81±1.8	0.72	75.63±42.1	81.38±47.1	0.93
6	9.66±0.74	9.65±0.7	0.83	57.66±1.9	58.82±1.8	0.65	327.30±42.1	274.20±47.1	0.38

P < 0.05, comparing treatment LS means at each time point
ND = Not Detected

Table 2.3: Apparent Efficiency of Absorption (AEA, %)[1] in the first 6-hours of life for IgG and BLG in bull calves receiving unheated or heat-treated colostrum

	AEA of IgG			AEA of BLG		
Age (Hr)	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value
0	0.06±2.0	0.05±1.65	0.99	ND	ND	ND
3	0.15±2.0	0.12±1.65	0.99	1.18±1.06	1.66±1.06	0.76
6	26.02±2.0	28.31±1.65	0.39	5.43±1.06	5.83±0.95	0.78

P < 0.05, comparing treatment LS means at each time point
[1]AEA = ((birth weight × 0.095 × serum IgG)/total IgG or BLG fed) × 100
ND = Not Detected

Table 2.4: Plasma IgG (mg/mL), TP (mg/mL) and BLG (µg/mL) concentrations in the first 12 hours of life in bull calves receiving unheated or heat-treated colostrum

	IgG			TP			BLG		
Age (Hr)	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value
0	0.43±1.4	0.75±1.34	0.79	47.25±1.62	47.12±1.53	0.95	ND	ND	ND
3	1.61±1.4	0.80±1.46	0.55	48.22±1.73	48.42±1.62	0.93	110.76±53.1	96.74±53.1	0.75
6	10.30±1.37	8.47±1.34	0.13	62.01±1.62	57.29±1.53	0.04	324.34±52	239.90±51	0.04
9	14.72±1.37	12.37±1.37	0.06	68.16±1.62	62.35±1.62	0.01	258.23±52	126.32±51.9	0.0023
12	15.83±1.37	12.89±1.37	0.02	68.06±1.62	68.88±1.53	0.71	114.65±52	98.24±52	0.7

$P < 0.05$, comparing treatment LS means at each time point
ND = Not Detected

Table 2.5: Apparent Efficiency of Absorption (AEA, %)[1] in the first 12-hours of life for IgG and BLG in bull calves receiving unheated or heat-treated colostrum

	AEA of IgG			AEA of BLG		
Age (Hr)	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value
0	1.41±2.4	1.61±2.2	0.95	ND	ND	ND
3	4.54±2.4	2.40±2.8	0.55	1.64±0.7	1.83±0.72	0.79
6	25.11±2.25	24.53±2.23	0.85	4.94±0.67	4.26±0.67	0.31
9	35.49±2.25	35.35±2.4	0.97	4.0±0.67	2.30±0.7	0.02
12	38.23±2.25	38.17±2.4	0.99	1.81±0.67	1.48±0.7	0.63

P < 0.05, comparing treatment LS means at each time point
[1]AEA = ((birth weight × 0.095 × serum IgG)/total IgG fed) × 100
ND = Not Detected

Table 2.6: Ileum villi height (μm), crypt depth (μm) and goblet cell count[1] in 6 and 12-hour old bull calves

	Villi Height			Crypt Depth			Goblet Cell Count		
Age (Hr)	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value
6	551.97±28.1	581.72±28.1	0.48	101.04±6.3	104.44±6.4	0.38	ND	ND	ND
12	639.1±55.8	695.4±54.2	0.37	176.16±12.3	188.23±12.3	0.51	48.76±3.3	63.42±3.3	0.02

P < 0.05, comparing treatment LS means
[1]Average number of goblet cell was calculated for each calf. Only 12-hour bulls were used for this analysis
ND = Not Determined

Table 2.7: Growth of ileum villus height (μm) and crypt depth (μm) in the first 12 hours after birth

Age (Hr)	Villus Height	Crypt Depth
0	486.43±54.1[a]	113.53±7.8[a]
6	566.84±29.6[a]	101.63±4.3[a]
12	677.85±25.4[b]	182.2±6.2[b]

P < 0.05, comparing treatment LS means within each column

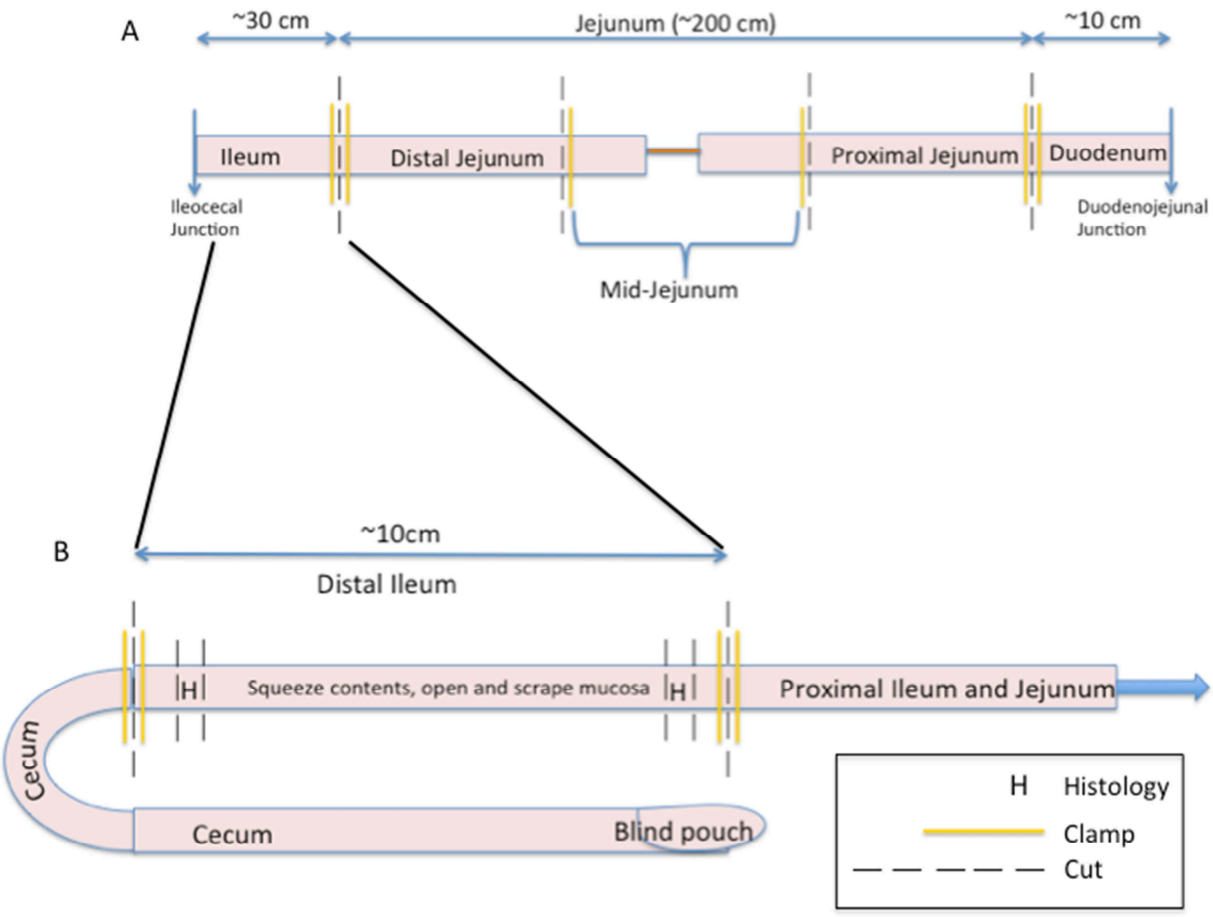

Figure 2.1: Schematic showing the anatomical sections of the small intestine (A) and specific dissection of the distal ileum for tissue sample collection (B). Only the ileum was used for the present study, but other sections were collected for later use.

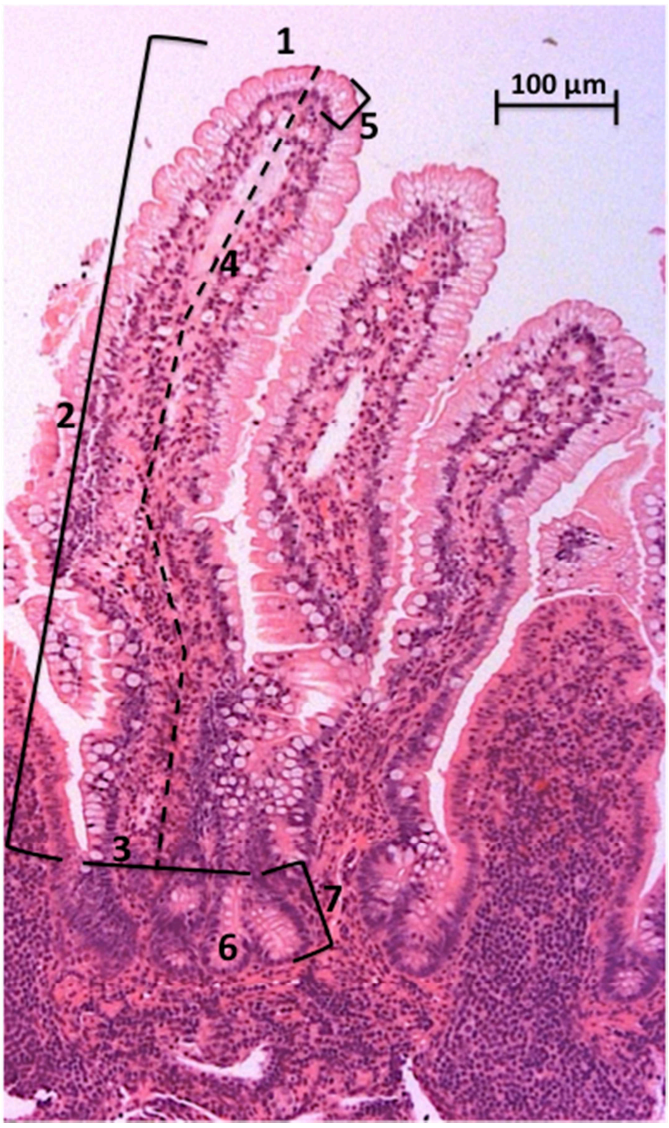

Figure 2.2: Criteria for measuring villi and crypts include: 1) Leaf-, finger-, or tongue-like shape of villi. 2) A recognizable villus body and tip. 3) A villus-crypt junction. 4) The villus measurement starting at the tip and ending at the villus-crypt junction, and passing through the center of the lamina propria (indicated with a dashed line). 5) Epithelial cells lining the tip. 6) Pouch-like crypt structure. 7) The crypt measurement starting at the villus-crypt junction and ending at the crypt basement membrane. Slide stained with H&E, imaged at 10X (Ross et al., 2009).

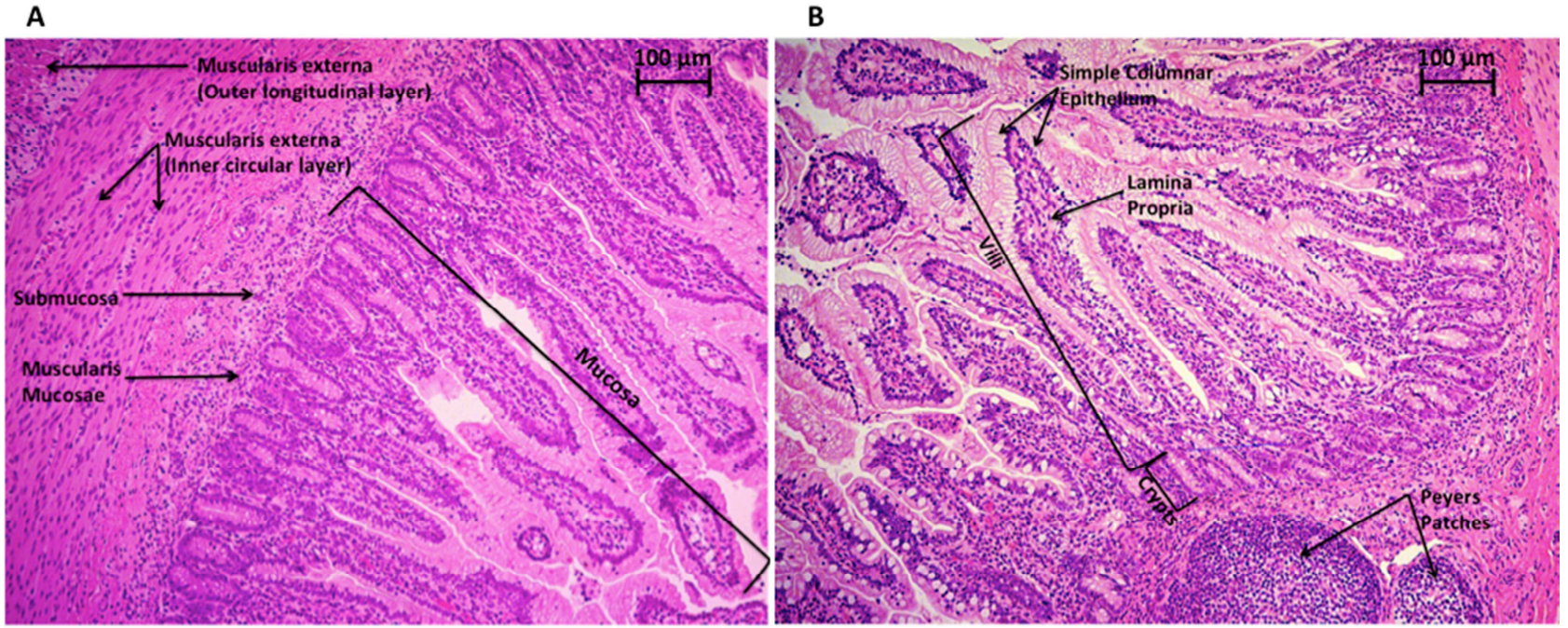

Figure 2.3: Layers of the small intestine (A) and common features of the ileum (B) in neonatal calves (≤ 12 hours old). Slides are H&E stained and imaged at 10X.

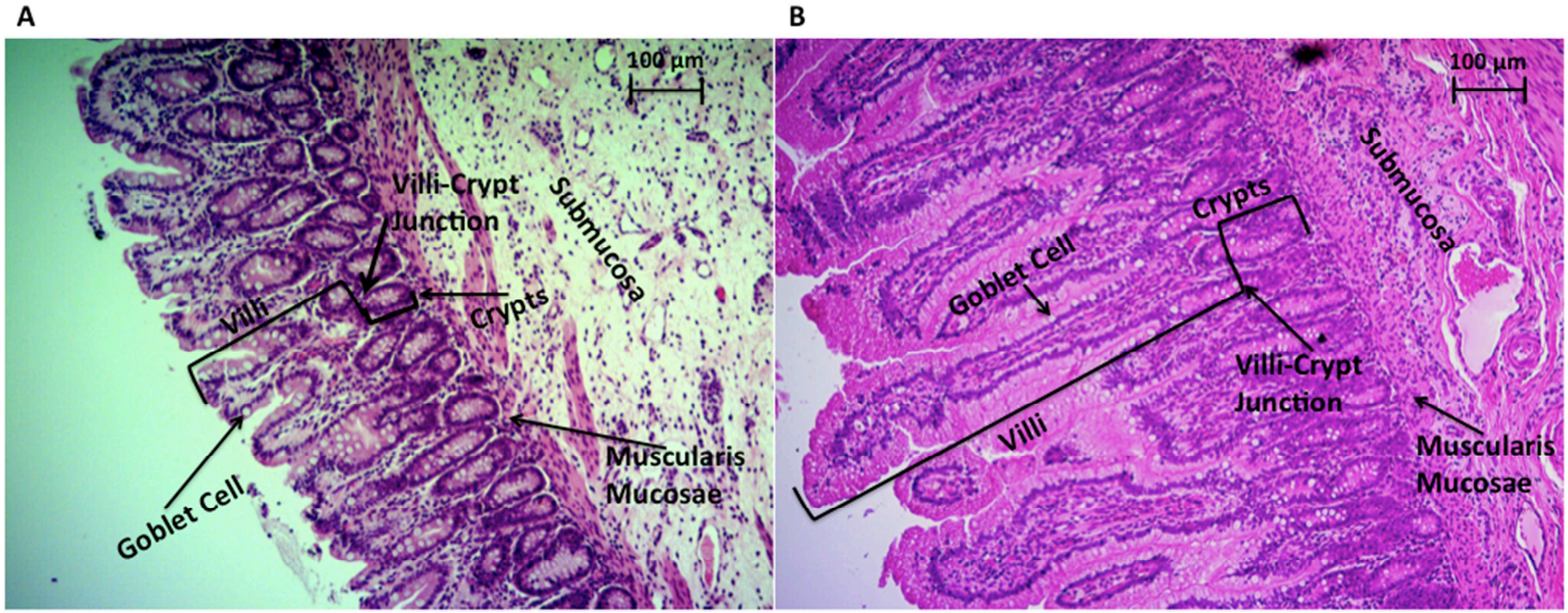

Figure 2.4: Example of ileum in a 0-hour calf, euthanized immediately after birth and prior to colostrum (A) compared to a treated calf at 6 hours after birth (B). Slides stained with H&E, imaged at 10X.

