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HEAT TREATMENT OF BOVINE COLOSTRUM: EFFECTS ON PHYSICAL AND CHEMICAL PROPERTIES AND ON NEONATAL BLOOD AND GROWTH PARAMETERS

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ABSTRACT

Studies were conducted to: 1) identify the optimal temperature and time, at which heat treatment of bovine colostrum would produce the least significant changes in viscosity and IgG concentrations yet produce a significant reduction in bacterial count, 2) describe the effect of heat treatment of colostrum at 60°C for 30 min on colostrum characteristics including bacterial counts, IgG_1 and IgG_2 concentrations (g/L), and viscosity (Pa·s), 3) describe the effects of feeding heat-treated (vs. unheated) colostrum to neonatal dairy heifers on passive transfer of colostral immune parameters, health, and growth characteristics to 8 wk of age, and 4) determine the effects of feeding heat-treated colostrum and unheated colostrum with two different bacterial counts on passive transfer of immunity in neonatal bull calves.

In the first study, 10-mL colostrum samples were heat-treated for 0, 30, 60, or 90 min at 63, 60 or 57°C and evaluated for IgG_1 and IgG_2 concentrations, standard plate (SPC) count, preliminary incubation count (PIC), coagulase-negative staphylococci (CNS) count, environmental streptococci (ES) count, coliform (CC) count, gram-negative noncoliform (NC) count, *Streptococcus agalactiae* (SAG) count, and *Staphylococcus aureus* (SA) count. The results of the study showed that all heat treatments resulted in a significant reduction of SPC, CC, NC, ES, CNS, SA, and PIC. Heat-treatment at 60°C and above resulted in significant denaturation of colostral IgG_1; however, colostral IgG_2 concentrations were not significantly reduced when the temperature was held at 60°C for 30 or 60 minutes. Viscosity was not significantly affected when temperature was held at 60°C for 30 or 60 minutes.

In the second study, unheated or heat-treated colostrum was fed to newborn Holstein heifer calves to determine the effect on immunoglobulins G absorption, serum total IgG and serum total protein concentrations, lymphocyte counts, health scores, growth measurements, and

starter intake. Batch heat treatment of colostrum at 60°C for 30 min resulted in lower colostrum bacteria concentrations while maintaining colostral IgG concentration and viscosity. Calves fed heat-treated colostrum had significantly ($P < 0.01$) greater IgG concentrations at 24 h, plus greater apparent efficiency of IgG absorption (IgG = 23.4 g/L; apparent efficiency of absorption = 33.2%) compared with calves fed unheated colostrum (IgG = 19.6 g/L; apparent efficiency of absorption = 27.7%). There was no difference between treatment groups when examining lymphocyte counts, growth measurements, calf starter intake, or health scores.

In the third study, heat-treated colostrum or unheated colostrum with two different bacterial concentrations were fed to newborn Holstein bull calves to determine the effect on immunoglobulins G absorption, serum total IgG and serum total protein concentrations. Batch heat treatment of colostrum at 60°C for 30 min resulted in lower colostrum bacteria concentration while maintaining colostral IgG concentration. Calves fed heat-treated colostrum had significantly ($P < 0.01$) greater serum total protein IgG concentrations at 24 h, plus greater apparent efficiency of IgG absorption (total protein = 62.5 g/L; IgG = 26.7 g/L; apparent efficiency of absorption = 43.9%) compared with calves fed unheated-low bacteria colostrum (total protein = 57.0 g/L; IgG = 20.2 g/L; apparent efficiency of absorption = 35.4%) or unheated-high bacteria (total protein = 56.2 g/L; IgG = 20.1 g/L; apparent efficiency of absorption = 32.4%). High bacteria load in colostrum did not interfere with IgG absorption.

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Chapter 1

INTRODUCTION

Raising replacement dairy heifers provides an excellent opportunity to build for the future, since they are the foundation of any dairy enterprise. Approximately 25 to 32% of the milking herd are replaced annually (National Animal Health Monitoring System, 2007). Therefore, to maintain herd size and improve genetic potential for high milk production, quality replacements must be continuously available. Good management is essential to raising healthy calves and it should start with an adequate supply of colostrum, which is of crucial importance for the health status of newborn calves.

The syndesmochorial placentation in the bovine prevents the transmission of immunoglobulins from the dam to the fetus (Arthur, 1996). Consequently, passive immune protection is achieved by the ingestion and absorption of adequate amounts of colostral immunoglobulins, until the calf's own immune system becomes completely functional (Robinson et al., 1988; Weaver et al., 2000). The small intestine of the newborn calf remains permeable to intact large molecules, such as immunoglobulins and other proteins for approximately 24 h after birth (Broughton and Lecce, 1970; Stott and Menefee, 1978; Stott et al., 1979; Larson et al., 1980; Morin et al., 1997; Hopkins and Quigley, III, 1997). For this reason, to ensure adequate protection against disease exposure, calves rely on the consumption of an adequate amount of high immunoglobulin concentration colostrum within the first hours of life (Stott et al., 1979; Stott et al., 1981; Weaver et al., 2000). However, many diseases, including Johne's disease (*Mycobacterium avium* ssp. *paratuberculosis*), can be transmitted to calves via colostrum (Stabel, 2001) and the presence of bacteria in the small intestine at the time of

colostrum administration has been suggested to interfere with systemic absorption of immunoglobulin molecules (James et al., 1981).

Preventing contamination during harvest, storage, and feeding processes should be the first control point in feeding clean colostrum (Stewart et al., 2005). Freezing, refrigeration, and the use of preservative agents such as potassium sorbate are some of the management strategies to prevent bacterial proliferation in stored colostrum (Stewart et al., 2005). One additional method for reducing or eliminating bacterial pathogens is to heat-treat fresh colostrum (McMartin et al., 2006). Pasteurization studies on bovine colostrum have been done using the same times and temperatures recommended for milk (Godden et al., 2003). However, laboratory and field studies investigating the practice of heat-treating colostrum have been limited and have reported varying results with respect to effect of pasteurization on colostral immunoglobulin levels, viscosity, and rates of failure of passive transfer. Furthermore, there is a lack of information on short- and long-term calf health and performance when they are fed heat-treated colostrum (Johnson et al., 2007). Consequently, the objectives of this study were:

1) Present a comprehensive review of the literature of bovine colostrum pasteurization, including the importance of colostrum for the neonate, IgG absorption, and effects of pasteurization on bacterial load, viscosity and IgG concentration.

2) Identify the optimal temperature and timing at which heat treatment of bovine colostrum would produce small changes in viscosity and IgG levels, and significantly reduce pathogenic microorganisms so that they are less likely to cause health problems in neonate calves.

3) Determine the effects of feeding pasteurized colostrum on growth characteristics and blood parameters in neonatal dairy heifers.

4) Determine the effects of feeding heat-treated colostrum or unheated colostrum with two different bacterial concentrations on passive transfer of immunity in neonatal bull calves.

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Chapter 2

HEAT TREATMENT OF BOVINE COLOSTRUM

2.1 ABSTRACT

The syndesmochorial placenta of the bovine prevents the transmission of immunoglobulins in utero and calves are born agammaglobulinemic; consequently, ingestion and absorption of adequate amounts of colostral immunoglobulins is essential for establishing immunity, until the calf's own immune system becomes completely functional. A successful colostrum management program should consider age of calf at first feeding, volume of colostrum administered, and immunoglobulin concentration of the colostrum ingested. However, since several bacterial pathogens can be transmitted in colostrum, heat-treating fresh colostrum has been suggested as a method for reducing or eliminating those pathogens. Early studies on pasteurization of bovine colostrum using the same times and temperatures recommended for milk, have reduced or eliminated important bacterial pathogens; however, this process reduced immunoglobulin concentration and increased viscosity. More recent studies using lower temperatures have shown no reduction on colostral immunoglobulin concentration or increase in fluidity and have concluded that the practice of feeding heat-treated colostrum can be successfully adopted on commercial dairy farms without interfering with passive transfer of immunity in dairy calves. The objective of this study is to present a comprehensive review of the literature on bovine colostrum pasteurization, including the importance of colostrum for the neonate, IgG absorption, and effects of pasteurization on bacterial load, viscosity and IgG concentrations.

2.2 INTRODUCTION

Colostrum feeding is a critical step in raising healthy calves as a result of the physiology and metabolism of the bovine species. This has to do with one significant aspect in the placentation and fetal growth in the bovine. The syndesmochorial placenta of the bovine forms a syncytium between the maternal endometrium and the fetal trophectoderm, separating the maternal and fetal blood supplies and preventing the transmission of immunoglobulins in utero (Arthur, 1996). Although newborn calves are capable of mounting an immune response, they are best characterized as being immunonaive (Barrington and Parish, 2001). Consequently, ingestion and absorption of adequate amounts of colostral immunoglobulins are essential for establishing immunity, until the calf's own immune system becomes completely functional (Robinson et al., 1988; Weaver et al., 2000).