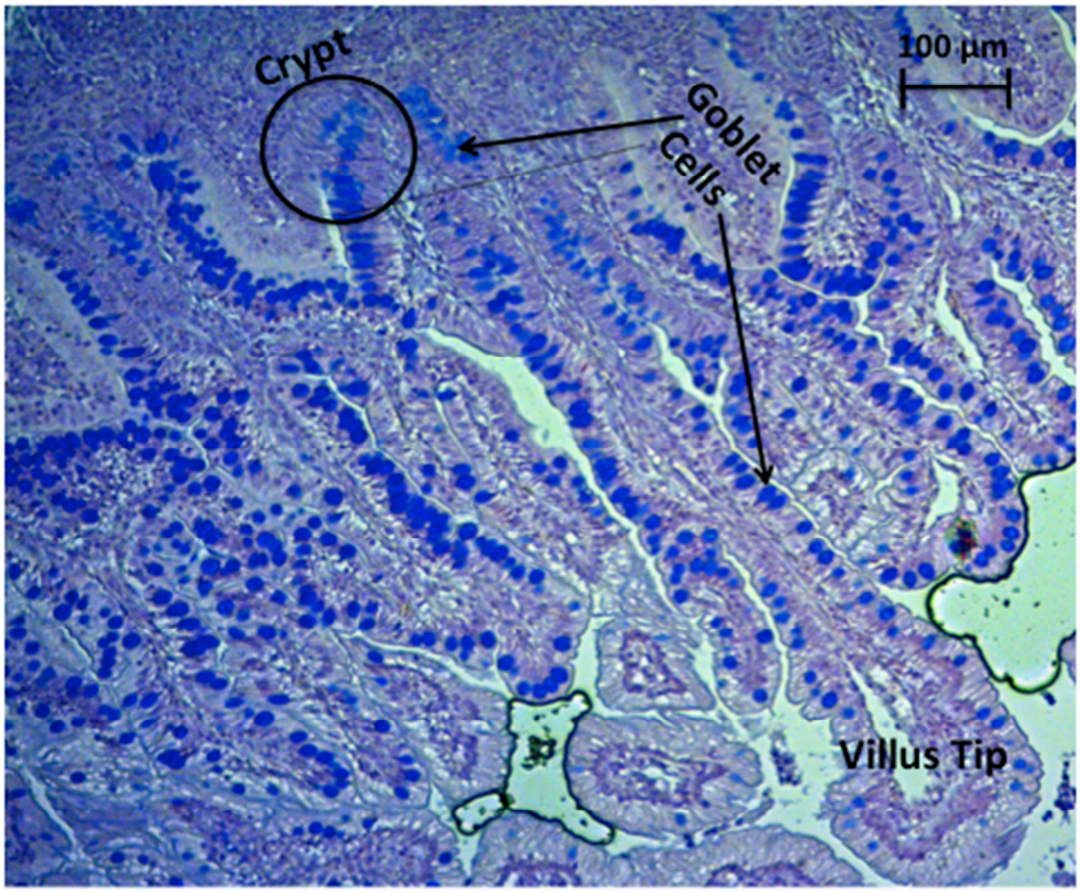

Figure 2.5: Villi stained with Alcian Blue and Nuclear Fast Red to show mucin within goblet cells (Slides imaged at 10X). Note the clustering of goblet cells in the crypts and towards the base of the villi.

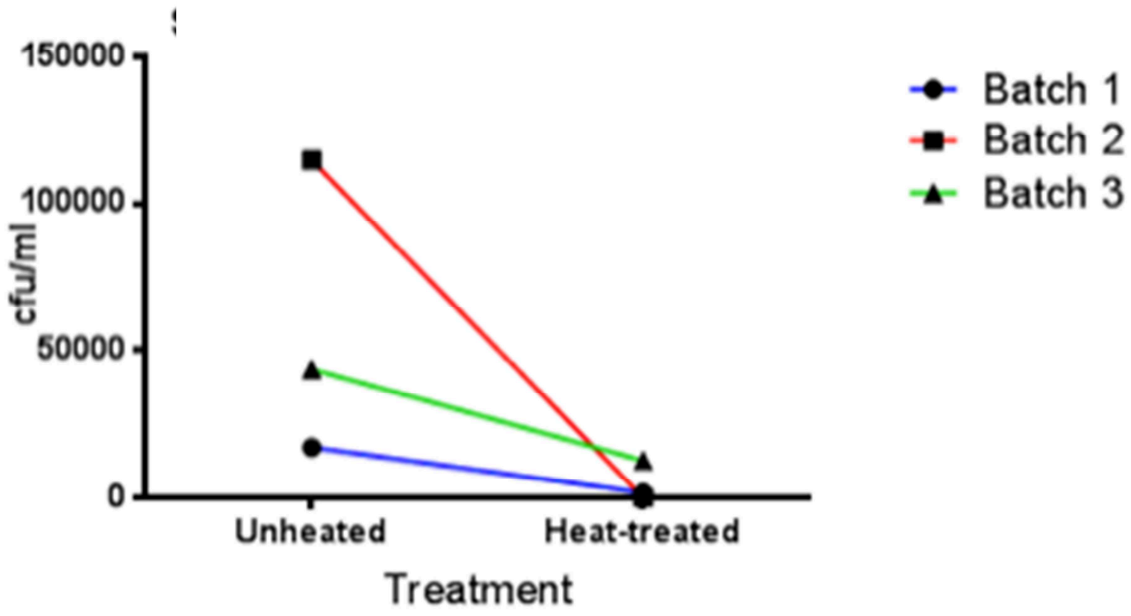

Figure 2.6: Total bacterial counts in unheated or heat-treated colostrum.

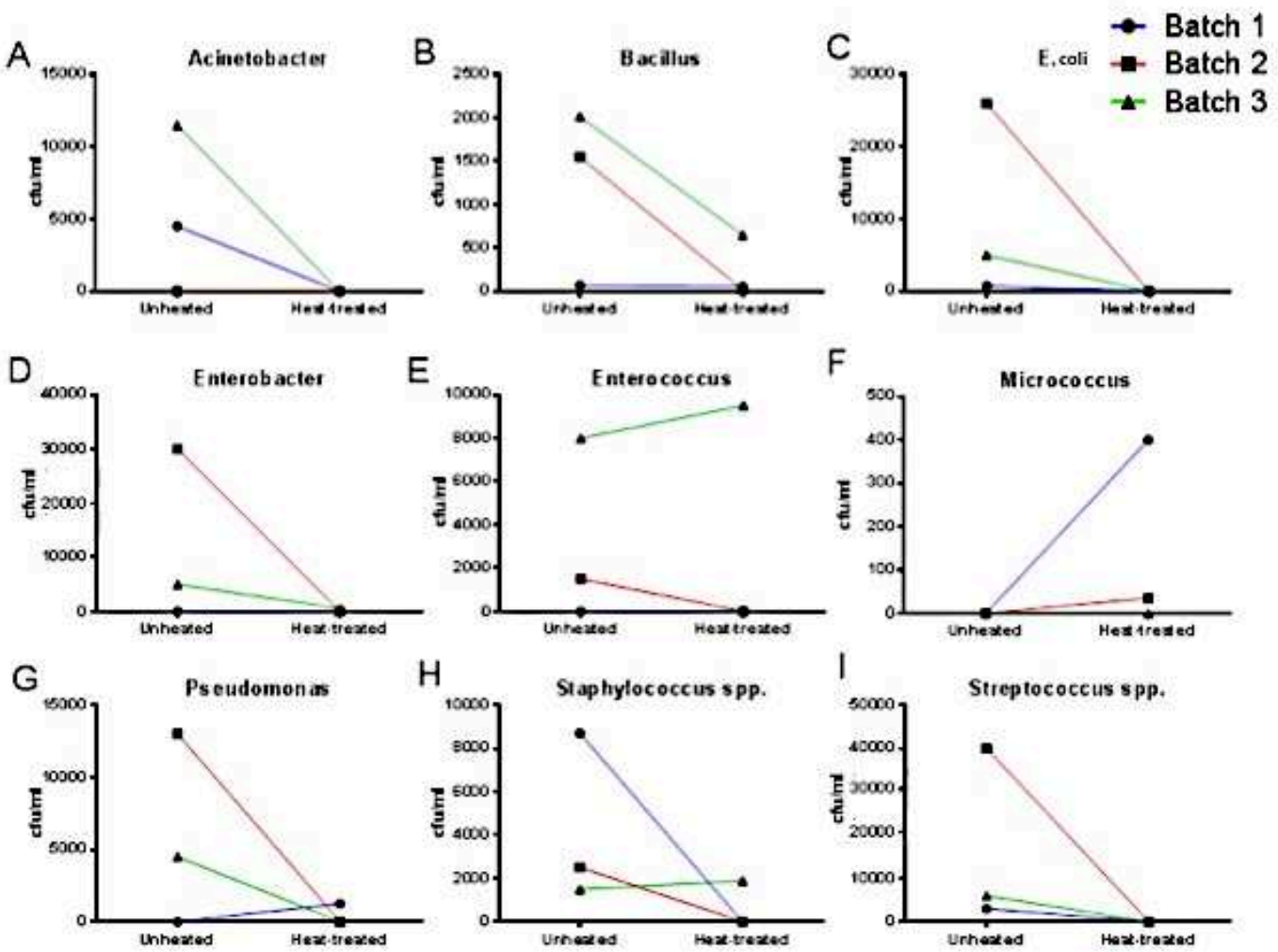

Figure 2.7: Levels of individual bacteria types in unheated and heat-treated colostrum.

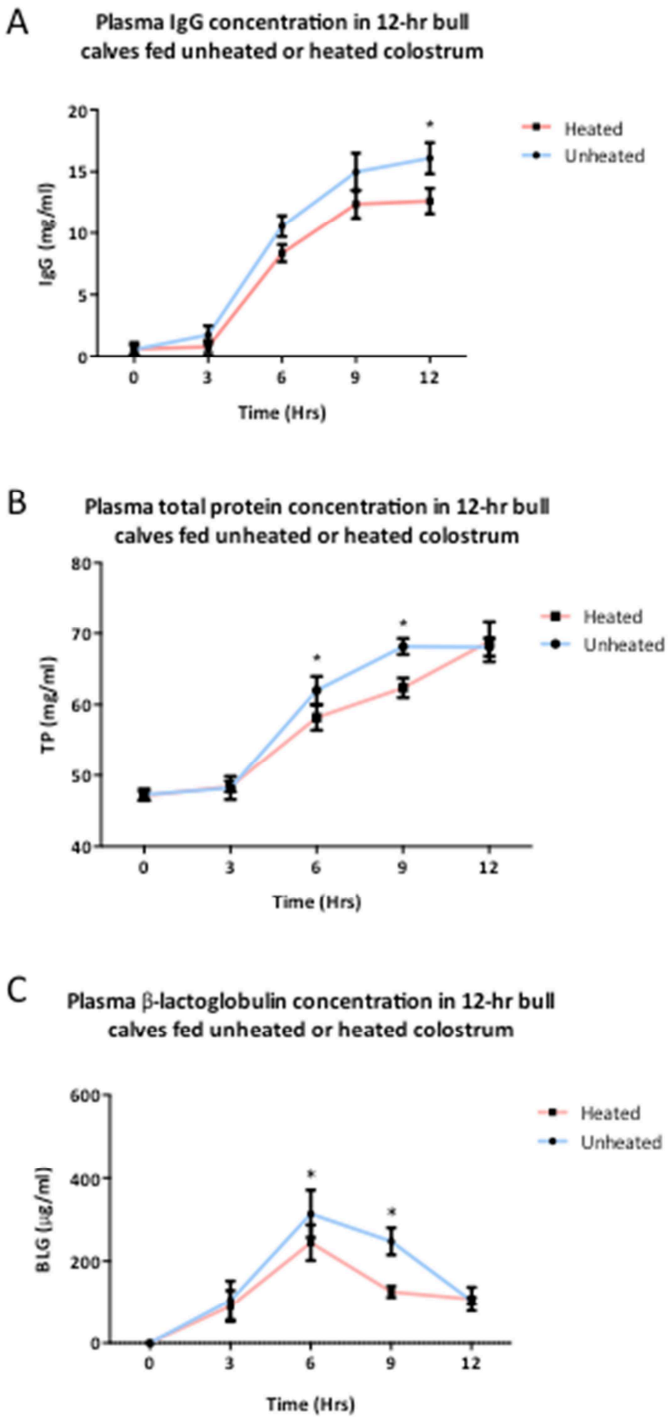

Figure 2.8: Plasma IgG (A), TP (B) and BLG (C) concentration in 12-hour bull calves receiving unheated or heat-treated colostrum ($P < 0.05$). Asterisks indicate significant differences.

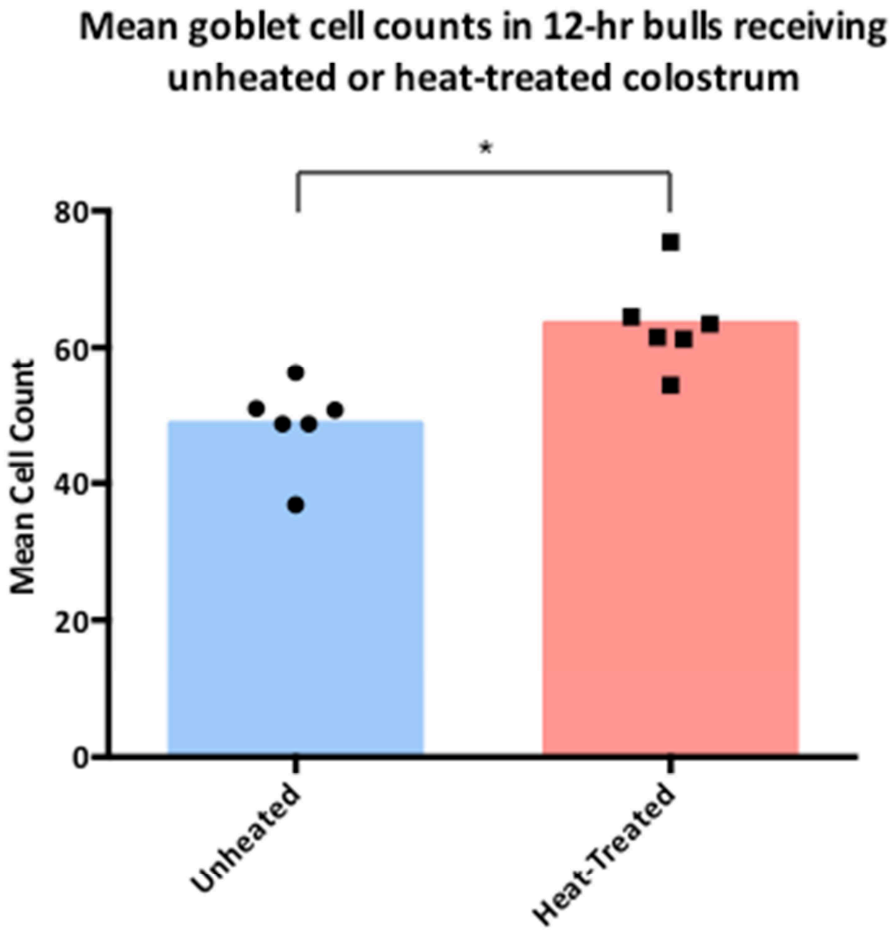

Figure 2.9: Mean goblet cell numbers in 12-hour bull calves receiving unheated (circles indicate standard error) or heat-treated (squares indicate standard error) colostrum ($P < 0.05$). Asterisk indicates significant difference.

2.5 References:

Blomhoff, R., and H. K. Blomhoff. 2006. Overview of retinoid metabolism and function. Journal of neurobiology 66: 606-630.

Blum, J. W., and H. Hammon. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livestock Production Science 66: 151-159.

Burrin, D. G., Y. Petersen, B. Stoll, and P. Sangild. 2001. Glucagon-like peptide 2: a nutrient-responsive gut growth factor. The Journal of nutrition 131: 709-712.

Chase, C. C., D. J. Hurley, and A. J. Reber. 2008. Neonatal immune development in the calf and its impact on vaccine response. The Veterinary clinics of North America. Food animal practice 24: 87-104.

Chatterton, D. E., D. N. Nguyen, S. B. Bering, and P. T. Sangild. 2013. Anti-inflammatory mechanisms of bioactive milk proteins in the intestine of newborns. The international journal of biochemistry & cell biology 45: 1730-1747.

Deplancke, B., and H. R. Gaskins. 2001. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. Am J Clin Nutr 73: 1131S-1141S.

Donovan, D. C., A.J. Reber, J.D. Gabbard, M. Aceves-Avila, K.L. Galland, K.A. Holbert, L.O. Ely, D.J. Hurley. 2007. Effect of maternal cells transferred with colostrum on cellular responses to pathogen antigens in neonatal calves. American journal of veterinary research 68: 778-782.

Dubé, P. E., and P. L. Brubaker. 2007. Frontiers in glucagon-like peptide-2: multiple actions, multiple mediators. American Journal of Physiology - Endocrinology And Metabolism 293: E460-E465.

Dunsford, B. R., W. E. Haensly, and D. A. Knabe. 1991. Effects of diet on acidic and neutral goblet cell populations in the small intestine of early weaned pigs. American journal of veterinary research 52: 1743-1746.

Elizondo-Salazar, J. A., and A. J. Heinrichs. 2009. Feeding heat-treated colostrum to neonatal dairy heifers: Effects on growth characteristics and blood parameters. Journal of dairy science 92: 3265-3273.

Enders, A. C., and A. M. Carter. 2004. What can comparative studies of placental structure tell us?--A review. Placenta 25 Suppl A: S3-9.

Ganessunker, D., H. R. Gaskins, F. A. Zuckermann, and S. M. Donovan. 1999. Total parenteral nutrition alters molecular and cellular indices of intestinal inflammation in neonatal piglets. JPEN. Journal of parenteral and enteral nutrition 23: 337-344.

Godden, S. 2008. Colostrum management for dairy calves. The Veterinary clinics of North America. Food animal practice 24: 19-39.

Godden, S., S. McMartin, J. Feirtag, J. Stabel, R. Bey, S. Goyal, L. Metzger, J. Fetrow, S. Wells, and H. Chester-Jones. 2006. Heat-Treatment of Bovine Colostrum. II: Effects of Heating Duration on Pathogen Viability and Immunoglobulin G. Journal of dairy science 89: 3476-3483.

Godden, S. M. S. Smith, J. M. Feirtag, L. R. Green, S. J. Wells, and J. P. Fetrow. 2003. Effect of on-farm commercial batch pasteurization of colostrum on colostrum and serum immunoglobulin concentrations in dairy calves. Journal of dairy science 86: 1503-1512.

Guimont, C., E. Marchall, J. M. Girardet, G. Linden, and H. Otani. 1997. Biologically active factors in bovine milk and dairy byproducts: Influence on cell culture. Critical Reviews in Food Science and Nutrition 37: 393-410.

Hae, D. J., and H. E. Swaisgood. 1990. Disulfide Bond Formation between Thermally Denatured Beta-Lactoglobulin and Kappa-Casein in Casein Micelles. Journal of dairy science 73: 900-904.

Izumi, H., S. Ishizuka , A. Inafune, T. Hira, K. Ozawa, T. Shimizu, M. Takase, H. Hara. 2009. α-Lactalbumin Hydrolysate Stimulates Glucagon-Like Peptide-2 Secretion and Small Intestinal Growth in Suckling Rats. The Journal of nutrition 139: 1322-1327.

Johnson, J. L., S. M. Godden, T. Molitor, T. Ames, and D. Hagman. 2007. Effects of Feeding Heat-Treated Colostrum on Passive Transfer of Immune and Nutritional Parameters in Neonatal Dairy Calves. Journal of dairy science 90: 5189-5198.

Lakritz, J., J.W. Tyler, D.E. Hostetler, A.E. Marsh, D.M Weaver, J.M. Holle, B.J. Steevens, J.L. Denbigh. 2000. Effects of pasteurization of colostrum on

subsequent serum lactoferrin concentration and neutrophil superoxide production in calves. American journal of veterinary research 61: 1021-1025.

Lecce, J. G., and D. O. Morgan. 1962. Effect of Dietary Regimen on Cessation of Intestinal Absorption of Large Molecules (Closure) in the Neonatal Pig and Lamb. The Journal of nutrition 78: 263-268.

Levieux, D., and A. Ollier. 1999. Bovine immunoglobulin G, β-lactoglobulin, α-lactalbumin and serum albumin in colostrum and milk during the early post partum period. Journal of Dairy Research 66: 421-430.

Longenbach, J. I., and A. J. Heinrichs. 1998. A review of the importance and physiological role of curd formation in the abomasum of young calves. Animal Feed Science and Technology 73: 85-97.

Madureira, A. R., C. I. Pereira, A. M. P. Gomes, M. E. Pintado, and F. X. Malcata. 2007. Bovine whey proteins - Overview on their main biological properties. Food Res Int 40: 1197-1211.

McCracken, B. A., H. R. Gaskins, P. J. Ruwe-Kaiser, K. C. Klasing, and D. E. Jewell. 1995. Diet-dependent and diet-independent metabolic responses underlie growth stasis of pigs at weaning. The Journal of nutrition 125: 2838-2845.

McMartin, S., S. Godden, L. Metzger, J. Feirtag, R. Bey, J. Stabel, S. Goyal, J. Fetrow, S. Wells, and H. Chester-Jones. 2006. Heat Treatment of Bovine Colostrum. I: Effects of Temperature on Viscosity and Immunoglobulin G Level. Journal of dairy science 89: 2110-2118.

Molenaar, A. J., Y. M. Kuys, S. R. Davis, R. J. Wilkins, P. E. Mead, And J. W. Tweed. 1996. Elevation of lactoferrin gene expression in developing, ductal, resting, and regressing parenchymal epithelium of the ruminant mammary gland. Journal of dairy science 79: 1198-1208.

Pakkanen, R., and J. Aalto. 1997. Growth factors and antimicrobial factors of bovine colostrum. International dairy journal / published in association with the International Dairy Federation 7: 285-297.

Parris, N., J. M. Purcell, and S. M. Ptashkin. 1991. Thermal-Denaturation of Whey Proteins in Skim Milk. J Agr Food Chem 39: 2167-2170.

Perez, M. D., L. Sanchez, P. Aranda, J.M. Ena, R. Oria, M. Calvo. 1990. Synthesis and evolution of concentration of β-lactoglobulin and α-lactalbumin from cow and sheep colostrum and milk throughout early lactation. Cellular and Molecular Biology 36: 205-212.

Prioult, G., S. Pecquet, and I. Fliss. 2004. Stimulation of interleukin-10 production by acidic beta-lactoglobulin-derived peptides hydrolyzed with Lactobacillus paracasei NCC2461 peptidases. Clinical and diagnostic laboratory immunology 11: 266-271.

Quigley, J. 2002. Passive immunity in newborn calves. Adv Dairy Technol 14: 273-292.

Reber, A. J., D.C. Donovan, J. Gabbard, K. Galland, M. Aceves-Avila, K.A. Holbert, L. Marshall, D.J. Hurley. 2008. Transfer of maternal colostral leukocytes promotes development of the neonatal immune system Part II. Effects on neonatal lymphocytes. Veterinary immunology and immunopathology 123: 305-313.

Ross, M. H., W. Pawlina, and T. A. Barnash. 2009. Atlas of descriptive histology. Sinauer Associates, Sunderland, Mass.

Sangild, P. T., K.A. Tappenden, C. Malo, Y.M. Petersen, J. Elnif, A.L. Bartholome, R.K. Buddington. 2006. Glucagon-like peptide 2 stimulates intestinal nutrient absorption in parenterally fed newborn pigs. Journal of pediatric gastroenterology and nutrition 43: 160-167.

Shing, C. M., D. C. Hunter, and L. M. Stevenson. 2009. Bovine colostrum supplementation and exercise performance: potential mechanisms. Sports medicine 39: 1033-1054.

Sutton, L. F., and B. Alston-Mills. 2006. β-lactoglobulin as a potential modulator of intestinal activity and morphology in neonatal piglets. The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology 288A: 601-608.

Taylor-Edwards, C. C., D. G. Burrin, J. J. Holst, K. R. McLeod, and D. L. Harmon. 2011. Glucagon-like peptide-2 (GLP-2) increases small intestinal blood flow and mucosal growth in ruminating calves. Journal of dairy science 94: 888-898.

Tyler, J. W., D. D. Hancock, J. G. Thorne, C. C. Gay, and J. M. Gay. 1999. Partitioning the mortality risk associated with inadequate passive transfer of colostral

immunoglobulins in dairy calves. Journal of veterinary internal medicine / American College of Veterinary Internal Medicine 13: 335-337.

Wagner, C. L., S. N. Taylor, and D. Johnson. 2008. Host factors in amniotic fluid and breast milk that contribute to gut maturation. Clinical reviews in allergy & immunology 34: 191-204.

Wong, K. F., N. Middleton, M. Montgomery, M. Dey, and R. I. Carr. 1998. Immunostimulation of murine spleen cells by materials associated with bovine milk protein fractions. Journal of dairy science 81: 1825-1832.

Xu, R. 1996. Development of newborn GI tract and its relation to colostrum/milk uptake a review. Reprod. Fertil. Dev. 8: 35-48.

Chapter 3. Effects of heat-treatment of colostrum on absorption, growth and health of heifer calves in the pre-weaned period

3.0 Introduction

Calves are born agammaglobulinemic as a result of bovine placentation type, which prevents transfer of immunoglobulins from dam to fetus *in utero*. Therefore, consumption of colostrum by the calf is required in order to achieve passive transfer of immunity (Enders and Carter, 2004) Immunoglobulins from colostrum, especially IgG, are critical for reducing early calf mortality and morbidity (Godden, 2008). In addition to IgG, colostrum also contains many other bioactive components that have important functions such as facilitating gut development, promoting maturation of the immune system, mediating nutrient uptake, stimulating enzymatic activity and providing early protection from disease (Pakkanen and Aalto, 1997). Although some bioactives, such as lactoferrin, have been studied extensively, there are many other components of colostrum for which there is little knowledge. More research is required to fully understand how these molecules influence growth and development of the calf.

Most bioactive components are found at higher concentrations in colostrum compared to mature milk, suggesting that they are essential for normal development, growth and health of the newborn calf (Blum and Hammon, 2000). In addition, effects of colostral bioactivity may extend into pre and post-weaned calves or even mature animals (Soberon et al., 2012). Evidence from previous studies suggested that growth and development are influenced by different feeding regimens. Most studies have focused on how the nutrient intake and quantity of milk can have a marked influence on the animal's growth and productivity as an adult (Bascom et al., 2007; Drackley et al., 2007). Faber et al. (2005), fed calves either 2 or 4 liters of colostrum and found that the volume of colostrum fed influenced growth and future milk production. Calves that were fed 4 liters

of colosturm produced 955 kg more milk in their first lactation compared to calves that received 2 liters. Other previous studies have shown a relationship between the amount of Ig received and future milk yield (DeNise et al., 1989; Robison et al., 1988; Soberon et al., 2012). Researchers speculated that the increased Ig may have allowed for more nutrients and energy to be utilized for growth and development, whereas calves receiving less Ig may have diverted more energy to immune protection important for fighting disease.

Some studies have investigated the differences between feeding whole milk versus milk replacer. Moallem et al. (2010) found that calves fed whole milk and started supplemented with soy protein produced approximately 725 kg more milk in their first lactation than calves fed milk replacer. The milk replacer contained soy as one of the protein sources, which may have influenced the results. The calves receiving whole milk also had higher body weights at weaning than the calves fed milk replacers. The authors suggested that as well as quality of protein, whole milk may also provide bioactive factors absent in milk replacer that contributed to greater growth and may aid development of the gastrointestinal tract and mammary gland.

The effects of feeding regimens on calves' health and future milk production are evident, but fewer studies have focused on the influence of bioactives on the pre-weaned calf. Development of the immune system in the pre-weaned calf has been of interest in recent years. In a study by Reber et al. (2008), calves were fed colostrum with or without maternal cells and surface expression of cellular markers as measured over the four-week period after birth. Results showed that calves fed colostrum with maternal cells had a lower number of lymphocytes expressing CD11a, an adhesion molecule involved in regulating leukocyte mobilization, on their surface at two weeks of age, and a higher density of MHC class I cell surface expression in the first week. These results indicate that maternal cells in colostrum promote development of the calf's immune system as shown by the higher antigen presenting capacity and lower expression of markers associated with activation of lymphocytes. The results of this study provide evidence for signaling from dam to offspring via colostrum. This effect may have important implications for future health, growth and productivity of the adult animal.

Other recent studies have investigated the effects of feeding colostrum replacer versus maternal colostrum on health and growth of the pre-weaned calf. Calves fed maternal colostrum were found to have higher serum IgG (14.8 versus 5.8 mg/ml,) in a study by Swan et al. (2007), and they had a significantly higher rate of successful passive transfer compared to those fed colostrum replacer. This study did not find any significant treatment effect on calf health, and did not measure any growth parameters. In another study (Priestley et al., 2013), calves were fed maternal colostrum, colostrum-derived replacer or plasma-derived replacer and similar results as previous studies were found; serum IgG concentration and passive transfer were greater in calves that received maternal colostrum compared to the other two treatment groups. Morbidity rate was significantly higher in calves fed colostrum replacer compared to those fed maternal colostrum. Growth rate and weaning weights were higher in calves fed maternal colostrum compared to calves fed plasma-derived colostrum replacer, but not colostrum-derived replacer.

Increased health and growth in calves fed whole colostrum may be attributed to higher rates of passive transfer of immunity (Robison et al., 1988), however bioactive components other than IgG may contribute significantly to better performance in the pre-weaned period (Pakkanen and Aalto, 1997, Reber et al., 2008). Colostrum replacer, especially blood-derived replacer, normally does not have high levels of bioactive compounds, whereas whole maternal colostrum does (Priestly et al., 2013). However, little is known about the bioactivity of colostrum beyond its reported effects on immunoglobulins and passive transfer.

Pasteurization of colostrum prior to feeding is becoming a popular and effective management practice that is effective for reducing transmission of pathogens to the calf, while still providing an adequate IgG concentration (Godden et al., 2006). Pasteurization of colostrum was found to increase absorption of IgG by the calf (Johnson et al., 2007), suggesting that heat-treatment alters components of colostrum and influences their bioactive functions in the calf. A study by Elizondo-Salazar and Heinrichs (2009b) also found higher serum IgG concentration in calves that had received heat-treated colostrum, but no significant differences were seen in growth or health during the pre-weaned period. Heat-treatment of colostrum, in this study, also reduced bacterial load below the

level of detection. The above studies focused on the effects of heat-treatment on IgG absorption. Johnson et al. (2007) also measured serum concentrations of some other colostral compounds including IgA, IgM, vitamin A, vitamin E, β-carotene and cholesterol, but no significant differences of treatment were detected.

β-lactoglobulin (BLG) is the most abundant whey protein in milk and is found at high concentrations in colostrum (approximately 14 mg/ml versus 4.2 mg/ml in milk) (Levieux and Ollier, 1999). Studies in non-bovine species have suggested that BLG is an important immunomodulator and plays a role in gut development (Prioult et al., 2004; Sutton and Alston-Mills, 2006; Wong et al., 1998). However, little is known about the role in transport or the effects of BLG in the pre-weaned calf.

The objective of this study was to examine the effects of heat-treated colostrum on absorption of colostral protein, specifically IgG and BLG. β-lactoglobulin was selected as a protein of interest because it is abundant in colostrum and little is known about the absorption of BLG in neonatal calves. Along with absorption of protein in the neonatal period, absorptive capacity, as measured by xylose absorption, was analyzed at 4 weeks of age. Another aim of this experiment was to investigate the effects of heat-treatment of colostrum on the growth and health in pre-weaned heifer calves, and on the response to dehorning, a common management procedure that causes acute stress. A minor objective in this study was to investigate whether bull calves are suitable replacements for heifers in research.

We hypothesized that pasteurization of colostrum would alter gut absorption resulting in higher levels of IgG and lower levels of BLG in blood, as stated in chapter 2 for bull calves. It was also hypothesized that heat-treatment would lead to increased absorptive capacity (as measured by D-xylose challenge), increased body growth, and health during the pre-weaned period, due to the expected increase in colostral IgG absorption.

3.1 Materials and Methods

3.1.1 Colostrum Management

First-milking colostrum was collected from primi- and multiparous cows and was tested at room temperature using a hydrometer. Colostrum with an IgG concentration of 50 mg/ml or greater was aliquoted into 1-liter volumes in plastic freezer bags, which were immediately laid flat on wire racks and frozen at -20°C. Two batches, consisting of ~56 liters each, were collected. Batch 1 was collected from 6 cows from May to mid-June and Batch 2 was collected from 10 cows from mid-June to late-July. Once 56 liters was collected, colostrum was placed in a 4°C cold room and thawed slowly for 24 hours. After thawing, colostrum was mixed thoroughly. Half of the colostrum was subsequently aliquoted into 1-liter volumes and refrozen in plastic freezer bags. Several subsamples were collected for later analysis. The other half of the colostrum was pasteurized for 60 minutes at 60°C using on-farm pasteurizer (Dairy Tech Inc, Greeley, CO, USA). The temperature was carefully monitored and did not fluctuate more than 1.5 degrees for the entire 60 minutes. At the end of the pasteurization cycle, colostrum was rapidly cooled, aliquoted into 1-liter volumes and refrozen in plastic freezer bags. Subsamples were taken for later analysis.

3.1.2 Heifer Calf Management

Protocols for this study were approved by the University of Alberta Animal Care and Use Committee for Livestock and conducted in accordance with the Canadian Council of Animal Care (Ottawa, ON, Canada). Seventeen heifer calves were enrolled in the study as they were born and assigned in a semi-random fashion to receive either heat-treated (n=8) or unheated (n=9) colostrum. Near parturition, cows were placed in individual maternity pens and monitored closely via video cameras. At birth, cows were allowed to lick the calf dry. Calves were removed from the cow within 30 minutes of

calving or before suckling could occur. Calves were moved to individual pens bedded with fresh wood shavings, tagged with an identification ear tag, and their navels were dipped with 7% (v/v) iodine. Calf pens were located inside a mechanically ventilated room, and were freshly bedded every other day. Calves remained in their designated pens throughout the entire study. Each calf was fed 2 liters of either heat-treated or unheated colostrum via esophageal tube feeder within 1 hour after birth. For the second feeding at 12 hours after birth, calves received 400g in 1.5 liters of colostrum replacer (Immu-Start 50, Imutek Animal Health, Inc., Fort Collins, CO, USA). At the third feeding, all calves received milk replacer twice daily according to manufacturer instructions (COOP Purlac, Saskatoon, SK, Canada), and calves were offered starter grain (23% Accelerated Calf Starter, Wetaskiwin Co-operative Association, Wetaskiwin, AB, Canada) and ad libitum water. Grain intake was measured daily. Milk replacer was reduced starting on day 29 and calves were weaned when they were between 42 and 46 days of age. Calves remained in their pens for 72 hours after weaning and then moved to a group pen.

3.1.3 Sample Collection

All animal experiments were approved by the Animal Care and Use Committee at the University of Alberta. Prior to colostrum feeding, approximately 8.5 ml of blood was collected to be used to determine initial plasma concentrations of IgG, BLG and TP. Blood samples were also collected at 3, 6, 9, 12, 24 and 48 hours after birth and once per week until weaning. The 12-hour blood sample was taken prior to feeding colostrum replacer. All blood was collected in heparinized tubes, stored on ice and centrifuged at 3000 x g at 4°C for 20 minutes. Plasma was aliquoted into 1.5 ml microcentrifuge tubes and frozen at -80°C for later analysis.

Birth weight was recorded for each calf, and calves were weighed once per week until weaning. Height at the hips and withers were also measured once per week. Daily grain intake was recorded and average daily gain was calculated based on the weekly body weights. Body temperature was taken each day at the afternoon feeding, and calves were scored each day for scour consistency, respiratory score and overall health status according to the scoring system outlined below. The scoring system used was adapted

from Lesmeister and Heinrichs (2004) and was as follows: for scours consistency, 1 = Normal, 2 = Soft to loose, 3 = Loose to watery, 4 = Very watery, mucus or blood present, 5 = Severe diarrhea; for respiratory scoring, 1 = Normal, 2 = Slight cough, 3 = Moderate and persistent cough, 4 = Severe cough, 5 = Severe and chronic cough; for overall appearance, 1 = Normal and alert, 2 = Ears slightly drooped, 3 = Ears and head drooped, dull eyes, slightly lethargic, 4 = Ears and head drooped, dull eyes, dehydrated and lethargic, 5 = Severe lethargy and dehydration, eyes sunken. Calves with loose manure were offered electrolytes at least once in between milk feedings. Those with a respiratory score of 3 or more were treated with antibiotics (Excenel, Zoetis, Florham Park, NJ). Calves were monitored carefully and those that became sick were treated as appropriate. There were no calves that became severely sick at any point during the study. Calves that became showed mild symptoms were not removed from the study, since health was one of the parameters being measured. All health events were recorded and are summarized in Table 3.9.