The transfer of immunoglobulins from the dam to the neonate, termed passive transfer, is important in the protection of the newborn from infectious disease. A condition that predisposes the neonate to the development of disease has been termed failure of passive transfer (FPT) (Weaver et al., 2000). It has been estimated that as many as 35% of dairy calves in the US suffered from FPT (Stott et al., 1979a; Brignole and Stott, 1980), making FPT a major economic consideration for dairy producers (Morein et al., 2007). Recent National Animal Health Monitoring System dairy studies reported that mortality rate among pre-weaned dairy heifers on United States farms averaged 7.9% (National Animal Health Monitoring System, 2007), and a great proportion of these deaths could be attributed to failure of passive transfer. Measuring serum total proteins (an indirect measure of colostral immunoglobulin absorption) in calves within the first 2 days of life is a relatively simple method for evaluating passive transfer;

however, only 2.1% of all operations in the United States routinely measured passive transfer via serum proteins (National Animal Health Monitoring System, 2007).

The small intestine of the newborn calf possesses the capacity to absorb intact large molecules, such as immunoglobulins and other proteins (Stott and Menefee, 1978; Larson et al., 1980; Morin et al., 1997; Hopkins and Quigley, III, 1997). The cessation of macromolecule absorption is termed closure and occurs at different times depending on the species (Broughton and Lecce, 1970). The exact mechanism behind closure has yet to be elucidated, but it has been proposed that it probably reflects a combination of exhaustion of pinocytotic capability and enterocyte replacement by a more mature population of gut epithelial cells (Broughton and Lecce, 1970). In calves, closure occurs at approximately 24 hours postpartum (Stott et al., 1979a). For this reason, the absorption of sufficient immunoglobulins that provide passive immunity to the calf must happen before this process occurs.

It has been known for many years that the cornerstones of a successful colostrum management program are age of calf at first feeding, volume of colostrum administered and immunoglobulin concentration of the colostrum ingested (Stott et al., 1979b,c,d). However, it has also been suggested that the presence of bacteria in the small intestine at the time of colostrum administration could interfere with systemic absorption of immunoglobulin molecules (James et al., 1981).

Several bacterial pathogens can be transmitted in colostrum and milk, whether by direct shedding from the mammary gland, postharvest contamination, or bacterial proliferation in improperly stored colostrum (Stewart et al., 2005). Some of the possible pathogens are *Campylobacter* spp., *Escherichia coli*, *Listeria monocytogenes*, *Mycoplasma* spp., *Mycobacterium avium* ssp. *paratuberculosis*, and *Salmonella* spp. (Doyle et al., 1987).

Preventing contamination during the harvest, storage, and feeding processes should be the first control point in feeding clean colostrum (Stewart et al., 2005). Some management strategies to prevent bacterial proliferation in stored colostrum include freezing, refrigeration, and the use of preservative agents such as potassium sorbate in refrigerated fresh colostrum (Stewart et al., 2005). One additional method for reducing or eliminating bacterial pathogens is to heat-treat fresh colostrum (McMartin et al., 2006). The adoption of commercial on-farm pasteurization systems for the purpose of pasteurizing nonsaleable milk has been reported to result in significant health and economic benefits for calves and producers, respectively (Jamaluddin et al., 1996). Pasteurization studies on colostrum have been done using the same times and temperatures recommended for milk (Godden et al., 2003). However, laboratory and field studies investigating the practice of heat-treating colostrum have been limited and have reported varying results with respect to effect of pasteurization on colostral IgG levels, viscosity, and rates of failure of passive transfer. Furthermore, there is a lack of information on short- and long-term calf health and performance (Johnson et al., 2007). Therefore, the objective of this study is to present a comprehensive review of the literature on bovine colostrum pasteurization, including the importance of colostrum for the neonate, IgG absorption, and effects of pasteurization on bacterial load, viscosity, and IgG concentrations.

2.3 IMPORTANCE OF COLOSTRUM

The immune system of the calf at birth does not possess sufficient capacity to produce antibodies to help fight infections (Morein et al., 2007). In turn, colostrum, the first secretion produced by the mammary gland after parturition, is especially rich in immunoglobulins

(Oyeniyi and Hunter, 1978; Foley and Otterby, 1978; Barrington et al., 2001; Madsen et al., 2004), which provide the calf immune protection during the first weeks of life (Nousiainen et al., 1994).

Colostrum not only provides passive immunity for the newborn calf, but it can also have profound effects on the development of the neonatal intestine, since it contains a number of bioactive and growth-promoting substances (Table 2.1) such as peptide hormones, growth factors, cytokines, steroid hormones, thyroxine, nucleotides, polyamines, enzymes, lactoferrin, lysozymes, insulin, cytokines, IGF-1, and IGF-2 (Koldovsky, 1989; Pakkanen and Aalto, 1997; Hagiwara et al., 2000). It has been demonstrated that villous circumference, area, height, and height/crypt depth ratio in the duodenum are higher for calves fed colostrum compared with colostrum-deprived calves (Buhler et al., 1998; Blattler et al., 2001). Calves fed colostrum also have higher plasma xylose concentrations after oral administration of xylose compared with calves fed milk replacer, suggesting enhanced absorptive capabilities in colostrum-fed animals (Hammon and Blum, 1997; Kuhne et al., 2000).

Colostrum is also important as the first source of nutrients for the calf after birth. It contains proteins, essential and nonessential amino acids, fatty acids, lactose, vitamins, and minerals (Table 2.1). Except for lactose, colostrum contains nutrients in higher concentrations than does mature milk (Foley and Otterby, 1978; Koldovsky, 1989; Blum and Hammon, 2000). Contents of energy, protein, fat and some minerals are well known to be markedly higher in colostrum than in mature milk (Davis and Drackley, 1998). It is important to emphasize that the concentration of proteins and peptides diminishes quickly after the onset of the lactation (Hadorn and Blum, 1997; Madsen et al., 2004). Likewise, the concentration of immunoglobulins is

significantly reduced in subsequent milkings (Oyeniyi and Hunter, 1978; Stott et al., 1981a; Davis and Drackley, 1998).

2.4 IMMUNOGLOBULINS IN COLOSTRUM AND THEIR IMPORTANCE

Colostrum contains large quantities of immunoglobulins (Kehoe et al., 2007) that are transferred from the cow's bloodstream (Larson, 1958; Sasaki et al., 1977; Larson et al., 1980; Butler, 1983; Barrington et al., 1997a). Transport of immunoglobulins from the serum to the mammary gland begins several weeks before parturition and reaches a peak 1 to 3 days before parturition in the cow (Sasaki et al., 1976; Barrington et al., 1997b). There are three major types of immunoglobulins in bovine colostrum: G, M and A (Butler, 1969; Pakkanen and Aalto, 1997). The distribution of the different classes of immunoglobulins in colostrum is very variable among cows (Stott et al., 1981b; Petrie, 1984). The IgG, IgA and IgM typically account for 85%, 5% and 7% of the total of immunoglobulins in colostrum, respectively (Sasaki et al., 1976; Larson et al., 1980). Bovine IgG can be divided into two subclasses: IgG_1 and IgG_2 (Butler, 1969). IgG_1 comprises more than 90% of the total IgG (Pakkanen and Aalto, 1997; Barrington et al., 2001). Although IgG_1 and IgG_2 are present at approximately equal concentration in ruminant blood, only the IgG_1 subclass is transported in large amounts from the maternal plasma across the alveolar epithelial cells into the mammary secretions (Larson et al., 1980; Baintner, 2007), facilitated by receptors present on these cells (Butler, 1983; Barrington et al., 1997b). Glandular epithelial cells cease expressing this receptor at the beginning of lactation (Butler, 1983; Barrington et al., 1997b). The bovine neonatal Fc receptor for IgG (FcRn) was recently cloned and its expression was demonstrated in multiple tissues, including the mammary gland and the

small intestine (Kacskovics et al., 2000); however, how FcRn is involved in mammary IgG transport has not been directly assessed (Mayer et al., 2005).

In spite of the fact that the other classes of immunoglobulins have important physiological roles, the predominant quantity of IgG makes the measurement of the concentration of total IgG or IgG_1 in the neonate blood serum an adequate indicator of the transfer of passive immunity (Besser and Gay, 1985).

Increased neonatal mortality and morbidity is a well-accepted consequence of failure of passive transfer. Virtala et al. (1999) showed that low postcolostral serum IgG level is a significant risk factor for the development of pneumonia in heifer calves. A study carried out by Wells et al. (1996) concluded that lack of colostral feeding was highly associated with neonatal death loss in the United States. Donovan et al. (1998), in a prospective study to determine calf-level factors that affected calf health status between birth and six months of age, showed that there was a clear association between serum total protein and mortality. Calves with low serum total protein values (< 50 g/L) were 3 to 6 times more likely to die within the first 6 months of life than those with serum total protein concentrations > 60 g/L. In a study by Nocek et al. (1984), calves deprived of colostrum gained poorly and suffered severe and long scour episodes and high mortality. Calves fed colostrum with high immunoglobulin concentration gained weight from birth to day 4 while those fed colostrum with low immunoglobulin concentration lost weight. Overall severity and duration of scours were less for calves fed colostrum with high compared to low immunoglobulin concentrations. In another study, Besser and Gay (1985) demonstrated that calves with high serum IgG concentrations had lower mortality rates from both enteritis and respiratory disease than calves with serum IgG concentrations of less than 10 g/L. Robinson et al. (1988) evaluated the effects of 24 to 48 h serum immunoglobulin

concentration on growth and survival of 1,000 Holstein heifer calves. They concluded that maternally derived antibody has a significant role in providing protection to the calf. Insufficient serum immunoglobulin concentrations at 24 to 48 h could necessitate an immune response by the calf before it is immunologically capable of handling an invasion of pathogenic organisms. Illnesses often associated with such invasions detract from the normal growth and development of the calf. Calves with adequate serum immunoglobulins often are able to inactivate pathogenic invasions earlier than calves with lower serum immunoglobulins that must mount an immune response for defense. Therefore, calves having adequate serum immunoglobulins will continue to grow normally and not be deterred as would calves with insufficient immunoglobulins (Robinson et al., 1988).

2.5 IgG ABSORPTION IN THE NEONATAL DAIRY CALF

As it has been stated, newborn calves obtain maternal antibodies solely from colostrum (Stott et al., 1976; Stott et al., 1979b). Maternal IgG and other constituents of colostrum are transported across the neonatal intestinal epithelium within the first 24 h of life (Kacskovics, 2004), travel through the lymphatics, and enter blood circulation via the thoracic duct (Balfour and Comline, 1962; Besser and Gay, 1994; Radostis et al., 2007). There is some controversy on how IgG and other proteins are taken up in the small intestine of the newborn. Non-selective pinocytosis has been pointed out as the mechanism for IgG to be transported across the intestinal epithelium (Besser and Gay, 1985; Baintner, 2002). However, recent evidence has pointed toward a role for the neonatal Fc receptor (FcRn) in these processes. The FcRn is composed of two subunits, β_2-microglobulin and an integral membrane polypeptide homologous to the Major

Hystocompatability Complex (MHC) class I proteins (Simister and Mostov, 1989). It binds IgG in a pH-dependent manner and was first described as an IgG transporter in the neonatal gut of rodents (Rodewald, 1976). The bovine neonatal Fc receptor (bFcRn) has been characterized, and its expression has been found in multiple tissues, including the mammary gland, small intestine, kidney and liver (Kacskovics et al., 2000). In previous studies, the obvious change in the subcellular localization of the receptor in the mammary epithelial cells around the time of parturition in ewes and its presence in the crypt epithelial cells of the neonatal lamb (Mayer et al., 2002) as well as in the lower respiratory tract (Mayer et al., 2004), led to the hypothesis that this receptor is involved in IgG transport across these barriers and by analogy with the human and mouse FcRn, it is expected to protect circulating IgG from catabolism. This hypothesis is further supported by the fact that allotypic variants of both the heavy and the light chains of the bFcRn influence serum IgG concentration in newborn calves (Laegreid et al., 2002; Clawson et al., 2004).

2.6 PATHOGENS IN MILK AND COLOSTRUM

Several bacterial pathogens can be transmitted in colostrum and milk, whether by direct shedding from the mammary gland, postharvest contamination, or bacterial proliferation in improperly stored colostrum (Stewart et al., 2005). Numerous studies have demonstrated that pasteurization of milk and colostrum effectively kills pathogens such as *Mycoplasma bovis*, *Mycoplasma californicum*, *Escherichia coli*, *Salmonella spp., Listeria monocytogenes*, and others (Butler et al., 2000; Stabel, 2001; Stabel et al., 2004). However, there is a major concern with *Mycobacterium avium* ssp. *paratuberculosis,* which causes a chronic, progressive enteric

disease in ruminants known as Johne's disease or paratuberculosis (Sung and Collins, 1998; Grant et al., 2005; McDonald et al., 2005).

Cattle become infected with *M. paratuberculosis* as calves but often do not develop clinical signs until 2 to 5 yr of age (Stabel, 2001). An important aspect is that *M. paratuberculosis* is shed in the colostrum and milk of clinically infected cows (Stabel, 2001). Therefore, it is imperative that once Johne's disease is diagnosed in a herd, management techniques are employed to further prevent the spread of this disease (Stabel, 2001). A recommendation for controlling the spread of infectious disease within a herd is to feed colostrum from non-infected dams. However, these recommendations come at considerable expense to the producer who must dump colostrum because it may be a source of *M. paratuberculosis* or other pathogens and purchase colostrum or milk replacer from outside sources. This recommendation can also limit colostrum availability if a high percentage of the herd is infected. Also, in most situations producers do not know which cows are infected.

Today, producers are willing to implement control measures such as on-farm pasteurization of colostrum to destroy potential pathogens (Stabel, 2001); however, technical issues inherent in pasteurization may be one reason that dairies have been slow to adopt this management practice, as demonstrated by the recent National Animal Health Monitoring System (2007) dairy studies, which report that only 0.8% of operations that hand-fed colostrum pasteurized the colostrum before feeding it to calves. A higher percentage of large operations (6.4%) pasteurized colostrum compared to medium and small operations (0.9 and 0.2%, respectively).

An on-farm batch pasteurizer unit (65.5°C for 30 min) was demonstrated to destroy *M. paratuberculosis* in waste milk (Stabel, 2001). On-farm pasteurization of waste milk held at

65°C for 10 min also destroyed common mastitic mycoplasma such as *M. bovis, M. californicum*, and *M. canadense* (Butler et al., 2000). In another study, Stabel et al. (2004) evaluated the efficacy of a commercial high temperature-short time (HTST) pasteurizer unit in the destruction of *M. paratuberculosis, Salmonella* spp., and *Mycoplasma* spp. in raw milk and *M. paratuberculosis* in colostrum. They showed that a commercial HTST (71.7°C for 15 s) unit was effective in the destruction of *M. paratuberculosis, Salmonella* spp., and *Mycoplasma* spp. in raw milk. Heat treatment of colostrum in a low temperature range (63.9 to 66.7°C) did not immediately destroy the *M. paratuberculosis* present. However, recovery of *M. paratuberculosis* from colostrum was reduced by 2 $\log_{10}$ after 10 min and achieved a nadir of < 3 cfu/mL after 30 min. Increasing the temperature range (68.3 to 70.8°C) completely abrogated recovery of viable *M. paratuberculosis* from the colostrum. Stabel et al. (2004) concluded that HTST pasteurization is effective for the destruction of *M. paratuberculosis, Salmonella* spp., and *Mycoplasma* spp. in raw milk and effectively destroys *M. paratuberculosis* in colostrum, providing dairy producers with an alternative to purchasing commercial replacement products and resulting in reduced costs. More importantly, pasteurization of waste milk and colostrum significantly reduces calf morbidity and helps break the transmission cycle of infectious pathogens on-farm.

2.7 PASTEURIZATION OF COLOSTRUM

Pasteurization is the process of heating liquids for the purpose of destroying viruses and harmful organisms such as bacteria, protozoa, molds, and yeasts (Jay, 2000). Unlike sterilization, pasteurization is not intended to kill all microorganisms. Instead, pasteurization aims to achieve a reduction in the number of viable organisms, reducing their number so they are unlikely to cause

disease. There are two common methods of pasteurizing: batch pasteurization and high temperature-short time (HTST) continuous flow pasteurization.

Batch pasteurization is accomplished when a batch (usually a vat or tank) of colostrum is heated to 63°C (145°F) for 30 minutes (Jay, 2000). Thereafter, the colostrum is cooled and can be fed to calves. Batch pasteurizers must be equipped with an agitator to allow for even heating. Batch size affects pasteurization time, since very large batches may take several hours to reach the desired temperature, and it is a concern that some bacteria may become heat resistant and survive the pasteurization process.

High temperature-short time pasteurization is different from batch pasteurization. In this method, colostrum is circulated through a network of heated coils, rapidly heated to 72°C (161°F) and held there for 15 seconds (Jay, 2000). This type of system is equipped to automatically cool the colostrum quickly to feeding or storage temperature. Continuous flow pasteurization is much more rapid than batch pasteurization and offers more opportunities for energy conservation. Continuous flow systems are generally more difficult to clean, requiring a cleaning procedure similar to that used in milking systems, yet in many cases the cleaning process can be automated.