3.1.4 Colostrum and Plasma Analysis of IgG and BLG Concentrations

Colostrum aliquots were analyzed for total IgG and β-lactoglobulin concentrations by enzyme-linked immunoassay (ELISA) (Bethyl Laboratories, Montgomery, TX). Affinity purified coating antibodies for bovine IgG or bovine BLG were diluted in coating buffer (0.05 M Sodium Carbonate-Bicarbonate, pH 9.6) and 100 µl was added to each well of a 96-well plate. The plate was incubated at room temperature for one hour and wells were washed five times, using an automated plate washer, with Tris-buffered saline (TBS) (50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, pH 8.0). This was used as the blocking, washing and sample buffer. After washing, 200 µl of blocking buffer was added to each well and the plate was incubated at room temperature for 30 minutes or overnight at 4°C. Samples were thawed on ice. Plasma samples were centrifuged at 3000 x g at 4°C for 5 minutes. All samples were diluted in sample buffer based on the predicted concentration of the target analyte so as to fall within the range of the standards. Standard dilutions from 7.8 to 500 ng/ml for IgG and 1.95 to 125 ng/ml for BLG were prepared. The 0 (buffer only) standard was used as the blank and this absorbance measurement was subtracted from all the other standards and

unknowns. Following vigorous mixing, 100 µl of each standard and sample was added to assigned wells and incubated at room temperature for one hour. The plate was then washed five times, 100 µl of horseradish peroxidase (HRP) conjugated bovine detection antibody and the plate was incubated at room temperature for an hour. The concentration of the IgG- and BLG-specific HRP antibodies was optimized (1:100,000 and 1:30,000 for IgG and BLG, respectively) to result in an optical density of 1.8-2.2 O.D. for the lowest dilution of standard. The plate was again washed five times, followed by the addition of 100 µl of the enzyme substrate, 3,3',5,5'-Tetramethylbenzidine (TMB) to each well. The plate was incubated at room temperature for 15 minutes in the dark. The enzymatic reaction develops as a blue color. After 15 minutes, 100 µl of stop solution (0.18 M H_2SO_4) was added, stopping the reaction and causing the solution to change from blue to yellow. Absorbance was measured on a plate reader (Molecular Devices LLC, Sunnyvale, CA, USA) at 450nm.

3.1.5 Colostrum and Plasma Total Protein

Total protein (TP) quantification of plasma was determined using a bicinchoninic acid (BCA) assay (Pierce Biotechnology, Rockford, IL, USA). The assay was performed using the microplate procedure according to the manufacturer's directions. Briefly, standards were prepared using bovine serum albumin (BSA) in water to achieve final concentrations of 2000, 1500, 1000, 750, 500, 250, 125, 25 and 0 µg/ml. Plasma samples were diluted 1:100. The working reagent was prepared from Reagent A and B, supplied with the kit, at a 50:1 ratio. Twenty-five microliters of each standard and sample was pipetted in duplicates into a 96-well microtiter plate and 200 µl of working reagent was added to each well using a multichannel pipette. The plate was incubated at 37°C for 30 minutes and absorbance was read at 562nm using a microplate reader (Molecular Devices LLC, Sunnyvale, CA, USA). The 0 standard was used as the blank and its absorbance measurement was subtracted from all the other standards and unknowns.

3.1.6 Colostrum bacteria analysis

Colostrum samples from each batch were analyzed for total bacterial plate count and for the levels of individual bacteria types in unheated and heat-treated colostrum. See Appendix I.

3.1.7 Xylose feeding and blood collection

The xylose challenge was performed on calves (described by Shea et al., 2009) when they reached 4 weeks after birth ± 3 days. D-xylose (Sigma-Aldrich, St Louis, MO, USA) was fed at 0.5g/kg BW by mixing with MR at the morning feeding. Calves had ad libitum access to water but grain and MR were withheld after the morning feeding for the duration of the sample collected. A baseline blood sample (0 hr) was obtained prior to and at 2, 4, 6, 8 and 12 hr after feeding. Blood samples were collected in vacuum tubes containing 10.8 mg EDTA via jugular venipuncture and immediately centrifuged at 3000 x g at 4°C for 20 minutes. Plasma was aliquoted into two 2-mL microcentrifuge tubes and frozen at -80°C for later analysis of D-xylose concentration.

3.1.8 Xylose assay

Plasma concentration of D-xylose was measured using methods adapted from Merritt and Duelly (1983) and Eberts et al. (1979). Phloroglucinol (1,3,5-trihydroxybenzene) was obtained from Sigma-Aldrich, St Louis, MO, USA. A stock solution consisting of 10 parts glacial acetic acid and 1 part 12M hydrochloric acid was prepared ahead of time. Immediately prior to the assay, a 0.5% (w/v) solution of phloroglucinol in the acid mixture was prepared. Standards were prepared by dissolving D-xylose in water to make 1, 0.5, 0.25, 0.125, 0.0625 and 0 mg/ml concentrations. The phloroglucinol-acid reagent (500 µl) was added to 5 µl of each standard and sample in 1.5 ml microcentrifuge tubes. Cap locks were placed over the tubes to prevent the lids from opening. The tubes were places in a heat block (Thermo Fisher Scientific, Waltham, MA, USA) set to 100°C and heated for exactly 4 minutes. Following heating, tubes were transferred to a water bath at room temperature and cooled for 5 minutes. The tubes were

vortexed and 200 µl of each standard and sample were transferred to a 96-well microtiter plate. Absorbance was read at 554nm using a microplate reader (Molecular Devices LLC, Sunnyvale, CA, USA). The 0 standard was used as the blank and this absorbance measurement was subtracted from all the other standards and unknowns. Absorbance of some non-specific analytes occurred, therefore the absorbance value of the 0-hour sample (pre-xylose administration) for each calf was subtracted from the other time points.

3.1.9 Statistical Analysis

Colostral concentrations of IgG, TP and BLG of each subsample were used to calculate the mean sample concentration in heat-treated and unheated colostrum for each batch (the experimental unit). Colostrum measurements were analyzed by paired, one-tailed paired t-test. Blood and growth observations were analyzed by two-way ANOVA (treatment by time) and repeated measures of time using the MIXED procedure of SAS 9.3 (SAS Institute Inc. 2012). Calves from both treatment groups receiving colostrum (within each batch) from the same original pool of colostrum, therefore batch was considered a random effect. The statistical model used for this analysis was:

$$Y_{ijk} = \mu + C_i + T_j + (CT)_{ij} + Batch_l + e_{ijk}$$

Where Y_{ijk} = dependent variables, μ = overall mean, C_i = fixed effect of colostrum treatment i, where i = heat-treated or unheated, T_j = repeated measure of time j, $(CT)_{ij}$ = effect of treatment by time interaction, $Batch_l$ = random effect of batch l, and e_{ijk} = residual. Birth weight and minutes to first feeding were offered as covariates. Both were found to contribute significantly to the variation, and therefore they were left in the final model. Grain intake, health scores and body temperature observations were also analyzed by two-way ANOVA using the MIXED procedure of SAS, but without repeated measures.

3.2 Results

3.2.1 Colostral total IgG, β-lactoglobulin concentrations, and Bacterial Counts

Heifer calves in this study were fed from the same batches as the bull calves. Colostral IgG and BLG concentrations in heated colostrum were compared to unheated colostrum. Refer to results section in chapter 2.

3.2.2 Plasma IgG, BLG and total protein concentrations in heifer calves

The plasma IgG and TP concentrations in the 0-hour (pre-colostrum) samples were low and did not differ with respect to treatment. As expected, BLG was not detectable at 0-hour and its concentration was presumed to be zero. As shown in Figure 3.1A, at 3 hours the plasma IgG concentration was not significantly different with respect to colostrum treatment. However, the concentrations were higher in heifers fed heat-treated versus unheated colostrum at 6 hours (13.54 ± 1.65 versus 10.41± 1.6 mg/ml; p=0.05), 9 hours (17.21± 1.7 versus 14.12 ± 1.6 mg/ml; p=0.05), 12 hours (17.46 ± 1.7 versus 14.76 ± 1.6 mg/ml; p=0.09) and 24 hours (17.73 ± 1.7 versus 13.95 ± 1.6; p=0.02). At 48 hours there was no difference in plasma IgG concentration between the two treatment groups. The concentration in week 1 of life was significantly higher in calves fed heat-treated colostrum (14.15 ± 1.3 mg/ml) compared to those fed unheated colostrum (11.38 ± 1.3 mg.ml; p=0.03), but at 2 and 3 weeks of age there was no longer a difference. The plasma BLG and TP concentrations were not statistically significant at any time point (see Figure 3.1B and C).

The AEA for IgG and BLG were calculated and the results are presented in Table 3.3. The AEA for IgG was significantly higher in calves fed heat-treated colostrum compared to unheated colostrum at 6 hours (36.55 ± 2.7 versus 26.83 ± 2.5%; p=0.008), 9 hours (45.78 ±2.7 Versus 35.62 ± 2.5%; p=0.0056) and at 12 hours (46.78 ± 2.6 versus 37.21 ± 2.5%; p=0.009). There was no difference in AEA of BLG at any time point.

3.2.3 Plasma IgG and BLG concentrations of heifers compared to bull calves

Circulating levels of IgG and BLG in heifer calves, as well as the apparent efficiency of absorption, were compared to bull calves, irrespective of colostrum treatment. Overall, heifer calves appeared to absorb higher amounts of IgG and BLG at some time points. Concentrations of IgG (shown in Figure 3.2A) were significantly higher in heifer versus bull calves at 6 hours (12.21 ± 1.24 versus 9.22 ± 1.26 mg/ml; p=0.006) and 9 hours (15.90 ± 1.24 versus 13.49 ± 1.3 mg/ml; p=0.03), and tended to be higher at 12 hours (16.34 ± 1.24 versus 14.41 ± 1.3 mg/ml; p=0.08) The AEA (see Table 3.4.) was also significantly higher in heifer versus bull calves at 6 hours (31.87 ± 1.65 versus 23.92 ± 1.75%; p=0.001) and 9 hours (40.88 ± 1.65 versus 34.20 ± 1.83%; p=0.007), and tended to be higher at 12 hours (42.18 ± 1.65 versus 37.5 ± 1.83%; p=0.06).

Plasma BLG concentrations (see Figure 3.2B) did not differ significantly at any time point. However, there was a tendency for heifer calves to have higher BLG levels compared to bulls at 6 hours (356.54 ± 40.99 versus 271.70 ± 42.4 μg/ml; p=0.05). The AEA (see Table 3.5.) at 6 hours was significantly higher in heifers compared to bulls (6.06 ± 0.5 versus 4.45 ± 0.52%; p=0.03). At 9 and 12 hours, however, the concentrations and the AEA were the same for both heifer and bull calves.

Similar patterns over time for plasma IgG, TP and BLG concentrations were observed in heifers as seen in bulls (see results in chapter 2). In heifers, plasma IgG and TP remained above 10 mg/ml and 60 mg/ml, respectively, through 48 hours after birth. However, the plasma BLG concentration decreased to close to zero as the calves approached 48 hours of age.

3.2.4 Plasma Xylose Concentration in Heifers at 4 weeks of age

To determine if heat-treatment of colostrum influenced absorptive capacity of the small intestine at 4 weeks of age, D-xylose was administered at a single feeding and plasma concentrations were measured at various time points over a 12-hour period. Results for plasma D-xylose concentration are shown in Figure 3.3. Concentrations increased rapidly in the first 2 hours after feeding and reached a plateau at approximately

4 hours at an average concentration of 32 mg/ml. Concentrations then began to decrease gradually, reaching 14.8 mg/ml at 12 hours after feeding. Calves that received unheated colostrum had numerically higher D-xylose concentrations compared to those that received heated colostrum. However, no statistically significant differences in plasma D-xylose concentration were seen with respect to colostrum treatment.

3.2.5 Growth and Health of Heifer Calves in the Pre-Weaned Period

To determine if heat-treatment of colostrum had a significant influence on growth in the pre-weaned calf, starter grain intake, body weight, hip and wither height and average daily gain (ADG) were measured until weaning at approximately 42 days of age. Results are presented in Figure 3.4. No significant differences in body weight were observed. Figure 3.4C shows ADG in pre-weaned calves. No significant differences were found with regard to colostrum treatment in ADG at any time point. However, between week 5 and 6 the ADG of calves that had received heat-treated colostrum dropped and had a tendency to be lower than calves fed unheated colostrum (0.54 ± 0.1 versus 0.75 ± 0.09 kg/d; p=0.09). Calves fed heated colostrum continued to have numerically lower ADG between week 6 and weaning, but there was no statistical significance. As shown in Table 3.7, at 4 weeks of age, heifers receiving heat-treated colostrum were significantly higher at the withers than calves receiving unheated colostrum (85.12 ± 0.9 versus 83.74 ± 0.88, respectively; p=0.04). There was no statistical difference for starter intake between the two treatment groups for at any time point.

Rectal temperature, and health scores (scours, respiratory and overall appearance) were recorded daily. Daily temperatures (Figure 3.5A) were averaged for every 7 days from birth until day 42 of age. For weeks 1 through 4 and week 6 average temperatures were not statistically different between the treatment groups. The average temperature for week 5 was statistically higher in calves receiving heat-treated versus those receiving unheated colostrum (39.49 ± 0.08 versus 39.24 ± 0.08°C, respectively; p=0.01). To determine if this difference was a result of dehorning, body temperature was analyzed during the 3 days before and after dehorning (see Figure 3.5B). In the days prior to dehorning there was no significant difference in body temperatures between treatment groups, and temperature was normal. On the day of dehorning, as well as the following

day, calves that had been fed heat-treated colostrum had significantly higher body temperatures compared to those fed unheated colostrum, and on the second day after dehorning had occurred there was still a tendency for the heat-treated group to have higher temperatures. Although both groups increased in temperature in response to dehorning, the increase in the heat-treated group corresponds to a mid-grade fever, suggesting that the response to dehorning is not only statistically significant, but may also be biologically relevant (Data in Table 3.8b).

Health scores were not affected by colostrum treatment at any time point (see Table 3.9).

3.3 Discussion

Recent hypotheses suggest that the bioactive factors in colostrum not only affect the newborn, but may also influence longer-term growth, health and productivity of animal (Soberon et al., 2012). This suggests that colostrum may facilitate a kind of communication from the dam to the offspring. For example, research has shown that different feeding strategies, including those of colostrum, for dairy calves can affect growth in the pre-weaned period (Robison et al., 1988) and also productivity of the adult animal (Soberon et al., 2012). Differences in long-term outcomes attributed to colostrum are likely a result, at least in part, to alterations in its bioactive functions.

As expected, plasma IgG concentrations at 0 and 3 hours were low and not significantly different between treatment groups. By 6 hours after birth, however, levels of circulating IgG increased substantially and calves that received heat-treated colostrum had significantly higher plasma IgG concentrations compared to those that were fed unheated colostrum. This effect was also seen at 9, 12 and 24 hours, and is consistent with previous research that found a similar effect in calves at 24 hours after birth (Johnson et al., 2007). Another study by Elizondo-Salazar and Heinrichs (2009b) also found that calves fed heat-treated colostrum versus unheated colostrum had significantly higher concentrations of IgG from 4 through 48 hours, and also from week 1 through week 5. At week 6 and beyond, the concentration was the same for both treatment

groups. In the present study a treatment difference was detected at week 1, but not at 48 hours, or beyond 1 week of age. The reason for this variation is unknown. Previous studies of this nature used twice as many calves. The smaller sample size used in the current study could have resulted in the differences observed by not providing enough statistical power to detect a difference.

The authors of the two studies mentioned above give several explanations for the demonstrated effect of heat-treatment. First, the immunoglobulins in colostrum may bind bacteria at the time of ingestion, but prior to absorption, which could prevent the uptake of Ig from unheated colostrum by the gut (Acres, 1985). In the heat-treated colostrum there would be more absorbable Ig available due to the lower bacterial load. Secondly, there may be some non-specific binding by bacteria to enterocyte receptors that may impede IgG absorption by the gut (James et al., 1981; Staley and Bush, 1985). To attempt to answer the question of whether bacteria could decrease IgG absorption by the calf, Elizondo-Salazar and Heinrichs (2009a) fed calves one of three treatments: Heat-treated colostrum, unheated colostrum with a low bacterial load or unheated colostrum with a high bacterial load. Calves that received heat-treated colostrum had higher circulating IgG than either of the unheated groups. Total serum IgG concentration in the low-bacteria and high-bacteria groups was identical. These results suggest that bacterial load does not impede IgG absorption by the calf, as previously hypothesized.

Another explanation for higher IgG in the heat-treated group is that there are proteins in colostrum that are altered or denatured during heat-treatment, which would otherwise inhibit IgG absorption. Level of activity of brush border enzymes, such as lactase and maltase, is considered to be a marker of gut maturation in the neonate, and it is known that there are factors in colostrum that can stimulate enzymatic activity (Jensen et al., 2001). Heat-treatment of colostrum may alter these factors and subsequently affect the activity of these brush border enzymes, slowing maturation of the gut and promoting increased absorption of IgG. It is currently unknown whether this hypothesis may be true and more research is required. Previous research has suggested that BLG may influence brush border enzymes (Sutton and Alston-Mills, 2006), among other factors in the neonatal gut. However, evidence for this effect is limited. In the present study, there was

no statistical difference in the plasma BLG concentration in heifer calves that received heat-treated versus unheated colostrum.

In the present study, IgG and BLG levels in heifer and bull calves were also compared. In the previous research that studied the effects of heat-treatment on IgG absorption (Elizondo-Salazar and Heinrichs, 2009b; Johnson et al., 2007), heifer calves were used for the analysis. Our study demonstrated that heat-treatment of colostrum did not have the same effect on bull calves as it did on the heifer calves, with bulls in the heat-treated group having lower IgG levels than the calves fed unheated colostrum. Further analysis also revealed that bull calves had significantly lower circulating IgG than heifer calves that had received heat-treated colostrum, but not heifers fed unheated colostrum. Results from calculating the AEA showed that at 6 and 9 hours, heifers were more efficient at absorbing IgG and BLG from colostrum. In some previous work, authors suggested that because bull calves are, in general, larger in body weight, they have a larger blood volume, which would dilute the circulating IgG (Quigley, 2002). However, in the present study there was no difference in birth weight between male and female calves.