Different laboratory and field studies have investigated the practice of pasteurizing colostrum. In order to simulate pasteurization of colostrum under laboratory conditions, Meylan et al. (1996) heated 5-mL volumes of a total of 18 colostrum samples to 63°C for 30 min. Mean (± SD) IgG values for fresh and pasteurized samples were 44.4 (± 30.3) g/L and 37.2 (± 23.8) g/L, respectively. This study reported a mean loss of immunoglobulins after pasteurization of 12.3 ± 8.7%. The authors concluded that this 12.3% loss was manageable; assuming that the quality of colostrum was determined by a colostrometer prior to heat treatment and the amount

fed was adjusted to ensure successful passive transfer of immunity. One field study, Godden et al. (2003) investigated the effect of on-farm commercial batch pasteurization on IgG concentrations and the fluid and feeding characteristics of colostrum and compared serum IgG concentrations in calves fed fresh versus pasteurized colostrum. Results showed that batch pasteurization (63°C for 30 min) reduced colostrum IgG concentration, with the percentage reduction averaging 58.5 and 23.6% for 95-L and 57-L batches, respectively. The pasteurization of 57-L batches produced lower levels of IgG denaturation and colostrum consistency was normal or mildly thickened. Serum IgG concentrations were higher for calves fed fresh colostrum than for calves fed pasteurized colostrum and for calves with a shorter time interval (≤ 6 h) between first and second colostrum feedings. After controlling for the time interval between feedings, serum IgG concentrations were significantly higher for 40 calves fed unpasteurized (19.1 g/L) vs. 55 calves fed pasteurized colostrum (9.7 g/L) for calves fed 2 L at first feeding. By contrast, there was no difference in serum IgG concentrations between 8 calves fed unpasteurized (16.1 g/L) and 20 calves fed pasteurized colostrum (13.5 g/L) when calves were fed 4 L at the first feeding. However, it should be pointed out that there were some weaknesses in the study's design in the sense that pooled batches of colostrum were not split each time after batch assembly so that half the calves could be fed pasteurized colostrum and the other half unpasteurized colostrum. Therefore, colostrum batch was confounded by treatment group. For this reason, the authors indicated that the results were preliminary and should be interpreted with caution. In another study, McMartin et al. (2006) wanted to identify the critical temperature, at or below which heat treatment of bovine colostrum would produce no significant changes in viscosity, IgG concentration, or immunoglobulin activity. They presented results of preliminary work, using a Rapid Visco Analyzer (RVA) to heat 50-mL aliquots from 6 unique batches of

bovine colostrum at 59, 60, 61, 62, and 63°C, which suggested that colostrum could be heated to 60°C for up to 120 min without changing viscosity or IgG concentration. They later wanted to confirm the finding by heating 50-mL aliquots from 30 unique batches of colostrum in an RVA for 120 min at 60 and 63°C. They showed that heating colostrum to 63°C resulted in a 34% decrease in IgG concentration and 33% increase in viscosity. However, there was no difference in IgG concentration between pre-heat-treated (73.4 ± 26.5 g/L) and post-heat-treated (74.5 ± 24.3 g/L) samples after heating colostrum to 60°C in an RVA for 120 min. Similarly, viscosity was unaffected after heating colostrum to 60°C in an RVA for 120 min. High quality colostrum (≥ 73.0 g of IgG/L) suffered greater losses of IgG and greater viscosity changes when heated to 63°C than did moderate quality colostrum (< 73.0 g of IgG/L). However, the effects of colostrum quality were minor if high quality colostrum was only heated to 60°C. The results of a bovine viral diarrhea serum neutralization assay suggested that antibody activity was unchanged after heating colostrum to either 60 or 63°C. However, these results were interpreted as being inconclusive due to a high proportion of missing results because of the congealing of many samples after heat treatment. The results of their study indicated that 50-mL volumes of bovine colostrum may be heat-treated at 60°C for up to 120 min in an RVA without affecting IgG concentration or viscosity (McMartin et al., 2006).

In one experiment, Godden et al. (2006) inoculated 30-L batches of first-milking bovine colostrum with *Mycoplasma bovis* (10^8 cfu/mL), *Listeria monocytogenes* (10^6 cfu/mL), *Escherichia coli* O157:H7 (10^6 cfu/mL), *Salmonella enteritidis* (10^6 cfu/mL), and *Mycobacterium avium* subsp. *paratuberculosis* (MAP; 10^3 cfu/mL). The colostrum batches were heat-treated at 60°C for 120 min in a commercial on-farm batch pasteurizer system. Sub-samples of colostrum were collected at 15-min intervals throughout the heat treatment process for the

purpose of bacterial culture and for measurement of IgG concentration (g/L) and antibody activity [log_2(bovine viral diarrhea virus type 1 serum neutralization titer)]. They found no effect of heating moderate- to high-quality colostrum at 60°C for at least 120 min on mean IgG concentration (pre = 60.5 g/L; post = 59.1 g/L). Similarly, there was no effect of heat treatment on the mean log_2 bovine viral diarrhea virus type 1 serum neutralization titer (pre = 12.3; post = 12.0). Viable *M. bovis, L. monocytogenes, E. coli* O157:H7, and *S. enteritidis* added to colostrum could not be detected after the colostrum was heat-treated at 60°C for 30 min. Their average bacteria counts showed that MAP was not detected when batches were heated at 60°C for 60 min. Although the authors stated that heat-treating colostrum at 60°C for 60 min should be sufficient to eliminate MAP from colostrum in most situations. They concluded that further research is needed to determine whether these findings may be replicated, given that variability was observed in MAP culture results.

One field study, using a HTST pasteurization method (72°C for 15 s), reported that total colostral IgG mass received by 150 calves fed pasteurized colostrum (mean = 151.4 g) was significantly lower than for 150 calves fed unpasteurized colostrum (mean = 203.1 g) (Jamaluddin et al., 1996). Yet, there was no statistical difference in the number of calves experiencing FPT (based on < 10 g/L of total serum IgG measured at 48 to 96 h after colostrum intake) between treatment (16.2%) and control (19.5%) groups. Similarly there was no difference in mean serum IgG concentrations between treatment (14.76 g/L) and control (14.35 g/L) groups. The lack of difference is likely due to the large quantity of IgG fed to both groups of calves.

In another study, with the objective to describe the effect of feeding heat-treated (60°C for 60 min) colostrum versus raw colostrum on passive transfer of colostral immune and nutritional parameters in neonatal calves, Johnson et al. (2007) found that calves fed heat-treated

colostrum had significantly greater serum total protein and IgG concentrations at 24 h and therefore greater apparent efficiency of IgG absorption (total protein = 63 g/L; IgG = 22.3 g/L; apparent efficiency of absorption = 35.6%) compared with calves fed raw colostrum (total protein = 59 g/L; IgG = 18.1 g/L; apparent efficiency of absorption = 26.1%). The authors found no effect of treatment on serum concentrations of IgA, IgM, vitamin A, vitamin E, cholesterol, ß-carotene or vitamin E to cholesterol ratio, or on serum bovine viral diarrhea virus type I serum neutralization titers. There was no difference between treatment groups when examining calf plasma total leukocyte counts, neutrophil counts, lymphocyte counts, or neutrophil opsonization activity. However, the latter results were considered inconclusive since it has yet to be determined if neonatal calves can absorb non-dam colostral leukocytes and if passive absorption of colostral cellular immune fractions or functions of these cell fractions are affected by heat-treating colostrum.

2.8 HYPOTHESIS ON WHY FEEDING HEAT-TREATED COLOSTRUM INCREASES IgG ABSORPTION

It has not been demonstrated why feeding heat-treated colostrum increases IgG absorption. The current hypothesis suggests that the presence of bacteria in the small intestine at the time of colostrum administration could interfere with systemic absorption of immunoglobulin molecules (James and Polan, 1978; James et al., 1981; Staley and Bush, 1985). Possible mechanisms for this effect could include competition between microbes and IgG molecules for common receptors on the intestinal epithelial cells, or physical binding of colostral IgG by microbes within the intestinal lumen, thus decreasing the availability of transportable IgG (James and Polan, 1978; James et al., 1981; Staley and Bush, 1985). There may be more than one

possible explanation to this phenomenon. Therefore, it is necessary to carry out more research in order to establish the mechanisms behind the increase in IgG absorption.

2.9 CONCLUSIONS

It has been established that heat treatment of bovine colostrum utilizing the same times and temperatures recommended for milk resulted in denaturation of colostral IgG and significant increases in viscosity; however, heat treatment at 60°C is enough to reduce bacterial load with low reduction in IgG levels and no increase in viscosity. Although these are important findings describing effects of heat treatment on colostrum, there is still a lack of information on the short- and long-term calf health and performance of feeding heat-treated colostrum on commercial dairy farms.

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Table **2.1**: Composition of bovine colostrum and mature milk.

	Colostrum milking			Mature
Nutrient or compound	1	2	3	milk
Dry matter, g/L	245.00	190.00	160.00	122.00
Crude ash, g/L	18.00	10.00	10.00	7.00
Gross energy, MJ/L	6.00	4.80	3.90	2.80
Crude fat, g/L	64.00	56.00	46.00	39.00
Crude protein, g/L	133.00	85.00	62.00	32.00
Solids-non-fat, g/L	167.00	122.00	98.00	88.00
Casein, g/L	48.00	43.00	38.00	25.00
Lactose, g/L	27.00	39.00	44.00	50.00
Ash, g/L	11.10	9.50	8.70	7.40
Immunoglobulins, g/L	81.00	58.00	17.00	< 2.00
Lactoferrin, g/L	1.84	0.86	0.46	ND
Trasferrin, g/L	0.55	0.44	0.39	ND
γ-Glutamyltransferase, μkat/L[1]	509.00	284.00	145.00	52.00
TNF-α, μg/L	5.00	ND	ND	< 2.00
Insulin, μg/L	65.00	35.00	16.00	1.00
Glucagon, μg/L	0.16	0.08	0.08	0.01
Prolactin, μg/L	280.00	180.00	150.00	15.00
Growth hormone, μg/L	1.40	0.50	< 1.00	< 1.00
IGF-I, μg/L	310.00	195.00	105.00	< 2.00
IGF-II, μg/L	150.00	ND	ND	ND
Retinol, μg/g	4.90	-	-	0.37
Tocopherol, μg/g	2.92	-	-	0.28
β-Carotene, μg/g	0.68	-	-	0.27
Thiamin, μg/mL	0.90	-	-	0.45
Riboflavin, μg/mL	4.55	-	-	1.75
Niacin, μg/mL	0.34	-	-	0.90
Vitamin B_{12}, μg/mL	0.60	-	-	0.01
Ca, mg/kg	4,716.10	-	-	1,300.00
Mg, mg/kg	733.24	-	-	100.00
Na, mg/kg	1,058.93	-	-	400.00
K, mg/kg	2,845.89	-	-	1,500.00
Zn, mg/kg	38.10	-	-	3.50
Fe, mg/kg	5.33	-	-	0.75
Cu, mg/kg	0.34	-	-	0.10
Mn, mg/kg	0.10	-	-	0.01

[1]μKat/L = microkattal/L.

Source: Foley and Otterby (1978); Blum and Hammon (2000); Kehoe et al. (2007).

Chapter 3

HEAT TREATMENT OF BOVINE COLOSTRUM: EFFECTS ON BACTERIAL COUNT, VISCOSITY, AND IMMUNOGLOBULIN G CONCENTRATION

3.1 ABSTRACT

A study was conducted to identify the optimal temperature and time, at which heat treatment of bovine colostrum would produce the least significant changes in viscosity and IgG concentrations yet produce a significant reduction in bacterial count. First milking colostrum of good quality (> 50 g of IgG/L measure with a colostrometer) was collected from 30 Holstein cows and frozen at -20°C. Each sample was thawed at 4°C, thoroughly mixed and ten 10-mL aliquots were taken in sterile 15-mL screw-cap centrifuge tubes. Samples were heat-treated for 0, 30, 60, or 90 min at 63, 60 or 57°C using a water bath. Samples were evaluated for standard plate count (SPC), preliminary incubation count (PIC), coagulase-negative staphylococci (CNS) count, environmental streptococci (ES) count, coliform (CC) count, gram-negative noncoliform (NC) count, *Streptococcus agalactiae* (SAG) count, and *Staphylococcus aureus* (SA) count. IgG_1 and IgG_2 concentrations were determined in all samples using radial immunodiffusion. Viscosity was also measured using a digital viscometer. The results of the study showed that all heat treatments resulted in a significant reduction of SPC, CC, NC, ES, CNS, SA, and PIC compared to the control. Heat treatment at 60°C and above resulted in significant denaturation of colostral IgG_1; however, colostral IgG_2 concentration was not significantly reduced when the temperature was held at 60°C for 30 or 60 minutes. Viscosity was not significantly affected when temperature was held at 60°C for 30 or 60 minutes. The findings of the study suggest that heat treatment of bovine colostrum at 60°C for a period of time 30 or 60 min may be used as an optimal

temperature and timing, at which heat treatment of bovine colostrum would produce no significant changes in viscosity, a small reduction in IgG concentration, and a significant reduction in bacterial count.

3.2 INTRODUCTION

The bovine neonate is born agammaglobulinemic and depends on colostral immunoglobulin intake to obtain an adequate passive immunity (Besser and Gay, 1994; Weaver et al., 2000). Thus, early ingestion of colostrum by the newborn is critical for its survival. Failure of passive transfer (**FTP**) of colostral immunoglobulins is associated with increased morbidity and mortality from neonatal diseases. It has been estimated that as many as 35% of dairy calves in the United States suffered from FTP (Stott et al., 1979; Brignole and Stott, 1980), and indeed, more than 40% of United States dairy heifer calves had a serum IgG concentration lower than 10 g/L in a USDA survey (USDA, 1993).

Some disease-causing pathogens that can be transferred to newborns by colostral and milk secretions, either by direct shedding from the mammary gland or from post-harvest contamination, include *Mycobacterium avium* subsp. *paratuberculosis* (Sweeney et al., 1992; Streeter et al., 1995; Grant et al., 1996), *Listeria monocytogenes* (Doyle et al., 1987), *Campylobacter jejuni* (Lovett et al., 1983), *Salmonella* spp. (Spier et al., 1991), and *Escherichia coli* (Steele, 1997). Recovery of these and other pathogens from colostrum and milk of cows has raised concerns about transmission of diseases from the dam to the calf immediately after birth. Most programs for controlling the spread of infectious disease within a herd recommend feeding colostrum from non-infected dams only. However, this recommendation can limit colostrum availability if a high percentage of the herd is infected, and it creates additional expense for producers who must dispose of colostrum and purchase commercial colostrum substitutes (Stabel et al., 2004).

Pasteurization is one possible measure to reduce the transfer of potential pathogens. Nonetheless, some constituents of colostrum are thermolabile. In addition, decreased serum IgG,

lactoferrin concentration and neutrophil function have been observed in calves fed pasteurized bovine colostrum, indicating that immunological status can be compromised due to heat treatment of colostrum (Meylan et al., 1996; Tyler et al., 2001; Godden et al., 2003). However, the IgG molecule is known to be very heat stable at temperatures higher than those used in the pasteurization process (Li-Chan et al., 1995; Dominguez et al., 1997; Mainer et al., 1997).

When treating colostrum with heat, there are three goals that should be accomplished: 1) to reduce the bacterial load, 2) to maintain or minimally increase the viscosity, and 3) to maintain or minimally reduce immunoglobulin concentrations. Meylan et al. (1996) heated eighteen 5-mL colostrum samples to 63°C for 30 min to simulate pasteurization of colostrum under laboratory conditions. The study using single radial immunodiffusion (**RID**) reported a mean loss of 12.3% of immunoglobulins after pasteurization. In another study, McMartin et al. (2006) using a turbidometric immunoassay reported a 34% decrease in IgG concentration and 33% increase in viscosity after heating colostrum to 63°C for 120 min. A high increase in viscosity may cause colostrum to congeal into a thick, pudding-like consistency which creates a final product with unacceptable feeding and cleaning characteristics (McMartin et al., 2006). One field study investigating the effect of on-farm commercial batch pasteurization (63°C for 30 min) on IgG concentrations and the fluid and feeding characteristics of colostrum showed that pasteurization reduced colostrum IgG concentration (measured by RID), averaging 58.5 and 23.6% reduction for 95-L and 57-L batches, respectively (Godden et al., 2003). The authors also found that pasteurization produced colostrum of normal or only mildly thickened consistency that could be fed to calves. In another experiment, Godden et al. (2006) inoculated batches of colostrum with *Mycoplasma bovis*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Mycobacterium avium* subsp. *Paratuberculosis* (MAP). They showed that viable *M. bovis, L.*

monocytogenes, E. coli O157:H7, and *S. enteritidis* added to colostrum could not be detected after the colostrum was heat-treated at 60°C for 30 min. Meanwhile, MAP was not detected after 60 min of heating at 60°C.

Few laboratory and field studies have investigated the practice of heat-treating colostrum, and more research is needed. For this reason, the objectives of the study was to identify the optimal temperature and timing combination at which heat treatment of bovine colostrum would produce the least significant changes in viscosity and IgG concentration, yet significantly reduce bacterial load.

3.3 MATERIALS AND METHODS

3.3.1 Colostrum Management and Heat Treatment

First milking colostrum with an IgG concentration > 50 g/L measured by colostrometer (Biogenics. Mapleton, OR) was collected from 30 Holstein cows from the Pennsylvania State University dairy herd and frozen at -20°C for 1 to 3 mo prior to use in this study. Colostrum was thawed at 4°C and each sample was thoroughly mixed. Ten 10-mL aliquots from each colostrum sample were taken in sterile 15-mL screw-cap centrifuge tubes (total n = 30). Samples were heat-treated for 0, 30, 60, or 90 min at 63, 60 or 57°C in a preheated water bath, using the methodology described by Meylan et al. (1996). Samples were placed in the water bath, brought to temperature, and held for the corresponding time. The temperature was continuously monitored by 2 thermometers, 1 in the water bath and the other in a 10-mL sample of colostrum that served as an indicator. After heat treatment, samples were immediately cooled in an ice bath until they reached a temperature below 30°C. Microbial assays were immediately run afterwards.