Although the reason for the differences seen here are unknown, it is well documented that there are gender-specific differences in growth and development of both the fetus and neonate, which may affect many factors in the neonate that influence intestinal absorption or concentration of molecules in blood (Alfarawati et al., 2011; Godfrey et al., 2011; Xu et al., 1992). A study of fetal lambs (Edwards and McMillan, 2002) showed that singleton male lambs had higher circulating Adrenocorticotropic Hormone (ACTH) after 130 days of gestation compared to female singletons. This hormone is often associated with biological stress. It is unknown whether this same effect is seen in fetal calves; however the effects of ACTH on other species have been documented. For example, in an experiment performed by Bate et al. (1991) sows were administered either ACTH, isoflupredone (a synthetic glucocorticoid) or saline as a control in late gestation (~100 days). Piglets were subsequently analyzed post-partum. Those in the ACTH group had lower birth weights, a larger stomach and small intestine and lower circulating IgG. These results indicate that the fetal environment has

significant consequences in the neonatal period, and provides a hypothesis for the gender-specific differences shown in the current study.

To investigate whether heat-treatment of colostrum could affect other aspects of nutrient absorption (specifically sugar absorption capacity) during the pre-weaned period, a xylose challenge was administered to heifer calves at 4 weeks of age. D-xylose is an inert pentose sugar that can be easily detected using a simple colorimetric assay. Unlike glucose, D-xylose is not metabolized and is excreted rapidly from the body. It is actively transported by the gut using the same pathway as glucose, but may also be absorbed passively. Xylose is a proxy measurement of glucose uptake and absorption rate; it is considered a marker of absorptive function (Eberts et al., 1979; Fordtran et al., 1962). Previous studies have performed xylose challenges in calves (Hammon and Blum, 1997; Shea et al., 2009), but animals used were 5 days and 60 hours old, respectively. In the current study, the xylose challenge was conducted on 4-week old calves. There was no difference in plasma xylose concentration at any time point between calves receiving heat-treated or unheated colostrum, suggesting that pasteurization of colostrum did not significantly impact later absorptive capacity in the small intestine in pre-weaned calves.

In general, heat-treatment of colostrum did not influence body weight, and ADG. However, between week 5 and 6 calves that had been fed heat-treated colostrum tended to decrease ADG compared to the calves that were fed unheated colostrum. This time corresponds to the period after the calves were dehorned. Height at the hips and withers was also measured weekly. There was no effect of heat-treatment on hip height. At four weeks of age, the withers height was significantly higher in calves that were fed heat-treated colostrum. Although not statistically significant, prior to 4 weeks the withers height was also numerically higher in the calves fed heat-treated colostrum. In the weeks after dehorning, there were no differences in withers height between the two treatment groups. These results, with the ADG data, suggest that dehorning negatively affected the growth of calves receiving heat-treated colostrum. To investigate whether this effect on growth was due to a decrease in starter intake, daily grain consumption was analyzed. An average of intake was calculated for 7-day increments from birth to day 42 of age. There was no difference in grain consumption observed, and intake did not decrease in the week after dehorning.

As well as growth, calf health was carefully monitored. Daily body temperatures were recorded, and the average temperature for each week from birth to 42 days of age was calculated. Weekly body temperatures were statistically the same for both treatment groups, except during the week 5 at which time the calves receiving heat-treated colostrum had significantly higher body temperatures than calves receiving unheated colostrum. On the day of dehorning, as well as the following day, calves that had been fed heat-treated colostrum had significantly higher body temperatures compared to those fed unheated colostrum, and on the second day after dehorning had occurred there was still a tendency for the heat-treated group to have higher temperatures. Calves are considered to have a fever when they have a rectal temperature of 39.5°C or above. Both groups increased in temperature in response to dehorning, but calves that had received heat-treated colostrum had temperatures higher than 39.5°C, whereas those that were fed unheated colostrum did not.

Dehorning is a common management practice used extensively on dairy farms to decrease the risk of injury, both to humans and other cows. Although this practice is known to moderately increase acute stress in calves (Laden et al., 1985), the growth and body temperature data in the current study suggest that calves fed heat-treated colostrum respond differently to this stress compared to those fed unheated colostrum. A probable explanation for these differences is that even though consumption of starter remained the same, calves in the heat-treated group expended more energy mounting an immune response. However, these results are not indicative of an influence by IgG. In this experiment, heifer calves that received heat-treated colostrum had higher circulating IgG for at least the first 24 hours of life. Yet it was these calves that mounted a greater immune response due to dehorning. This suggests that maternal colostral IgG did not play a significant role in this effect.

Although not measured in the present study, colostral immune cells are known to be heat-labile (Liebhaber et al., 1977). Maternal leukocytes are transferred from colostrum across the calf's gut and have been shown to exhibit priming effects on the neonatal immune system (Reber et al., 2008). Heat-treatment of colostrum in the current study could have resulted in lower maternal immune cells in the colostrum, thus interfering with this priming effect, and influencing the calf's subsequent immune

response. Although the explanation for this effect is purely speculative, the findings strongly indicate a tangible, applicable effect of pasteurization on colostrum. Although IgG is critical for survivability and health of calves, other components of colostrum should no longer be overlooked as being important for the development of the young calf.

3.4 Summary and Conclusion

In this study, heifer calves that received heat-treated colostrum had significantly higher plasma IgG concentrations, even though colostral IgG was not significantly affected by heating. Concentrations of circulating BLG did not differ between treatment groups. These results suggest that although absorption of bioactive components may be affected by pasteurization of colostrum, there may also be differences between male and female neonates. Absorption of D-xylose at 4 weeks of age was not significantly altered by colostrum treatment, and there was no effect on body weight or starter intake. Results indicate that calves receiving heat-treated colostrum have lower ADG and differ in their response to dehorning. This study suggests that heat-treatment of colostrum has both a negative and positive influence on the pre-weaned heifer calf. Moreover, male and female calves appear to differ in their absorption of colostral protein. Future studies should be performed to elucidate the differences observed in the male versus heifer calves, along with the effect of heat-treatment of colostrum on the response to dehorning.

Table 3.1: Colostral IgG, total protein and β-lactoglobulin levels

	Treatment Group		
Components	Unheated	Heat-Treated	P-value
IgG, mg/mL	74.49±7.9	69.05±8.6	0.0671
TP[1], mg/mL	186.3±14.04	177±10.8	0.2101
BLG[2], mg/mL	11.2±1.6	10.14±1.44	0.0131

$P < 0.05$
[1]Total Protein
[2]β-Lactoglobulin

Table 3.2a: Plasma IgG (mg/mL), TP (mg/mL) and BLG (µg/mL) concentration in heifer calves receiving unheated or heat-treated colostrum

	IgG			TP			BLG		
Age (Hr)	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value
0	0.09±1.46	0.05±1.5	0.94	48.27±3.53	47.83±3.61	0.89	ND	ND	ND
3	1.11±1.5	0.10±1.5	0.65	51.29±3.12	49.59±3.61	0.61	133.49±39.3	96.39±39.3	0.51
6	10.41±1.46	13.54±1.5	0.05	63.27±3.53	64.06±3.61	0.80	314.30±37.03	377.83±39.3	0.24
9	14.12±1.46	17.21±1.5	0.05	70.59±3.53	70.51±3.61	0.98	214.79±37.03	227.38±39.3	0.82
12	14.76±1.46	17.46±1.5	0.09	69.88±3.53	71.28±3.61	0.66	102.62±37.03	100.81±39.3	0.97
24	13.95±1.46	17.73±1.5	0.02	70.66±3.53	70.94±3.61	0.93	6.25±37.03	8.74±39.3	0.96
48	12.57±1.46	14.12±1.6	0.36	66.02±3.53	70.99±3.85	0.15	0.93±37.03	1.11±45.35	0.99

$P < 0.05$, comparing treatment LS means at each time point
ND = Not Detected

Table 3.2b: Plasma IgG (mg/mL) in heifer calves receiving unheated or heat-treated colostrum 1, 2 and 3 weeks after birth

	IgG		
Age (Wk)	Unheated	Heat-Treated	P-value
1	11.38±1.24	14.15±1.25	0.03
2	10.04±1.24	10.53±1.22	0.69
3	9.05±1.32	10.58±1.27	0.26

P < 0.05, comparing treatment LS means at each time point

Table 3.3: Apparent Efficiency of Absorption (AEA, %)[1] in the first 12-hours of life for IgG and BLG in heifer calves receiving unheated or heat-treated colostrum

	AEA of IgG			AEA of BLG		
Age (Hr)	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value
0	0.24±2.5	0.12±2.66	0.77	ND	ND	ND
3	2.57±2.67	0.95±2.66	0.66	2.07±0.77	1.75±0.77	0.77
6	26.83±2.5	36.55±2.66	0.008	5.16±0.73	6.85±0.77	0.12
9	35.62±2.5	45.78±2.66	0.0056	3.54±0.73	4.16±0.77	0.56
12	37.21±2.5	46.78±2.66	0.009	1.72±0.73	1.85±0.77	0.90

$P < 0.05$, comparing treatment LS means at each time point
[1]AEA = ((birth weight × 0.095 × serum IgG)/total IgG or BLG fed) × 100
ND = Not Detected

Table 3.4: Plasma IgG (mg/ml) and AEA of IgG in heifer versus bull calves

	IgG			AEA of IgG		
Age (Hr)	Heifers	Bulls	P-value	Heifers	Bulls	P-value
0	0.09±1.24	0.40±1.3	0.78	0.46±1.65	1.30±1.81	0.73
3	0.48±1.25	0.89±1.33	0.73	1.93±1.7	3.18±1.98	0.63
6	12.21±1.24	9.22±1.26	0.0061	31.87±1.65	23.92±1.75	0.001
9	15.90±1.24	13.49±1.3	0.03	40.88±1.65	34.20±1.83	0.007
12	16.34±1.24	14.41±1.3	0.08	42.18±1.65	37.51±1.83	0.06

$P < 0.05$, comparing treatment LS means at each time point

Table 3.5: Plasma BLG (μg/ml) and AEA of BLG in heifer versus bull calves

	BLG			AEA of BLG		
Age (Day)	Heifers	Bulls	P-value	Heifers	Bulls	P-value
0	ND	ND	ND	ND	ND	ND
3	124.98±37.8	96.52±39.6	0.54	1.95±0.51	1.58±0.57	0.63
6	356.54±37.14	271.70±37.1	0.05	6.06±0.5	4.45±0.53	0.03
9	231.56±37.14	185.21±37.83	0.30	3.90±0.5	3.00±0.55	0.23
12	112.20±37.14	92.50±37.8	0.66	1.83±0.5	1.54±0.55	0.69

$P < 0.05$, comparing treatment LS means at each time point
ND = Not Detected

Table 3.6: Body weight (kg), starter intake (kg)[1] and average daily gain (ADG)[2] in heifer calves receiving unheated or heat-treated colostrum

	Weight			Intake			ADG		
Age (Wk)	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value
Birth	43.38±0.97	43.25±1.01	0.92	ND	ND	ND	ND	ND	ND
1	44.21±0.97	43.34±1.01	0.48	0.04±0.09	0.086±0.093	0.58	0.12±0.09	0.02±0.095	0.40
2	46.36±0.97	46.02±1.01	0.79	0.18±0.09	0.19±0.093	0.92	0.31±0.09	0.39±0.095	0.51
3	50.30±0.97	50.19±1.01	0.93	0.31±0.09	0.39±0.093	0.30	0.57±0.09	0.60±0.095	0.78
4	55.14±0.97	55.42±1.01	0.82	0.48±0.09	0.53±0.093	0.53	0.69±0.09	0.75±0.095	0.62
5	60.37±0.97	59.15±1.01	0.33	0.91±0.09	0.86±0.093	0.53	0.75±0.09	0.54±0.095	0.09
6	67.20±0.97	65.41±1.01	0.15	1.34±0.09	1.30±0.093	0.57	0.98±0.09	0.90±0.095	0.53

$P < 0.05$, comparing treatment LS means at each time point
[1]Intake = Average starter consumption per week
[2]ADG = Average daily gain (kg/day)
ND = Not Detected

Table 3.7: Height (cm) at the withers and hips in calves receiving unheated or heat-treated colostrum

	Withers Height			Hip Height		
Age (Wk)	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value
Birth	79.72±1.09	78.16±1.08	0.16	ND	ND	ND
1	80.45±0.9	80.26±0.9	0.77	82.94±0.84	83.24±0.84	0.78
2	81.75±0.88	82.50±0.9	0.25	84.13±0.8	85.14±0.84	0.33
3	82.95±0.88	83.77±0.9	0.21	85.61±0.8	86.89±0.84	0.22
4	83.74±0.88	85.12±0.9	0.04	87.20±0.8	88.57±0.84	0.19
5	85.43±0.88	85.99±0.9	0.39	89.32±0.8	89.86±0.84	0.61
6	87.56±0.93	88.01±0.94	0.57	91.37±0.9	91.63±0.96	0.82

$P < 0.05$, comparing treatment LS means at each time point
ND = Not Detected

Table 3.8a: Average body temperature (°C) per week in calves receiving unheated or heat-treated colostrum

	Body Temperature		
Week[1]	Unheated	Heat-Treated	P-value
1	38.93±0.08	38.89±0.75	0.70
2	38.99±0.08	39.02±0.75	0.77
3	39.11±0.08	39.08±0.75	0.75
4	39.11±0.08	39.09±0.75	0.85
5	39.24±0.07	39.49±0.75	0.01
6	39.20±0.08	39.18±0.75	0.78

$P < 0.05$, comparing treatment LS means at each time point
[1]Weekly temperature value is an average taken every 7 days from birth to day 42 of age

Table 3.8b: Average daily body temperature (°C) 3 days before and after dehorning in calves receiving unheated or heat-treated colostrum

	Body Temperature		
Day	Unheated	Heat-Treated	P-value
-3	38.92±0.14	39.09±0.14	0.35
-2	39.16±0.14	39.29±0.14	0.49
-1	39.19±0.14	39.39±0.14	0.29
Dehorned[1]	39.35±0.14	39.83±0.14	0.01
1	39.12±0.14	39.81±0.14	0.0002
2	39.36±0.14	39.66±0.14	0.09
3	39.34±0.14	39.62±0.15	0.14

$P < 0.05$, comparing treatment LS means at each time point
[1]Calves were dehorned mid-day. All daily temperatures were taken at evening feedings

Table 3.9: Health scores[1] (scours, respiratory and general appearance) of calves receiving unheated or heat-treated colostrum

	Scours			Respiratory			General Appearance		
Age (Wk)	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value	Unheated	Heat-Treated	P-value
1	1.35±0.08	1.22±0.08	0.24	1.00±0.06	1.00±0.06	1.00	1.02±0.02	1.00±0.02	0.43
2	1.10±0.08	1.16±0.08	0.55	1.00±0.06	1.04±0.06	0.66	1.00±0.02	1.00±0.02	0.99
3	1.12±0.08	1.11±0.08	0.91	1.00±0.06	1.00±0.06	1.00	1.00±0.02	1.00±0.02	0.99
4	1.14±0.08	1.13±0.08	0.87	1.06±0.06	1.02±0.06	0.58	1.02±0.02	1.02±0.02	0.92
5	1.00±0.08	1.04±0.08	0.75	1.19±0.06	1.05±0.06	0.10	1.03±0.02	1.00±0.02	0.18
6	1.00±0.08	1.00±0.08	1.00	1.11±0.06	1.14±0.06	0.75	1.03±0.02	1.00±0.02	0.18

$P < 0.05$, comparing treatment LS means at each time point
[1]Weekly averages

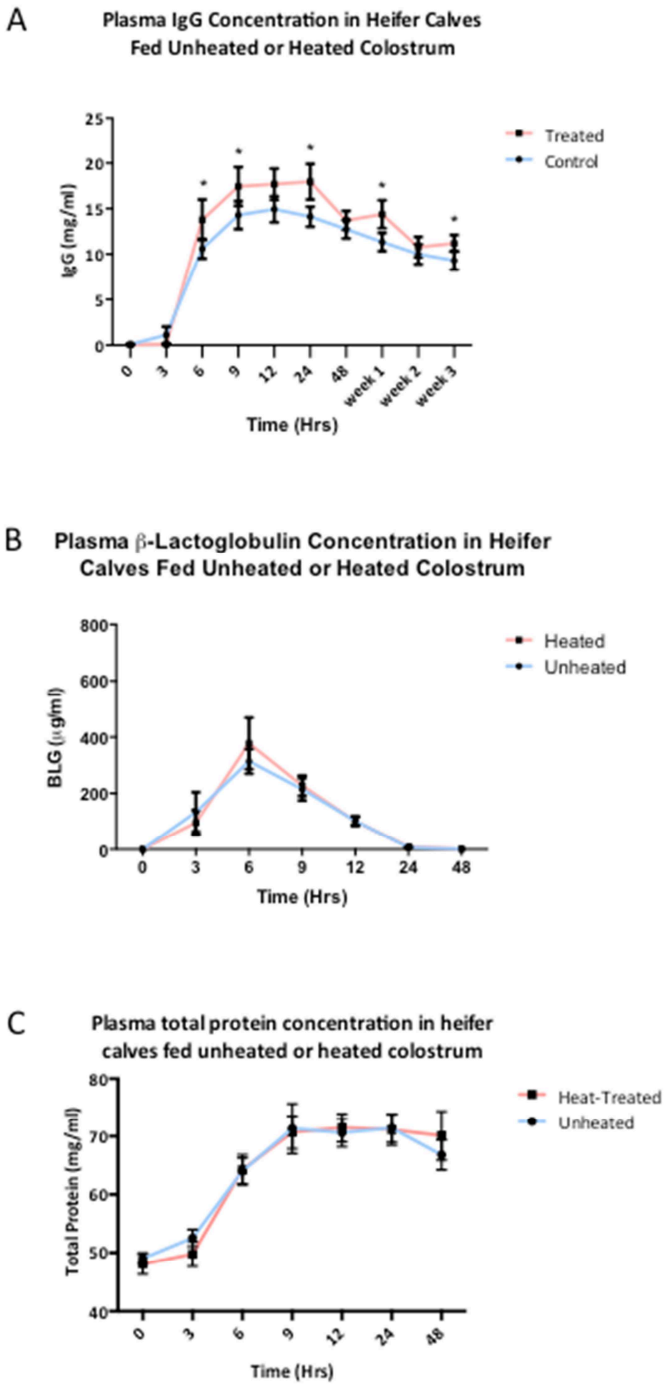

Figure 3.1: Plasma IgG (A), TP (B) and BLG (C) concentration in heifer calves receiving unheated or heat-treated colostrum ($P < 0.05$).