3.3.2 Colostrum Analysis

Samples were examined for standard plate count (**SPC**), preliminary incubation count (**PIC**), coagulase-negative staphylococci count (**CNS**), environmental streptococci count (**ES**), coliform count (**CC**), gram-negative noncoliform count (**NC**), *Streptococcus agalactiae* count (**SAG**), and *Staphylococcus aureus* count (**SA**) (Jayarao et al., 2004). Colostrum samples were thoroughly mixed by inverting the tube 20 to 25 times, then 50 μL were placed on selective and non-selective media using an inoculating loop. Plate count agar was used for enumeration of SPC and PIC. The ES and SAG in colostrum samples were estimated using modified Edward's agar supplemented with colistin sulfate and oxolinic acid (Sawant et al., 2002). MacConkey's agar no. 3 (Oxoid, Hampshire, England) was used to determine CC and NC. Baird Parker's agar (Difco, LePont de Claix, France) was used to determine CNS and SA. Plates for enumeration of SPC and PIC were incubated at 32°C for 48 h. Plates for enumeration of CNS, ES, CC, SAG, and NC were incubated at 37°C for 48 h.

IgG_1 and IgG_2 levels were determined in all samples by immunoprecipitation using single RID (VWRD, Pullman, WA) as described by Hadorn and Blum (1997). A monocular comparator (VMRD, Pullman, WA) was used to read the precipitin rings.

Viscosity was also measured at 39°C with a digital viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA) using a parallel plate geometry (plate diameter = 50 mm). The gap between the 2 plates was set at 0.50 mm to allow good contact between the sample and the plates. Shear rate was set at 1.0 rpm.

3.3.3 Statistical Analysis

IgG concentration (g/L), bacteriology (cfu/mL), and viscosity [($\log_{10}$(Pa·s)] were analyzed using the MIXED procedure of SAS 9.1 (SAS Institute., 2006). Viscosity measurements and bacteriology data were log-transformed to normalize residuals and obtain *P*-values. Statistical significance was declared at $P < 0.05$. Cow was used as the random effect. The statistical model used for the analysis was:

$$Y_{ijk} = \mu + T_i + W_j + (TW)_{ij} + cow_k + e_{ijk}$$

where:

Y_{ijk} = dependent variables,

μ = overall mean,

T_i = temperature effect i,

W_j = time effect j,

$(TW)_{ij}$ = effect of temperature by time interaction,

Cow_l = random effect of cow l,

e_{ijk} = residual.

3.4 RESULTS AND DISCUSSION

3.4.1 Effect of Heat Treatment and Time on Bacterial Load

Heat treatment is the most common means used to eliminate pathogenic organisms in foods, and this study was designed to determine its effect on bacterial load in bovine colostrum. Least square means of bacteria groups studied are presented in Table 3.1. The degree of bacterial

reduction increased with time and temperature of treatment as would be anticipated, since the use of high temperatures to preserve food is based on their destructive effects on microorganisms (Jay, 2000). The SPC, which provides an estimate of the total number of aerobic bacteria present, in colostrum started to decline at the lowest time and temperature combination of 57°C for 30 min (Figure 3.1). Environmental streptococci and CNS were also reduced after heating colostrum samples at 57°C for 30 min. Coliform organisms include *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp., while gram-negative non-coliform organisms include Pseudomonas and Serratia. These types of organisms in conjunction with SA and SAG were not detected after heating colostrum at 60°C for 30 min (Table 3.1), which corroborates that common pasteurization temperatures are sufficient to destroy gram-negative and many gram-positive bacteria (Jay, 2000). The PIC is an indicator of the number of psychrotrophic bacteria in raw milk. In the control, PIC values were close to 49,000 cfu/mL, and a significant reduction was seen after heating the colostrum samples at 57°C. Overall, it was demonstrated that heat treatment of colostrum significantly reduced bacterial counts when compared to the control.

In order to better understand the thermal destruction of microorganisms relative to heat treatment, it is necessary to understand one basic concept associated with this process; the **D Value**, which is the time required to destroy 90% of the organisms at a given temperature (Jay, 2000). Table 3.2 shows the D value (in minutes) for the different bacterial groups present in the colostrum samples calculated for each heating temperature. By increasing the temperature, there is a reduction in the time required to destroy pathogenic organisms. The higher D-values for bacterial destruction suggest higher resistance to thermal treatment. In this case, environmental streptococci are more resistant than the other groups of bacteria and the non-coliform organisms are the less resistant to thermal treatment.

Despite the fact that a large number of studies have investigated the effectiveness of pasteurization in reducing the number of pathogens in milk, only a few studies have reported on heat treatment of colostrum, which is much more viscous and has higher protein and fat levels (Kehoe et al., 2007). Early studies on pasteurization of bovine colostrum using the same times and temperatures recommended for milk, have reduced or eliminated important bacterial pathogens. Stabel et al. (2004) showed that heat treatment of colostrum in a low temperature range (63.9 to 66.7°C) did not immediately destroy *M. paratuberculosis*. However, recovery of *M. paratuberculosis* from colostrum was reduced by 2 $\log_{10}$ after 10 min of pasteurization and achieved a nadir of < 3 cfu/mL after 30 min. Increasing the temperature range (68.3 to 70.8°C) completely abrogated recovery of viable *M. paratuberculosis* from colostrum. The authors concluded that pasteurization in that temperature range effectively destroys *M. paratuberculosis* in colostrum, providing dairy producers with an alternative to purchasing commercial replacement products, which results in reduced costs and reduced calf morbidity by breaking the transmission cycle of infectious pathogens on-farm.

In one experiment, Godden et al. (2006) inoculated batches (30 L) of first-milking bovine colostrum with *Mycoplasma bovis* (10^8 cfu/mL), *Listeria monocytogenes* (10^6 cfu/mL), *Escherichia coli* O157:H7 (10^6 cfu/mL), *Salmonella enteritidis* (10^6 cfu/mL), and *Mycobacterium avium* subsp. *paratuberculosis* (MAP; 10^3 cfu/mL). Colostrum batches were heat-treated at 60°C for 120 min in a commercial on-farm batch pasteurizer system, and 50-mL subsamples were collected at 15-min intervals throughout the heat treatment process for bacterial culture. Viable *M. bovis, L. monocytogenes, E. coli* O157:H7, and *S. enteritidis* added to colostrum could not be detected after the colostrum was heat-treated at 60°C for 30 min. Average bacteria counts showed that MAP was not detected when batches were heated at 60°C for 60

min. In an on-farm pasteurization experiment, Johnson et al. (2007) significantly reduced bacterial counts by heat-treating colostrum at 60°C for 60 min. The present study has demonstrated, as well as others, that treating colostrum with heat can greatly reduce pathogenic bacteria that may act directly to cause diseases.

3.4.2 Effect of Heat Treatment and Time on Colostrum Viscosity and IgG Concentration

Heat treatment is extensively used to preserve foods, and knowing its effects on the components and characteristics of colostrum is essential. The potential disadvantage of heat-treating colostrum is that immunoglobulins may become denatured and viscosity may increase. Three major isotypes of immunoglobulins (IgA, IgG, and IgM) have been reported in bovine colostrum (Butler, 1969; Pakkanen and Aalto, 1997; Kehoe et al., 2007) with IgG, represented by subclasses IgG_1 and IgG_2 (Butler, 1969), accounting for 85% of the total immunoglobulins (Sasaki et al., 1976; Larson et al., 1980). IgG is a monomeric (150 kDa) glycoprotein consisting of two heavy and two light polypeptide chains linked by disulphide bonds. The polypeptide chains contain both constant (Fc) and variable (Fab) regions of amino acid sequence with the Fab regions linked to the Fc domain by a hinge region, which varies in length and flexibility according to antibody class and isotype. Antigen-binding activity is located in the Fab N-terminal region, whereas the Fc domain is responsible for effector functions of IgG (Kindt et al., 2007).

For this study, RID analysis for IgG_1 and IgG_2 concentrations, and viscosity of colostrum were compared for different temperatures and times, and the results can be found in Table 3.2. Total IgG concentration decreased as temperature and holding time increased (Figure 3.2). IgG_1

ranged from 27.7 to 71.6 g/L while IgG_2 ranged from 2.2 to 3.2 g/L, showing a significant decline as temperature and time increased. The mean IgG_1 and IgG_2 concentrations were not different between the control and colostrum heated at 57°C regardless of time. However, when colostrum was heated at 60°C there was a reduction in IgG, especially in IgG_1, even when colostrum was heated for just 30 min. The greatest reduction in IgG concentration was observed when colostrum was heated at 63°C. There were no differences in viscosity [($\log_{10}$(Pa·s)] between the control and post-heat-treated samples at 60°C for 30 or 60 min (Table 3.2). However, there was a significant increase in viscosity [($\log_{10}$(Pa·s)] when colostrum samples were heat-treated at 60°C for 90 min and at 63°C regardless of the time (Figure 3.1).