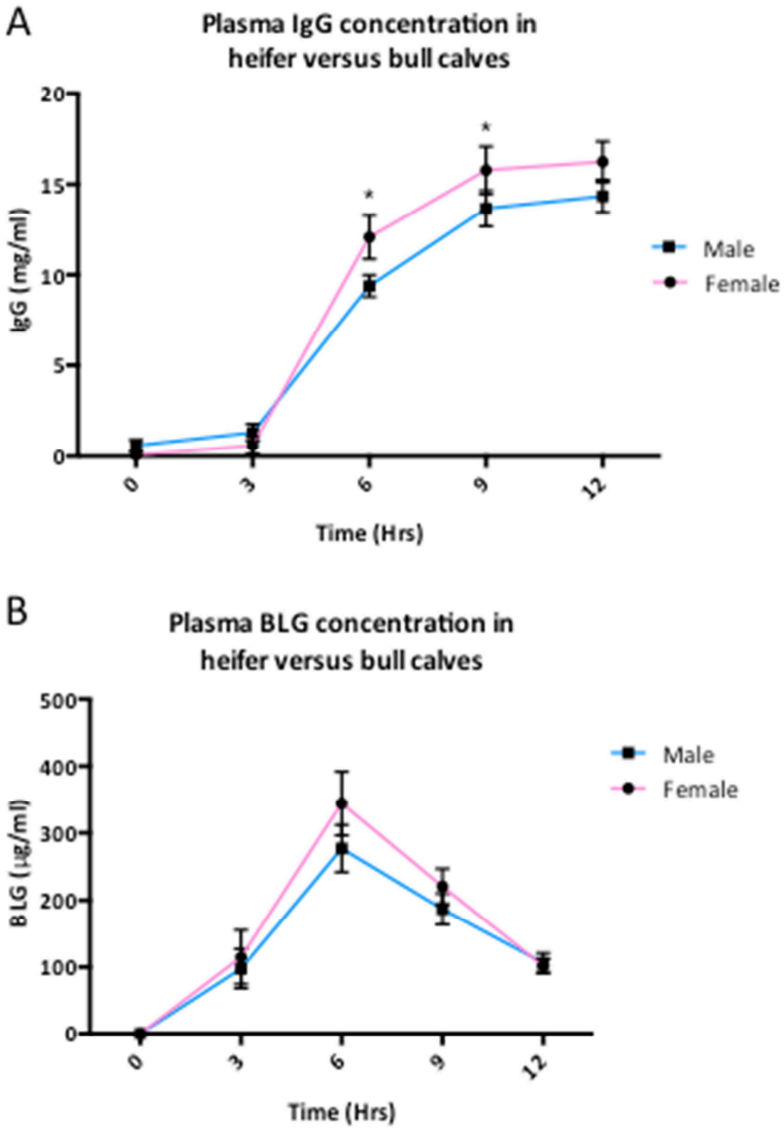

Figure 3.2: Plasma IgG (A) and BLG (B) concentration in heifer and bull calves ($P < 0.05$).

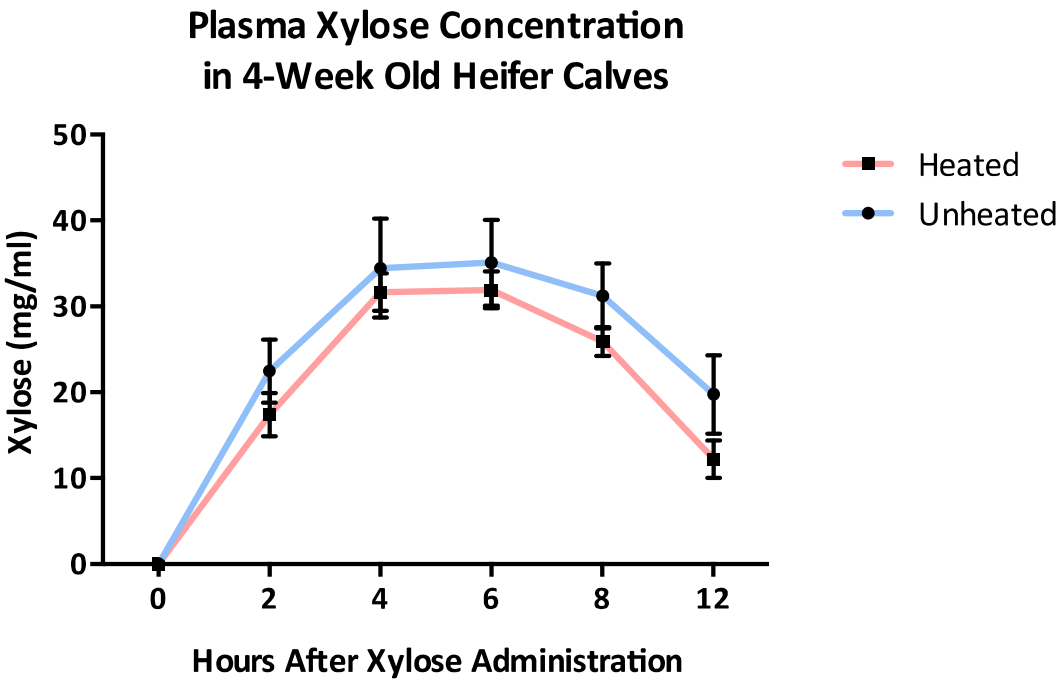

Figure 3.3: Plasma xylose concentration in 4-week old calves receiving unheated or heat-treated colostrum (P=0.05).

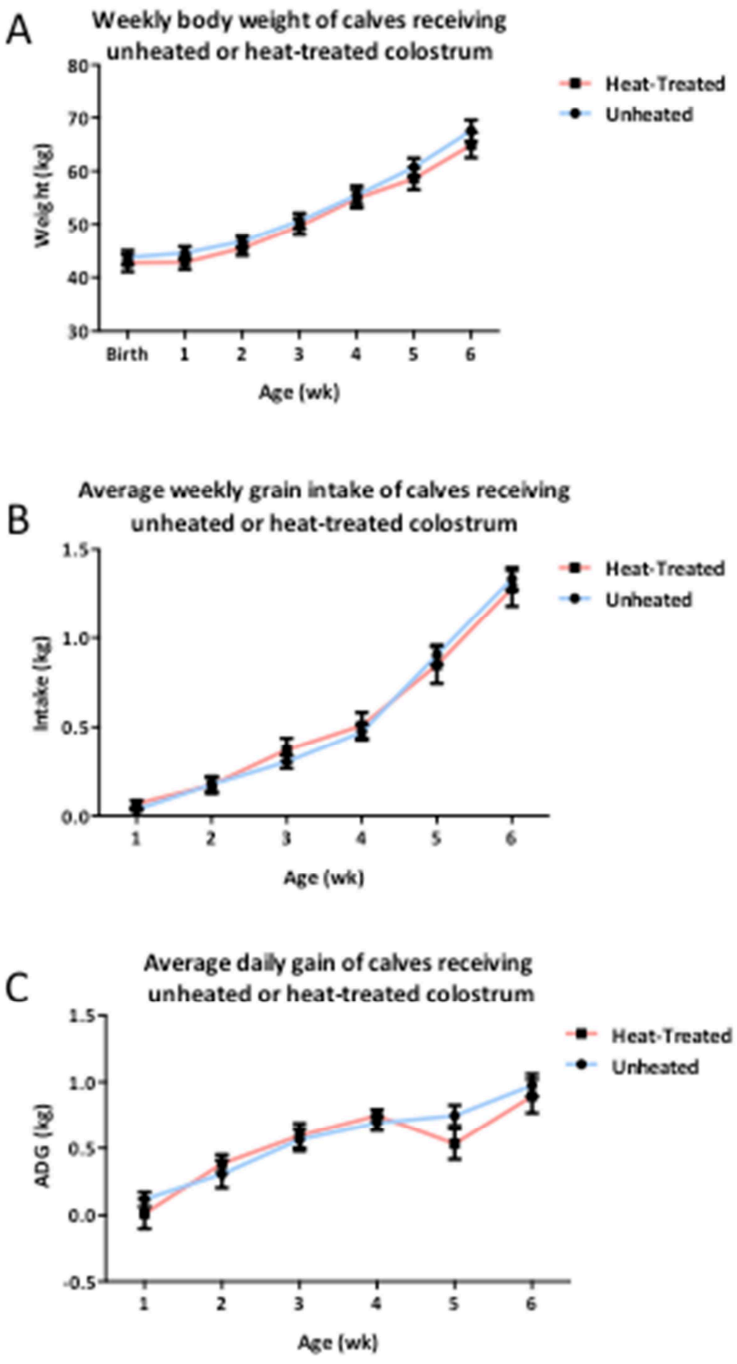

Figure 3.4: Weekly body weight (A), grain intake (B) and average daily gain (C) in calves receiving unheated or heat-treated colostrum.

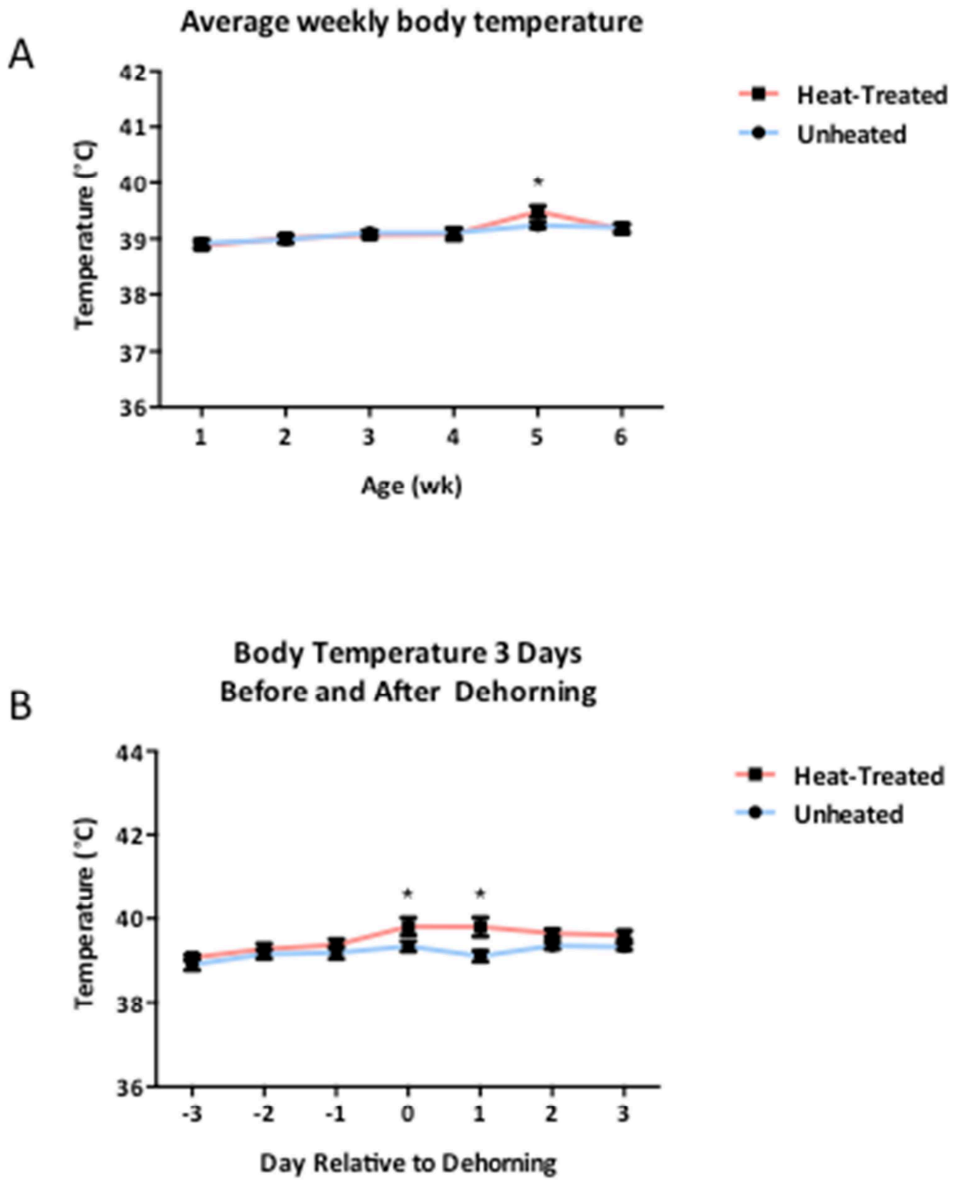

Figure 3.5: Average weekly body temperature (A) and daily body temperature 3 days before and after dehorning (B) in calves receiving unheated and heat-treated colostrum

3.5 References:

Acres, S. D. 1985. Enterotoxigenic Escherichia coli infections in newborn calves: a review. Journal of dairy science 68: 229-256.

Alfarawati, S. et al. 2011. The relationship between blastocyst morphology, chromosomal abnormality, and embryo gender. Fertility and sterility 95: 520-524.

Bascom, S. A., R. E. James, M. L. McGilliard, and M. Van Amburgh. 2007. Influence of dietary fat and protein on body composition of Jersey bull calves. Journal of dairy science 90: 5600-5609.

Bate, L.A., Ireland, W., Connell, B.J., Grimmelt, B., 1991. Development of the small intestine of piglets in response to prenatal elevation of glucocorticoids. Histol. Histopathol. 6: 207‑216.

Blum, J. W., and H. Hammon. 2000. Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. Livestock Production Science 66: 151-159.

DeNise, S. K., J. D. Robison, G. H. Stott, and D. V. Armstrong. 1989. Effects of passive immunity on subsequent production in dairy heifers. Journal of dairy science 72: 552-554.

Drackley, J. K., B. C. Pollard, H. M. Dann, and J. A. Stamey. 2007. First-lactation milk production for cows fed control or intensified milk replacer programs as calves. J. Dairy Sci 90: 614.

Eberts, T. J., R. H. Sample, M. R. Glick, and G. H. Ellis. 1979. A simplified, colorimetric micromethod for xylose in serum or urine, with phloroglucinol. Clinical chemistry 25: 1440-1443.

Edwards, L. J., and I. C. McMillen. 2002. Impact of maternal undernutrition during the periconceptional period, fetal number, and fetal sex on the development of the hypothalamo-pituitary adrenal axis in sheep during late gestation. Biology of Reproduction. 66: 1562–1569.

Elizondo-Salazar, J. A., and A. J. Heinrichs. 2009a. Feeding heat-treated colostrum or unheated colostrum with two different bacterial concentrations to neonatal dairy calves. Journal of dairy science 92: 4565-4571.

Elizondo-Salazar, J. A., and A. J. Heinrichs. 2009b. Feeding heat-treated colostrum to neonatal dairy heifers: Effects on growth characteristics and blood parameters. Journal of dairy science 92: 3265-3273.

Enders, A. C., and A. M. Carter. 2004. What can comparative studies of placental structure tell us?--A review. Placenta 25 Suppl A: S3-9.

Faber, S. N., N. E. Faber, T. C. McCauley, and R. L. Ax. 2005. Case Study: Effects of Colostrum Ingestion on Lactational Performance. The Professional Animal Scientist 21: 420-425.

Fordtran, J. S., P. H. Clodi, K. H. Soergel, and F. J. Ingelfinger. 1962. Sugar absorption tests, with special reference to 3-0-methyl-d-glucose and d-xylose. Annals of internal medicine 57: 883-891.

Godden, S. 2008. Colostrum management for dairy calves. The Veterinary clinics of North America. Food animal practice 24: 19-39.

Godden, S., S. McMartin, J. Feirtag, J. Stabel, R. Bey, S. Goyal, L. Metzger, J. Fetrow, S. Wells, and H. Chester-Jones. 2006. Heat-Treatment of Bovine Colostrum. II: Effects of Heating Duration on Pathogen Viability and Immunoglobulin G. Journal of dairy science 89: 3476-3483.

Godden, S. M. S. Smith, J. M. Feirtag, L. R. Green, S. J. Wells, and J. P. Fetrow. 2003. Effect of on-farm commercial batch pasteurization of colostrum on colostrum and serum immunoglobulin concentrations in dairy calves. Journal of dairy science 86: 1503-1512.

Godfrey, K. M., H. M. Inskip, and M. A. Hanson. 2011. The long-term effects of prenatal development on growth and metabolism. Seminars in reproductive medicine 29: 257-265.

Hae, D. J., and H. E. Swaisgood. 1990. Disulfide Bond Formation between Thermally Denatured Beta-Lactoglobulin and Kappa-Casein in Casein Micelles. Journal of dairy science 73: 900-904.

Hammon, H., and J. W. Blum. 1997. Prolonged colostrum feeding enhances xylose absorption in neonatal calves. Journal of animal science 75: 2915-2919.

James, R. E., C. E. Polan, and K. A. Cummins. 1981. Influence of administered indigenous microorganisms on uptake of [iodine-125] gamma-globulin in vivo by intestinal segments of neonatal calves. Journal of dairy science 64: 52-61.

Jensen, A. R., J. Elnif, D. G. Burrin, and P. T. Sangild. 2001. Development of intestinal immunoglobulin absorption and enzyme activities in neonatal pigs is diet dependent. The Journal of nutrition 131: 3259-3265.

Johnson, J. L., S. M. Godden, T. Molitor, T. Ames, and D. Hagman. 2007. Effects of Feeding Heat-Treated Colostrum on Passive Transfer of Immune and Nutritional Parameters in Neonatal Dairy Calves. Journal of dairy science 90: 5189-5198.

Laden, S. A., J. E. Wohlt, P. K. Zajac, and R. V. Carsia. 1985. Effects of stress from electrical dehorning on feed intake, growth, and blood constituents of Holstein heifer calves. Journal of dairy science 68: 3062-3066.

Lesmeister, K. E., and A. J. Heinrichs. 2004. Effects of corn processing on growth characteristics, rumen development, and rumen parameters in neonatal dairy calves. Journal of dairy science 87: 3439-3450.

Levieux, D., and A. Ollier. 1999. Bovine immunoglobulin G, β-lactoglobulin, α-lactalbumin and serum albumin in colostrum and milk during the early post partum period. Journal of Dairy Research 66: 421-430.

Liebhaber, M., N. J. Lewiston, M. T. Asquith, L. Olds-Arroyo and P. Sunshine. 1977. Alterations of lymphocytes and of antibody content of human milk after processing. J. Pediatrics. 91:897-900.

Merritt, A. M., and P. Duelly. 1983. Phloroglucinol microassay for plasma xylose in dogs and horses. Am. J. Vet. Res. 44:2184–2185.

Moallem, U. et al. 2010. Long-term effects of ad libitum whole milk prior to weaning and prepubertal protein supplementation on skeletal growth rate and first-lactation milk production. Journal of dairy science 93: 2639-2650.

Pakkanen, R., and J. Aalto. 1997. Growth factors and antimicrobial factors of bovine colostrum. International dairy journal / published in association with the International Dairy Federation 7: 285-297.

Priestley, D., J. H. Bittar, L. Ibarbia, C. A. Risco, and K. N. Galvao. 2013. Effect of feeding maternal colostrum or plasma-derived or colostrum-derived colostrum replacer on passive transfer of immunity, health, and performance of preweaning heifer calves. Journal of dairy science 96: 3247-3256.

Prioult, G., S. Pecquet, and I. Fliss. 2004. Stimulation of interleukin-10 production by acidic beta-lactoglobulin-derived peptides hydrolyzed with Lactobacillus paracasei NCC2461 peptidases. Clinical and diagnostic laboratory immunology 11: 266-271.

Quigley, J. 2002. Passive immunity in newborn calves. Adv Dairy Technol 14: 273-292.

Reber, A. J., D.C. Donovan, J. Gabbard, K. Galland, M. Aceves-Avila, K.A. Holbert, L. Marshall, D.J. Hurley. 2008. Transfer of maternal colostral leukocytes promotes development of the neonatal immune system Part II. Effects on neonatal lymphocytes. Veterinary immunology and immunopathology 123: 305-313.

Robison, J. D., G. H. Stott, and S. K. DeNise. 1988. Effects of passive immunity on growth and survival in the dairy heifer. Journal of dairy science 71: 1283-1287.

Shea, E. C., N. L. Whitehouse, and P. S. Erickson. 2009. Effects of colostrum replacer supplemented with lactoferrin on the blood plasma immunoglobulin G concentration and intestinal absorption of xylose in the neonatal calf. Journal of animal science 87: 2047-2054.

Soberon, F., E. Raffrenato, R. W. Everett, and M. E. Van Amburgh. 2012. Preweaning milk replacer intake and effects on long-term productivity of dairy calves. Journal of dairy science 95: 783-793.

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

Sutton, L. F., and B. Alston-Mills. 2006. β-lactoglobulin as a potential modulator of intestinal activity and morphology in neonatal piglets. The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology 288A: 601-608.

Swan, H. et al. 2007. Passive transfer of immunoglobulin G and preweaning health in Holstein calves fed a commercial colostrum replacer. Journal of dairy science 90: 3857-3866.

Wong, K. F., N. Middleton, M. Montgomery, M. Dey, and R. I. Carr. 1998. Immunostimulation of murine spleen cells by materials associated with bovine milk protein fractions. Journal of dairy science 81: 1825-1832.

Xu, K. P., B. R. Yadav, W. A. King, and K. J. Betteridge. 1992. Sex-related differences in developmental rates of bovine embryos produced and cultured in vitro. Molecular reproduction and development 31: 249-252.

4.0 General Discussion

4.1 Significance of study

Colostrum is one of the most important areas of management on a dairy farm, not only for calf health, but also for ensuring efficient production and longevity of the future cows in the herd. Previous work has focused heavily on studying immunoglobulin (Ig) absorption by the gut and the effects on calf mortality, morbidity and growth. Although Ig absorption is crucial to the health and survivability of the calf, rates of failure of passive transfer (FPT) continue to decrease (NAHMS, 2007). A large number of dairy farms are achieving more than adequate (higher than 10 mg/ml serum IgG in calves at 24 hours of life) rates of successful passive transfer and low mortality rates (NAHMS, 2007). Some current research is therefore turning its attention to the numerous other bioactive components in colostrum to investigate the role these may play in the calf.

Pasteurization of colostrum is a common management practice on dairy farms, and is used to prevent vertical transmission of disease to newborn calves. Recent research has demonstrated that heat-treatment of colostrum using low temperatures can positively influence uptake of IgG by the gut in the neonatal calf (Elizondo-Salazar and Heinrichs, 2009; Johnson et al., 2007). Limited research has studied the effect of pasteurization on other bioactives in colostrum, and the effect heat-treatment has on gut development, growth and health of the calf (Johnson et al., 2007; Lakritz et al., 2000). Therefore, the objective of the present study was to use the calf to investigate the effects of heat-treated colostrum on intestinal absorption and development, growth and health in neonatal and pre-weaned calves. This study found that pasteurization had a significant impact on absorption of colostral proteins in the gut. Absorption in both bull and heifer calves was affected by treatment. However, the effect of heat-treatment of colostrum resulted in different patterns of absorption, particularly with IgG. Whereas serum IgG concentration in bull calves appeared to be lower in those fed heat-treated colostrum, the concentration in heifer calves showed a contradictory effect. These results suggest that bull calves may not be appropriate to use in research when making inferences about heifer calves. The

bull calf experiment also suggested that heat-treatment didn't influence mucosal growth during the first 12 hours of life, as measured by villus height and crypt depth, but the number of goblet cells was increased by heat-treated colostrum. Moreover, the heifer experiment demonstrated that heat-treatment of colostrum may not influence gut absorptive capacity at 4 weeks of age, as shown by the measure of xylose absorption by the gut. Xylose absorption is used to estimate gut function, and is absorbed via glucose transporters (Hammon and Blum, 1997), indicating that it can be a measure of the uptake of glucose. Although there are many mechanisms by which nutrients and molecules are absorbed in the gut, the results of the present study suggest that heat-treatment of colostrum doesn't have lasting effects on uptake of glucose or similar small sugars. The heifer experiment also demonstrated an effect of heat-treatment on the response to management-induced stress, such as that which is caused by dehorning. Although the explanation for this result is not known, this finding indicates a difference in how calves respond to a stress or trauma when they are fed heat-treated or unheated colostrum.

4.2 Understanding the effects of heat-treated colostrum on absorption and gut development in the neonatal calf

Past research has focused on the effects of pasteurization of colostrum on IgG concentration and passive transfer of immunity (Johnson et al., 2007; McMartin et al., 2006). Investigation of the effects of heat-treatment on other bioactive compounds has mostly been done on human milk (Ewaschuk et al., 2011) and little is known about how the bioactivity of colostrum influences gut function in neonatal calves. The present study looked at the absorption of IgG as well as another common milk protein, β-lactoglobulin (BLG), that is thought to have an important influence on gut development and immunomodulation (Biziulevicius et al., 2006; Wong et al., 1998). The study also investigated the influence of heat-treated colostrum on mucosal growth and goblet cell numbers in the ileum of bull calves that were 6 or 12 hours of age. Analysis revealed that absorption of bioactive molecules by the calf and gut development could be significantly influenced by heat-treatment, in terms of goblet cell production. Measurement of gut

morphology also showed that there is considerable growth in the small intestine in the first 12 hours after birth.

Results from previous studies have shown that heifer calves receiving heat-treated, versus unheated, colostrum had significantly higher concentrations of circulating IgG, even though the colostral concentrations were not different (Elizondo-Salazar and Heinrichs, 2009). In the current study, bull calves receiving heat-treated colostrum had lower plasma IgG concentrations compared to those fed unheated-colostrum. Furthermore, when males and females were compared, regardless of colostrum treatment, analysis revealed that bull calves had lower circulating IgG. These results suggest that there are differences in absorption of IgG between male and female calves. The absorption of BLG also tended to be lower in bull versus heifer calves, further suggesting an effect of gender. Several factors could influence blood levels of metabolites, including blood volume, hormonal profiles or gender-specific prenatal development (Godfrey et al., 2011; Quigley, 2002), however these were not measured in the current study. More research is required to elucidate the exact cause of the differences seen in the present study. Although male calves are not usually kept on dairy farms, they are often used for research purposes. The present study provides evidence that caution should be used when using bull calves to make inferences about heifers. These results may also have implications for beef producers, who typically rear both male and female calves to weaning on cow-calf operations.

β-lactoglobulin is an abundant whey protein that has been shown to exhibit bioactive characteristics in neonatal animals (Prioult et al., 2004). Studies have shown immunomodulating functions, such as the stimulation of anti-inflammatory cytokines, as well as potential effects on gut development and gut uptake of other molecules (Prioult et al., 2004; Sutton and Alston-Mills, 2006). The effects of pasteurization on BLG have been investigated, but only with regard to the heating conditions used for processing bovine milk for human consumption. Heat-treatment of milk at these temperatures (65°C +) is known to cause aggregation of BLG to other molecules such as casein (Parris et al., 1991). In our study BLG concentration decreased by approximately 9%, suggesting that even when colostrum is heated at 60°C, there is still a negative effect on colostral BLG concentration, and likely other heat-labile constituents. Furthermore, calves that received

heat-treated colostrum had lower plasma BLG concentrations then those fed unheated colostrum. Analysis of the apparent efficiency of absorption (AEA) revealed that calves fed unheated colostrum only exhibited a higher percentage of colostral BLG at 9 hours, at which point calves receiving heat-treated colostrum significantly decreased in absorption. The results suggest that BLG was absorbed with equal efficiency in the first 6 hours, but by 9 hours the remaining BLG could have been bound to another molecule, such as casein and therefore was less efficiently absorbed. In combination with the difference in absorption efficiency, it is also likely, as the results from the current study indicate, that BLG has a short biological half-life, especially when compared to a protein such as IgG that has a much longer half-life.

As well as absorption of protein, the effects of feeding heat-treated colostrum on gut mucosal growth and goblet cell numbers were investigated. Bioactive compounds in colostrum, such as growth factors, are thought to be important for promoting growth and development of the intestinal mucosa (Pakkanen and Aalto, 1997; Yamashiro et al., 1989). Few studies have looked at the role of such bioactive factors in the calf in the first 12 hours of life, and, to our knowledge, no work has studied early effects of pasteurization of colostrum on gut development in the calf. The current study did not provide substantial evidence that heat-treatment altered villus or crypt growth during the first 12 hours of life. Numerically, at both 6 and 12 hours, villi in calves receiving heat-treated colostrum were higher. Due to the low sample size, a statistically significant different may not have been detectable. The current results suggest that heat-treatment of colostrum does not affect mucosal growth in neonatal calves in the first 12 hours.

There were higher numbers of goblet cells found in 12-hour bull calves receiving heat-treated colostrum. It was hypothesized that the bacteria levels in unheated colostrum would stimulate the proliferation of goblets cells, since previous research suggested that goblet cells and mucin production were increased as a result of bacteria in the gut (Deplancke and Gaskins, 2001). The results of the present study suggest that goblet cells may be influenced by other factors besides microbes. Goblet cell numbers can also be influenced by components of colostrum, as suggested by previous studies that show that feedstuffs can affect goblet cell and mucin production (Deplancke and Gaskins, 2001; McCracken et al., 1995).

4.3 Heat-treatment of colostrum influences passive transfer of immunity and immune response of pre-weaned heifer calves

In the present study, heifer calves that were fed heat-treated colostrum had significantly higher plasma IgG concentrations compared to those fed unheated colostrum. These results are in agreement with previous work that investigated pasteurization of colostrum (Elizondo-Salazar and Heinrichs, 2009; Johnson et al., 2007). Our study also measured the effect of heat-treatment on absorption of BLG in the heifer calves. Unlike the bull calves, there was no significant effect of colostrum treatment on heifer plasma BLG levels despite the 9% decrease in colostral BLG concentrations in heated versus unheated colostrum. Overall the heifers had higher AEA of BLG, especially at 6 hours, suggesting that the effect may be due to differences in blood volume between bull and heifer calves (Quigley, 2002). However, the birth weight of the bulls and heifers in the present study did not differ significantly. A more probable explanation is that there are specific developmental differences between males and females that contribute to differences in the interactions with bioactive components of colostrum (Bate et al., 1991). The exact physiological processes involved in the differences between the males and females in the current study are unknown, but the results suggest that bull calves may not be good representatives of heifer calves in research.

The pattern of uptake of IgG, TP and BLG over time in heifer calves was similar to that observed in the bull calves. Plasma IgG and TP peaked between 6 and 9 hours and subsequently began to level off, but remained at high levels over the first 12 hours and even into the first couple of weeks of life. The BLG plasma concentration, however, peaked at six hours, after which it decreased rapidly, reaching near zero levels by 48 hours. Although few studies (Kilshaw and Slade, 1980) describe the function, uptake and metabolism of BLG in the calf, the results of the present study show that it does not behave the same way as IgG, as seen by an apparently short biological half-life. Future

studies on colostrum should consider the numerous other compounds and their potential biological functions in the neonatal and pre-weaned calves.

An interesting finding in the heifer calf experiment was the influence of heat-treated colostrum on the response to the moderate, acute stress of dehorning. Dehorning is a commonly used practice that reduces the risk of injury, to other cows and humans. Even with the use of a local anesthetic, calves exhibit signs of moderate stress following the procedure (Doherty et al., 2007). Although overall body weight and height were not significantly affected by treatment over the course of the experiment, during the week post-dehorning, calves receiving heat-treated colostrum had lower average daily gain, despite the fact that grain intake during this period did not decrease. The average body temperature during the week after dehorning was also significantly higher in calves in the heat-treated group. To see whether this increase actually corresponded to dehorning, body temperature of the two treatment groups was analyzed for three days before and after dehorning occurred. During the three days prior, temperatures were normal and there were no significant differences between colostrum treatment groups. Body temperature on the day of dehorning (approximately 5 hours after the procedure) was significantly higher in the heat-treated group compared to that of the unheated group. The temperature remained higher for the following two days as well. Although both treatment groups increased in temperature following dehorning, calves in the heat-treated group exhibited a moderate fever, whereas the unheated group only had a low-grade fever. Although data from the current study did not suggest any longer-term affect, there may be implications for future growth and health. The above results indicate that heat-treated colostrum more negatively impacts the calf's response than the unheated colostrum. Previous research has demonstrated that there are large metabolic costs associated with activation of an immune response (Colditz, 2002). Because grain intake did not change, in the current study, it is possible that the effect on average daily gain was due to an increase in energy used to mount an immune response in the heat-treated group, in order to compensate for lower levels of important immune-modulating components, such as IgG or maternal leukocytes. Studies, in which calves were fed colostrum with and without cells, demonstrated that maternal cells are important stimulants of certain aspects of the calf's immune system, such as lymphocyte mobilization (Reber et al., 2008).

Colostrum is also known to stimulate cytokine production (Bessler et al., 1996), however it is unknown whether heat-treatment of colostrum could be involved in the mechanism of suppression or promotion of specific immune functions.

Overall, this study provides evidence that heat-treatment of colostrum can have a significant impact on gut development, absorption and health of calves in the neonatal and pre-weaned periods. These results emphasize the importance of considering the effects of bioactives in colostrum, not only with regards to immunoglobulins, but also with the hundreds of other components that help make up colostrum.

4.4 Future directions

Our study contributes to the knowledge on how heat-treatment of colostrum impacts absorption of protein, gut development, growth and health of the calf. However, many questions still remain. There are many bioactive factors in colostrum not analyzed in this study but that may have important implications for gut development and immune regulation, and may be affected by pasteurization. Although the goal of the present study was not to analyze the composition of colostrum, a more complete list of potential bioactives may have revealed other targets for future research. Future studies are also needed to investigate the mechanism by which heat-treatment of colostrum not only affects absorption of IgG in the calf, but also BLG and other bioactive compounds. Absorption and biological half-life are necessary to understand for determining the function and degree to which these compounds play a role in development of young calves.

The present study did not demonstrate an effect of heat-treated colostrum on mucosal growth, as measured by villus height and crypt depth, during first 12 hours of life. However, goblet cell numbers were increased when calves were fed heat-treated colostrum. Previous research has suggested that goblet cells and mucin production can be stimulated by components of colostrum and milk, and particularly certain proteins (Ganessunker et al., 1999; McCracken et al., 1995). A study investigating the specific mechanisms involved with goblet cell proliferation would better elucidate the impact of

bioactive compounds. Bacteria have also been indicated as a potential stimulator of goblet cell and mucin production (Kandori et al., 1996). One limitation of the present study was that the experiment was not designed for precise bacteriological measurements. The subsamples of colostrum had an extra freeze thaw cycle compared to the colostrum that was fed to the calves, which may have changed the microbial population, and may have affected the concentrations or properties of other bioactives. Also, results indicate that there was likely some contamination involved with collection and processing of the colostrum. Another limitation of the microbial analysis was that Standard Plate Count was used to determine a viable count of bacteria. This method provides an estimate of the level bacteria in a sample, but it is not optimized for every species of bacteria present.

Previous researchers have shown that heat-treatment of colostrum at 60°C for 60 minutes significantly reduces colostral bacteria (Elizondo-Salazar and Heinrichs, 2009; Johnson et al., 2007). However, future work should be designed for more precise and comprehensive bacteriological analysis of colostrum, under more controlled conditions, and how the microbes can affect gut development in the neonatal calf.

Future work should also include investigating the long-term effects of colostral bioactive factors, and whether some specific compounds may be beneficial when given to calves at a physiologically relevant concentration. For example, it is not known what component/s of colostrum may be responsible for the difference in response to dehorning observed in heifer calves. It is hypothesized that maternal cells in colostrum in unheated colostrum have an immune-modulating role in neonatal calves, influencing the way their immune system reacts to an acute "stressor", such as being dehorned. Future experiments could involve the administration of colostrum that is cell-free in order to answer some of the questions about the role of maternal cells that arise from this result. In the current study, calves fed heat-treated colostrum were studied until they reached the age of weaning. Along with the effects demonstrated during this period, bioactivity of colostrum may have important implications for long-term growth, health, productivity and longevity in cattle. Although the importance of passive transfer of immunity and IgG should not be overlooked, future work on colostrum should give significant consideration for the impacts of the other bioactive components essential for promoting growth and development of calves.

4.5 References:

Bessler, H., R. Straussberg, J. Hart, I. Notti, and L. Sirota. 1996. Human colostrum stimulates cytokine production. Biology of the neonate 69: 376-382.

Biziulevicius, G. A., O. V. Kislukhina, J. Kazlauskaite, and V. Zukaite. 2006. Food-protein enzymatic hydrolysates possess both antimicrobial and immunostimulatory activities: a "cause and effect" theory of bifunctionality. FEMS immunology and medical microbiology 46: 131-138.