Different studies have been published on the treatment of colostrum with heat, especially those concerning denaturation of antibodies and viscosity. Meylan et al. (1996) indicated a 12.3% loss of IgG after pasteurizing 5-mL volumes of colostrum samples at 63°C for 30 min. In one experiment, pasteurization of 3.8-L batches of bovine colostrum at 72°C for 15 s resulted in an average 28.4% loss of IgG, with pre- and post-pasteurized IgG concentrations being 58.5 g/L and 41.3 g/L, respectively (Green et al., 2003). McMartin et al. (2006) using a turbidometric immunoassay to test for IgG, reported a 34% decrease in IgG concentration and 33% increase in viscosity after heating colostrum to 63°C for 120 min. The authors also found no difference in IgG concentration between pre-heat-treated (73.4 ± 26.5 g/L) and post-heat-treated (74.5 ± 24.3 g/L) samples after heating colostrum to 60°C in an rapid visco analyzer (RVA) for 120 min. Similarly, viscosity was unaffected. Godden et al. (2006) found no effect of heating moderate- to high-quality colostrum at 60°C for 120 min on mean IgG concentration (pre = 60.5 g/L; post = 59.1 g/L). One on-farm batch heat treatment of colostrum experiment reported no significant

differences in colostral IgG concentration when colostrum was heat-treated at 60°C for 60 min (Johnson et al., 2007).

Since bovine IgG in colostrum has the potential to be utilized as an immunological supplement for infant formulas and other hyperimmune foods, its stability to thermal treatment has been widely studied in the food sciences using different experimental techniques (Dominguez et al., 1997; Chen et al., 2000; Kulmyrzaev et al., 2005; Cao et al., 2007). In general, these studies suggest that IgG denaturation involves an initial reversible unfolding of native structure, with loss of globular configuration, which can proceed further to irreversible denaturation and aggregation via hydrophobic and disulphide interactions (Indyk et al., 2008). deWit and Klarenbeek (1984) reported on the effects of heat treatment on the structure and solubility of the immunoglobulin fraction of whey. They showed that immunoglobulins are among the most heat stable whey proteins, which is attributed to the high content of disulfide bonds and components such as fats, lactose, carbohydrates, salts, and other proteins that help in the stabilization of antibodies during thermal treatment (Chen et al., 2000; Elfstrand et al., 2002; Indyk et al., 2008). Moreover, the immune-reactivity of IgG is the most thermo-resistant among the immunoglobulins (Mainer et al., 1997).

According to Price (2000), for a protein to display its biological activity, it must adopt its correct three-dimensional structure. For this reason, changes in secondary or tertiary structures may be responsible for the changes in biological activity upon heating (Li et al., 2005). However, it is important to notice that at the low temperatures used in the present study, some unfolding of the three-dimensional structure may occur, but this unfolding is reversible and native structure can be regained (Goto and Hamaguchi, 1982a; Goto and Hamaguchi, 1982b; Price, 2000). Another important aspect is that denaturation of a multi-domain protein (such as IgG) can be

described as a 2-state process in which individual domains can be affected independently and in different orders, depending on conditions (Vermeer and Norde, 2000). In this case, when IgG is subjected to thermal treatment the antigen binding site in the Fab fragment denatures more quickly or at a lower temperature than the Fc region (Vermeer and Norde, 2000; Cao et al., 2007). This agrees with Dominguez et al. (1997) who indicated that structural alterations in heated IgG are mainly located in Fab fragments, where the antigen-binding site is located, rather than in the Fc fragment. Dominguez et al. (1997) showed that the ability of IgG to bind an antigen, and thus to maintain its immunological activity, was maintained after a heat treatment of 63 to 65°C for 60 min. This is in agreement with the results of Li-Chan et al. (1995), who found that heating IgG at 62 ± 7°C for 30 min had no effect on its binding activity against bacterial lipopolysaccharides. Mainer et al. (1997) concluded that low temperature-long time pasteurization (63°C for 30 min) did not have any effect on IgG concentration in colostrum. Furthermore, Ustunol and Sypien (1997) showed that at 70°C IgG was the most heat stable immunoglobulin (compared to IgM and IgA) and heat treatment for 40 min did not reduce its activity. Lindstron et al. (1994) reported on the thermally induced unfolding of bovine milk immunoglobulins using differential scanning calorimetry (DSC) in the temperature range 25 to 100°C. They demonstrated that thermal unfolding of immunoglobulins at pH 6.6 took place at 80.9°C (determined by DSC). Among the individual immunoglobulin fractions, IgG_1 unfolded at 79.4°C and IgG_2 at 76.7°C. Li et al. (2005) showed a decrease in bovine IgG immunoactivity with changes in its secondary structure. They indicated that 72°C is the critical temperature point for IgG molecules to change their secondary structure, which is in agreement with Li-Chan et al. (1995), who reported that 73°C is the critical temperature for bovine IgG to lose its immunoactivity.

In general, research results have indicated that a substantial proportion of the antibody activity of IgG is retained after commercial processing with the exception of severe thermal treatment processes such as those encountered during production of canned evaporated milk and UHT-sterilized milk (Li-Chan et al., 1995).

The reported differences in IgG concentration after pasteurization could be attributed to the experimental techniques used to determine IgG concentration. For example, immunochemical methods such as ELISA, are based on the reaction between the IgG in colostrum or milk and the antibodies against them (Li-Chan et al., 1995). These methods determine structural changes caused by heating that occurred in different regions of the IgG molecule. Thus, the degree of denaturation of the whole IgG molecule can be estimated by measuring the loss of immunoreactivity during heating (Dominguez et al., 1997). Other more specific immunological methods are designed to estimate the antigen-binding activity of specific IgG against bacterial lipopolysacharides after heat treatment (Dominguez et al., 1997). In contrast for the RID test used in this trial, the tested antigen (IgG) is allowed to diffuse in anti-IgG antibody-containing agar and reacts with the antibody by its antigenic determinants, constant regions of IgG, to form complexes, and then IgG concentration is determined by measuring the diameter of the precipitation ring (Cao et al., 2007). Sensitivity of RID is between 10 and 50 μg antibody/mL, while ELISA sensitivity is between 0.0001 to 0.01 μg antibody/mL (Kindt et al., 2007).

As a consequence of the previous discussion, it seems that IgG is more heat tolerant than RID measures might suggest and further research is needed on this topic.

3.5 CONCLUSIONS

Heat treatment of colostrum significantly reduced the bacterial load in a variety of colostrum samples, indicating that heat treatment of colostrum could serve as an effective method for reducing pathogen exposure to newborn calves. Heat treatment of 10-mL of bovine colostrum at 60°C and above resulted in significant denaturation of colostral IgG_1 as measured by RID; however, colostral IgG_2 concentrations were not reduced when the temperature was held at 60°C for 30 or 60 min. Viscosity was not affected when temperature was held at 60°C for 60 or 30 min. The findings of this study suggest that heat treatment of bovine colostrum at 60°C for 30 to 60 min may be used as an optimal temperature and timing, at which heat treatment would produce no significant changes in viscosity, a small reduction in measured IgG concentrations, and a significant reduction in bacterial count. Additional research is needed to determine if these laboratory studies can be replicated using large-scale, commercial on-farm pasteurization systems and to determine if feeding heat-treated colostrum would affect health and growth performance in dairy calves.

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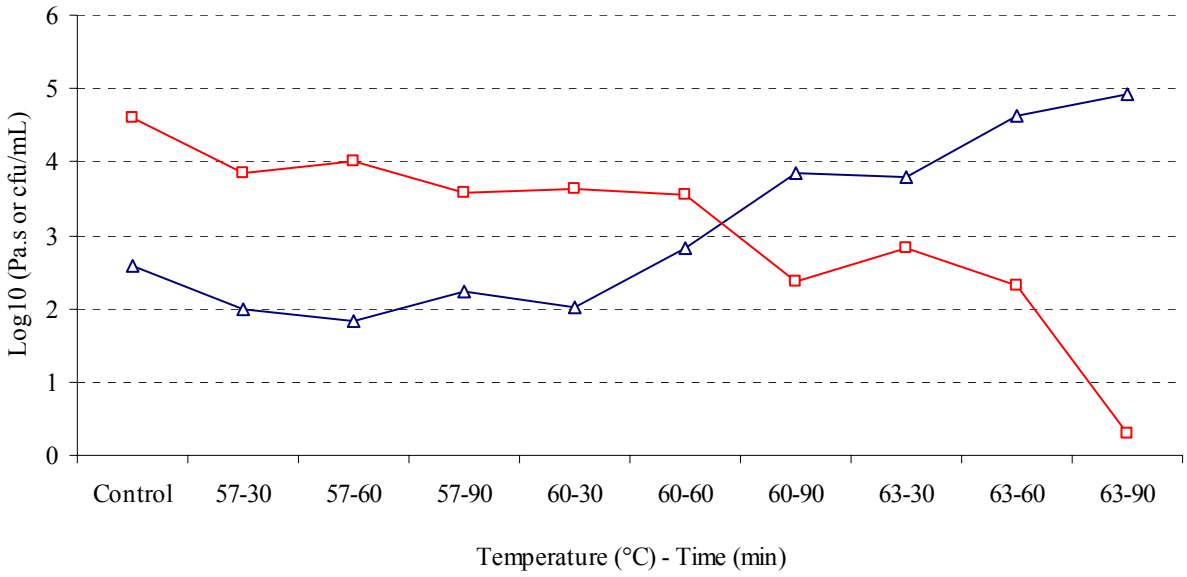

Figure **3.1**: Changes in viscosity (Δ) and standard plate count (□) in bovine colostrum samples after heat treatment.