Colditz, I. 2002. Effects of the immune system on metabolism: implications for production and disease resistance in livestock. Livestock production science 75: 257-268.

Deplancke, B., and H. R. Gaskins. 2001. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. Am J Clin Nutr 73: 1131S-1141S.

Doherty, T. J. et al. 2007. Effects of a concentrated lidocaine solution on the acute phase stress response to dehorning in dairy calves. Journal of dairy science 90: 4232-4239.

Elizondo-Salazar, J. A., and A. J. Heinrichs. 2009. Feeding heat-treated colostrum to neonatal dairy heifers: Effects on growth characteristics and blood parameters. Journal of dairy science 92: 3265-3273.

Enders, A. C., and A. M. Carter. 2004. What can comparative studies of placental structure tell us?--A review. Placenta 25 Suppl A: S3-9.

Ewaschuk, J. B., S. Unger, S. Harvey, D. L. O'Connor, and C. J. Field. 2011. Effect of pasteurization on immune components of milk: implications for feeding preterm infants. Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme 36: 175-182.

Ganessunker, D., H. R. Gaskins, F. A. Zuckermann, and S. M. Donovan. 1999. Total parenteral nutrition alters molecular and cellular indices of intestinal inflammation in neonatal piglets. JPEN. Journal of parenteral and enteral nutrition 23: 337-344.

Godfrey, K. M., H. M. Inskip, and M. A. Hanson. 2011. The long-term effects of prenatal development on growth and metabolism. Seminars in reproductive medicine 29: 257-265.

Hammon, H., and J. W. Blum. 1997. Prolonged colostrum feeding enhances xylose absorption in neonatal calves. Journal of animal science 75: 2915-2919.

Johnson, J. L., S. M. Godden, T. Molitor, T. Ames, and D. Hagman. 2007. Effects of Feeding Heat-Treated Colostrum on Passive Transfer of Immune and Nutritional Parameters in Neonatal Dairy Calves. Journal of dairy science 90: 5189-5198.

Kandori, H., K. Hirayama, M. Takeda, and K. Doi. 1996. Histochemical, lectin-histochemical and morphometrical characteristics of intestinal goblet cells of germfree and conventional mice. Experimental animals / Japanese Association for Laboratory Animal Science 45: 155-160.

Kilshaw PJ, Slade H. Passage of ingested protein into the blood during gastrointestinal hypersensitivity reactions: experiments in the preruminant calf. Clin Exp Immunol. 1980;44:575–582

Lakritz, J. et al. 2000. Effects of pasteurization of colostrum on subsequent serum lactoferrin concentration and neutrophil superoxide production in calves. American journal of veterinary research 61: 1021-1025.

McCracken, B. A., H. R. Gaskins, P. J. Ruwe-Kaiser, K. C. Klasing, and D. E. Jewell. 1995. Diet-dependent and diet-independent metabolic responses underlie growth stasis of pigs at weaning. The Journal of nutrition 125: 2838-2845.

McMartin, S. et al. 2006. Heat Treatment of Bovine Colostrum. I: Effects of Temperature on Viscosity and Immunoglobulin G Level. Journal of dairy science 89: 2110-2118.

National Animal Health Monitoring System. Dairy 2007. Heifer Calf Health and Management Practices on U.S. Dairy Operations. USDA:APHIS Veterinary Services, Ft. Collins, CO.

Pakkanen, R., and J. Aalto. 1997. Growth factors and antimicrobial factors of bovine colostrum. International dairy journal / published in association with the International Dairy Federation 7: 285-297.

Parris, N., J. M. Purcell, and S. M. Ptashkin. 1991. Thermal-Denaturation of Whey Proteins in Skim Milk. J Agr Food Chem 39: 2167-2170.

Prioult, G., S. Pecquet, and I. Fliss. 2004. Stimulation of interleukin-10 production by acidic beta-lactoglobulin-derived peptides hydrolyzed with Lactobacillus paracasei NCC2461 peptidases. Clinical and diagnostic laboratory immunology 11: 266-271.

Quigley, J. 2002. Passive immunity in newborn calves. Adv Dairy Technol 14: 273-292.

Reber, A. J., D.C. Donovan, J. Gabbard, K. Galland, M. Aceves-Avila, K.A. Holbert, L. Marshall, D.J. Hurley. 2008. Transfer of maternal colostral leukocytes promotes development of the neonatal immune system Part II. Effects on neonatal lymphocytes. Veterinary immunology and immunopathology 123: 305-313.

Shea, E. C., N. L. Whitehouse, and P. S. Erickson. 2009. Effects of colostrum replacer supplemented with lactoferrin on the blood plasma immunoglobulin G concentration and intestinal absorption of xylose in the neonatal calf. Journal of animal science 87: 2047-2054.

Sutton, L. F., and B. Alston-Mills. 2006. β-lactoglobulin as a potential modulator of intestinal activity and morphology in neonatal piglets. The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology 288A: 601-608.

Wong, K. F., N. Middleton, M. Montgomery, M. Dey, and R. I. Carr. 1998. Immunostimulation of murine spleen cells by materials associated with bovine milk protein fractions. Journal of dairy science 81: 1825-1832.

Yamashiro, Y., M. Sato, T. Shimizu, S. Oguchi, K. Maruyama, S. Kitamura. 1989. Possible biological growth factors in breast milk and postnatal development of the gastrointestinal tract. Acta paediatrica Japonica; Overseas edition 31: 417-423.

Appendices

Appendix I: Analysis of bacteria levels in colostrum

Duplicate samples were analyzed for bacteriology by Prairie Diagnostic Services, Saskatoon, Sk. The procedure used for analyses is outlined below. Standard Plate Count was requested, along with specific selection for *Streptococcus* spp., *Escherichia coli, Staphylococcus* spp., and *Bacillus* spp. Results were reported as the total cfu/ml for each species identified, and those counts were then used to estimate the total bacterial count in each of the colostrum samples.

1. Standard Plate Count (SPC) agar plates were used to detect a viable count of bacteria. Blood/MacConkey agar plates were also used to culture specific bacterial species in the colostrum samples. The plates were inoculated with 100 µl of colostrum by streaking.

2. Plates were incubated at 35°C for 48 hours.

3. Bacteria were identified based on whether they were gram positive of gram negative. Blood agar is used to grow *Streptococcus* and *Staphylococcus* spp., whereas MacConkey agar is selective for gram negative bacteria and can be used to differentiate lactose fermenters (*Bacillus* spp).

4. Colony forming units for all plate were determined. The counts of the individual bacteria species detected from the plates were used to estimate the total bacterial counts (cfu/ml) in colostrum.

Appendix II: Western blot for GLP-2 in gut tissue

In order to quantify the GLP-2 concentration in ileum mucosa, extracted gut protein was analyzed by Western blot. After numerous attempts to troubleshoot the procedure, detection of the peptide was unsuccessful. However, it is still hypothesized that GLP-2 secretion in the small intestine will be influenced by bioactives in colostrum.

Protein extraction from Ileum Mucosa

Frozen intestinal mucosa from Ileum was ground in liquid nitrogen using a chilled mortar and pestle. Tissue was weighed out (weights were typically 50 or 100mg, depending on the amount of ground tissue available) into 1.5 microcentrifuge tubes, which were kept on ice. A radio-immuno precipitation assay (RIPA) buffer (50 mM Tris-HCL, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 1% sodium deoxycholic acid, 0.1% SDS, 1 mM phenylmethylsulfonyl added fresh, protease inhibitors) was prepared and 500µl of the buffer was added to each tube, containing sample. A hand-held electric mortar (find actual name) was used to lyse the tissue for approximately 1 minute per sample. Samples were gently mixed for 15 minutes using a tube rotator in a cold room. Samples were centrifuged at 14,000 x g for 15 minutes at 4°C to pellet the cell debris. A second centrifugation was done if necessary to remove any floating particles. The supernatant was then transferred to a clean 1.5 mictrocentrifuge tube and a BCA assay was performed to determine total protein. Aliquots were stored at -80°C for later use.

Western Blot for GLP-2 Detection

For GLP-2 (3.9 kDa), 10-20% gradient tris-tricine precast gels (Mini-PROTEAN precast gel, 50 µl/well) (Gels and buffer formulas from Bio-Rad Laboratories, Inc., Hercules, CA, USA) were used as separating gels. Extracted protein from ileum mucosa (100 and 50 µg) was pipetted into wells with 20 µl sample buffer (200 mM Tris-HCl, pH 6.8, 2% SDS, 40% glycerol, 0.04% Coomassie Brilliant Blue G-250, 2% β-mercaptoethanol (added fresh)). A small protein ladder (Spectra Multicolor Low Range

Protein Ladder, Thermo Fisher Scientific Inc, Rockford, IL, USA) was run on each gel. A 1X running buffer (10x prepared: 1 M Tris, 1 M Tricine, 1% SDS, pH 8.3) used during electrophoresis. Gels were run at 50 volts for approximately 1 hour, after which the voltage was increased to 65-70 and run until the ladder was clearly separated and the dye front was about 2 cm from the bottom of the gel. Following electrophoresis, gels were allowed to equilibrate in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol) for 20 minutes, and proteins were then transferred onto hydrophobic polyvinylidene fluoride (Hybond-P, Amersham, GE Healthcare Life Sciences, Little Chalfont, UK) at 4ºC for 30 minutes at 100 volts (several different combinations of temperature and voltage were tried to find the ideal conditions for transferring small peptides).

Following the transfer, the membranes were temporarily stained with Ponceau S solution (0.1% w/v in 5% acetic acid; Sigma-Aldrich, St Louis, MO, USA) to verify transfer of protein onto the membrane. Membranes were then incubated in blocking solution, and followed by incubation with diluted primary antibody (Goat GLP-2 (C-20); Santa Cruz Biotechnology, Inc, Dallas, TX, USA) and diluted secondary antibody (donkey anti-goat IgG-HRP; Santa Cruz Biotechnology, Inc, Dallas, TX, USA). Optimal concentration of the primary and secondary antibodies was 1:1000 and 1:5000, respectively. Purified GLP-2 protein (Prospec-Tany TachnoGene Ltd., Israel) was used as a positive control. Table 5.1 outlines the various conditions that were tested in order to optimize the protocol for detection of GLP-2.

Five minutes prior to imaging, each membrane was covered in enhanced chemiluminescence (ECL) blotting substrate (Amersham, GE Healthcare Life Sciences, Little Chalfont, UK) for detection of horseradish peroxidase (HRP). A Typhoon Trio Variable Mode Imager (Amersham, GE Healthcare Life Sciences, Little Chalfont, UK) was used to visualize bands on the membrane.

Outcome of analysis

As shown in Table 5.1, many different conditions were tested. After some initial testing, it was assumed that there was non-specific binding between the antibodies and the milk (Carnation Instant Skim Milk Powder) used as the blocking reagent. Bovine

serum albumin (BSA) at 3% concentration resulted in a visible band at ~4 kDa, but lots of background (See Figure 5.1). Donkey serum (Sigma-Aldrich, St Louis, MO, USA) was tested and resulted in low background on the membranes, therefore was used for subsequent tests. The positive control was detectable (see Figure 5.2), but when added with gut sample it was no longer detectable. This indicated that the gut protein sample was impeding detection. Unfortunately, GLP-2 in gut tissue was not detected, regardless of conditions.

Table 5.1: Overview of conditions tested to optimize western blot for detection of GLP-2.

Date	Sample[1] (µg)	Positive control (µg)	Blocking solution[2]	Blocking time/temp	Time/temp in 1°Antibody[3]	Time/temp in 2°Antibody[3]	Quality of blot image[4]	Successful blot: CP/Sample[5]
20/01/2014	Not tested	15, 7, 1	5% Milk	1hr, RT	Overnight, RT	1hr, RT	Good	No
20/01/2014	Not tested	15, 7, 1	3% BSA	1hr, RT	Overnight, RT	1hr, RT	Poor	Yes
20/01/2014	Not tested	7	5% Milk	1hr, RT	Not Tested	1hr, RT	Good	No
06/02/2014	Not tested	10	2.5% Milk	0.5hr, RT	2.5hr, RT, (1%Milk)	1hr, RT, (1%Milk)	Poor	No
06/02/2014	100	10	5% DS	1hr, RT	1hr, RT	1hr, RT	Good	No/No
12/02/2014	100, 50	10	5% DS	2hr, RT	2hr, RT	1hr, RT	Good	Yes/No
12/02/2014	100, 50	10	5% DS	2hr, RT	Overnight, 4°C	1hr, RT	Good	Yes/No
12/02/2014	100, 50	10	10% DS	2hr, RT	2hr, RT	1hr, RT	Good	Yes/No
13/02/2014	Not Tested	10+100ug of sample	5% DS	2hr, RT	Overnight, RT	1hr, RT	Good	No

[1]Extracted protein from small intestine
[2]Blocking diluent: Tris Buffered Saline (10 mM Tris, 150 mM NaCl, pH 7.4) with 0.001% Tween.
[3]Antibodies were diluted with Tris Buffered Saline, no Tween
[4]Blots imaged using a Typhoon Trio Variable Mode Imager (Amersham, GE Healthcare Life Sciences, Little Chalfont, UK)
[5]Blots were considered successful if a band was detected at ~4 kDa
RT = Room Temperature
BSA = Bovine Serum Albumin
DS = Donkey Serum

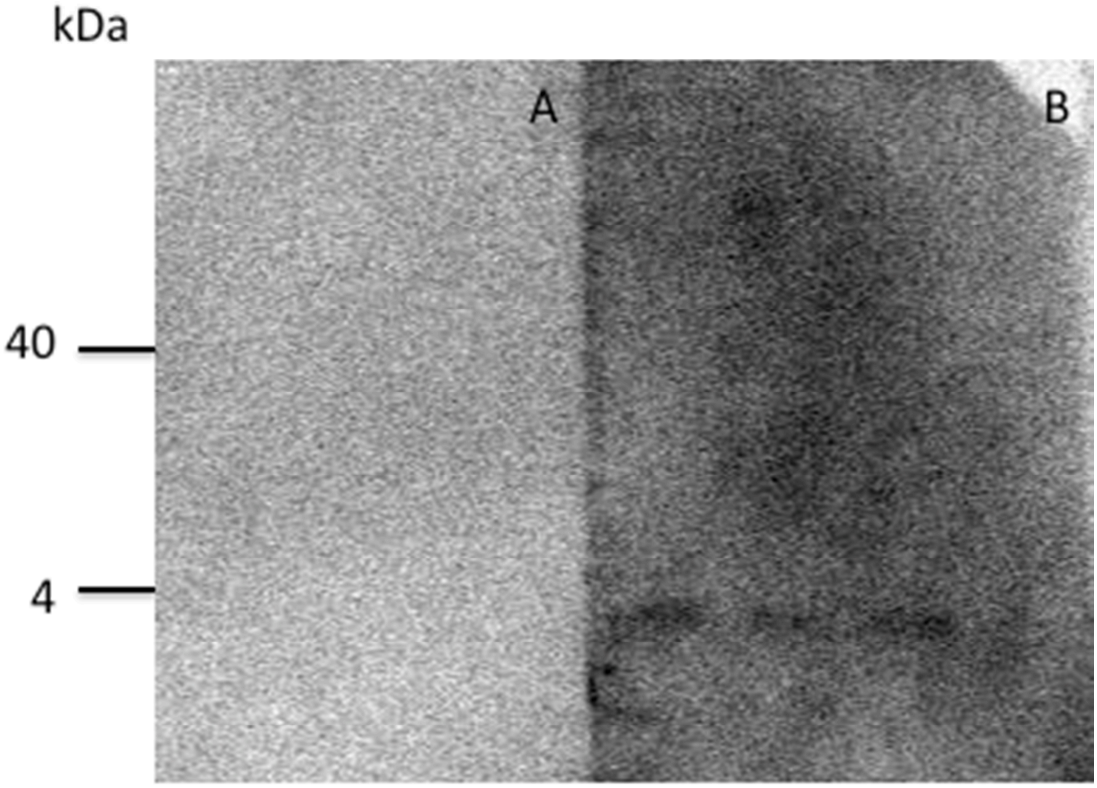

Figure 5.1: Western blot using 5% milk (A) and 3% BSA (B) as blocking solutions. Note the bands at ~4 kDa and the heavy background in B.

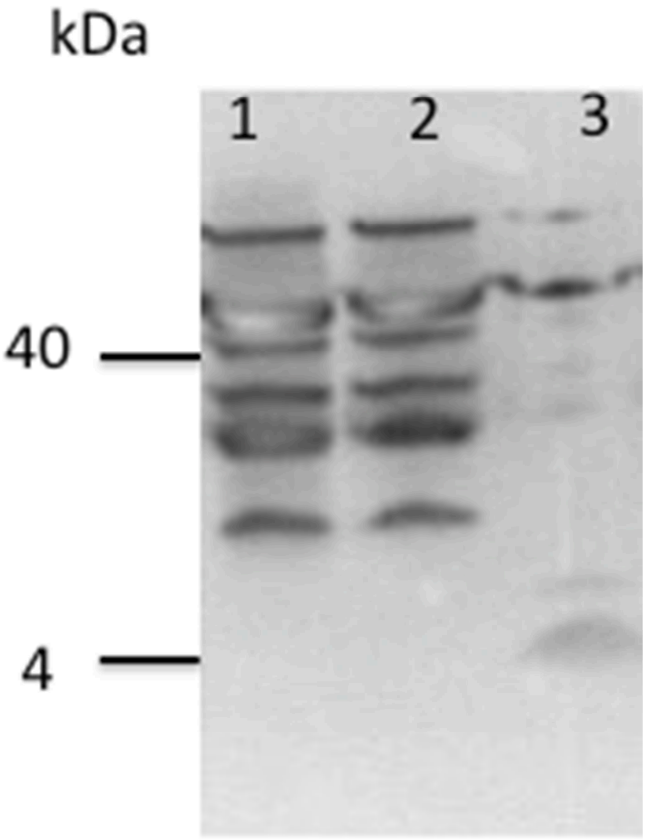

Figure 5.2: Lanes 1 and 2 are gut samples containing 100 and 50µg protein, respectively. No bands were detected at 4 kDa in gut samples. Lane 3 is the positive control, and has a band at ~4 kDa. Donkey serum was used as the blocking solution.