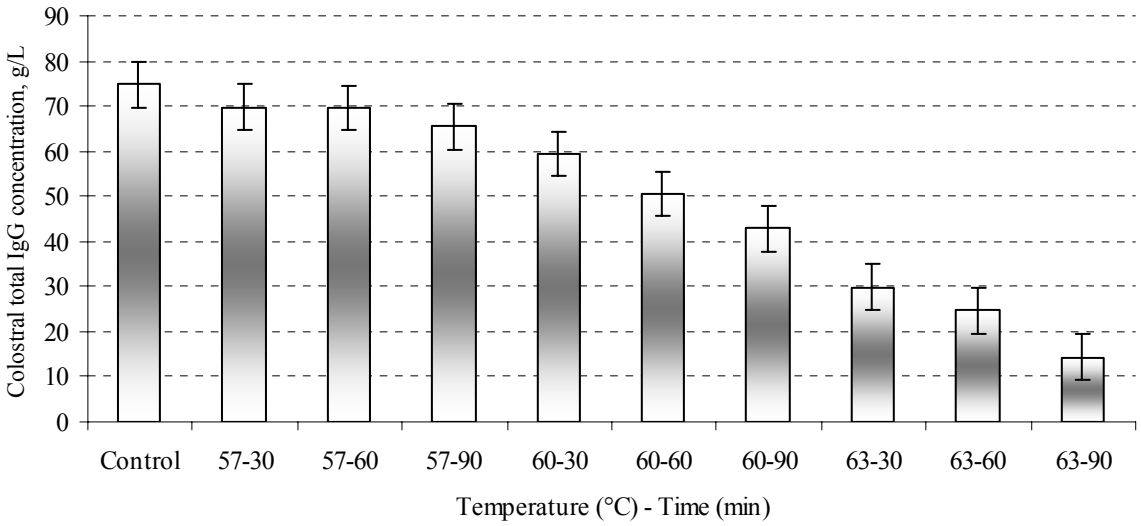

Figure **3.2**: Changes in total IgG concentration in bovine colostrum samples after heat treatment.

Table **3.1**: Least square means for bacterial load of bovine colostrum after heat treatment at 3 different temperatures for 0, 30, 60 or 90 min.

Temperature	Time	Bacteriology (cfu/mL)[1]							
°C	min	SPC[2]	ES[3]	SAG[4]	CNS[5]	SA[6]	CC[7]	NC[8]	PIC[9]
Control	0	4.60[a]	4.23[a]	1.33[a]	4.31[a]	4.31[a]	4.32[a]	4.58[a]	4.69[a]
57	30	3.85[b]	3.86[bc]	-0.52[b]	3.85[b]	1.15[b]	3.87[b]	3.85[b]	3.85[b]
57	60	4.02[b]	4.01[b]	0.00[b]	3.84[b]	0.48[b]	3.84[b]	3.53[bc]	4.02[b]
57	90	3.58[b]	3.53[bc]	0.00[b]	3.53[c]	0.00[b]	1.46[c]	0.00[c]	3.59[bc]
60	30	3.63[bcd]	3.85[bc]	0.00[b]	3.54[bc]	0.00[b]	0.48[c]	0.00[c]	3.61[bc]
60	60	3.55[bc]	3.63[bc]	0.00[b]	1.60[c]	0.00[b]	0.00[c]	0.00[c]	3.55[bc]
60	90	2.63[de]	3.53[bc]	0.00[b]	0.90[c]	0.00[b]	0.00[c]	0.00[c]	2.86[cd]
63	30	2.83[cde]	3.53[bc]	0.00[b]	1.46[c]	0.00[b]	0.00[c]	0.00[c]	3.52[bc]
63	60	2.31[e]	3.52[bc]	0.00[b]	0.00[c]	0.00[b]	0.00[c]	0.00[c]	2.22[d]
63	90	0.30[e]	0.70[c]	0.00[b]	0.00[c]	0.00[b]	0.00[c]	0.00[c]	0.00[d]

[a-e]Different superscripts within column indicate a significant difference ($P < 0.05$).

[1] Colony forming units/mL (log transformed values).

[2] Standard plate count.

[3] Environmental streptococci count.

[4] *Streptococcus agalactiae* count.

[5] Coagulase negative Staphylococci count.

[6] *Staphylococcus aureus* count.

[7] Coliform count.

[8] Non-coliform count.

[9] Preliminary incubation count.

Table **3.2**: D values (in minutes) for different bacterial groups present in bovine colostrum samples for each heating temperature.

Temperature	Bacteriology (cfu/mL)[1]					
(°C)	SPC[2]	ES[3]	CNS[4]	CC[5]	NC[6]	PIC[7]
57	34.09	65.22	40.00	37.04	30.93	34.09
60	25.64	54.55	36.14	32.26	10.00	28.66
63	23.50	45.92	23.08	10.75	10.00	22.96

[1] Colony forming units/mL (log transformed values).
[2] Standard plate count.
[3] Environmental streptococci count.
[4] Coagulase negative Staphylococci count.
[5] Coliform count.
[6] Non-coliform count.
[7] Preliminary incubation count.

Table **3.3**: Least square means of IgG_1 and IgG_2 concentrations and viscosity of bovine colostrum after heat treatment at 3 different temperatures for 0, 30, 60 or 90 min.

Temperature (°C)	Time (min)	IgG_1 (g/L)	SEM	IgG_2 (g/L)	SEM	Viscosity [(log_{10}(Pa·s[1])]	SEM
Control	0	71.6[a]	4.64	3.2[a]	0.22	2.59[de]	0.15
57	30	66.8[ab]	4.48	3.0[ab]	0.22	1.98[e]	0.09
57	60	66.7[ab]	4.25	3.0[ab]	0.23	1.83[e]	0.08
57	90	62.6[ab]	4.26	2.9[ab]	0.22	2.23[e]	0.11
60	30	56.7[bc]	4.01	2.7[abc]	0.21	2.03[e]	0.08
60	60	47.9[dc]	2.86	2.6[abc]	0.20	2.82[d]	0.15
60	90	40.5[d]	2.40	2.4[bcd]	0.19	3.85[c]	0.20
63	30	27.7[e]	1.79	2.2[cd]	0.19	3.80[c]	0.19
63	60	22.8[e]	1.55	1.9[de]	0.18	4.61[b]	0.24
63	90	12.9[f]	1.14	1.5[e]	0.17	4.92[a]	0.19

[a-e]Different superscripts within column indicate a significant difference ($P < 0.05$).

[1] $Pa \cdot s = kg \cdot m^{-1} \cdot s^{-1}$

Chapter 4

FEEDING HEAT-TREATED COLOSTRUM TO NEONATAL DAIRY HEIFERS: EFFECTS ON GROWTH CHARACTERISTICS AND BLOOD PARAMETERS

4.1 ABSTRACT

Newborn Holstein heifer calves were studied to compare absorption of immunoglobulins G (IgG_1 and IgG_2), total serum protein concentrations, lymphocyte counts, health scores, growth measurements, and intake from unheated or heat-treated colostrum. First milking colostrum with > 50 g IgG/L (measured by colostrometer) was collected from Holstein cows and frozen at -20°C until a total of 170 L were accumulated. Colostrum was thoroughly mixed at 4°C for about 20 min. Half of colostrum was transferred into 1.89 L plastic containers and frozen at -20°C until needed for feeding. The remaining half was heated at 60°C for 30 min, transferred into 1.89 L plastic containers, and then frozen at -20°C until needed for feeding. A total of 40 heifer calves weighing ≥ 32 kg at birth were systematically enrolled into 1 of the 2 treatment groups. Calves were separated from their dams at birth before suckling occurred. Before feeding colostrum, a jugular blood sample was collected from each calf. For the first feeding, 3.8 L of colostrum were bottle fed by 1.5 to 2 h of age. For the second and third feeding, pasteurized whole milk at 5% of birth BW was fed. Blood samples were collected until wk 8. Serum from samples was used to determine IgG concentrations, serum total protein and lymphocyte counts. Health scores were assigned daily for each calf to evaluate scours, respiration and general appearance. Growth measures including heart girth, hip height, BW, and withers height were taken weekly. Batch heat treatment of colostrum at 60°C for 30 min resulted in lower colostrum bacteria concentration while maintaining colostral IgG concentration and viscosity. Calves fed heat-

treated colostrum had significantly greater IgG concentrations at 24 h, plus greater apparent efficiency of IgG absorption (IgG = 23.4 g/L; apparent efficiency of absorption = 33.2%) compared with calves fed unheated colostrum (IgG = 19.6 g/L; apparent efficiency of absorption = 27.7%). There was no difference between treatment groups when examining growth measurements, calf starter intake, lymphocyte counts, or health scores.

4.2 INTRODUCTION

In ruminants, syndesmochorial placentation prevents the transmission of immunoglobulins in utero, and calves are essentially agammaglobulinemic at birth (Michanek et al., 1990; Loste et al., 2008). Thus calves depend on the ingestion and absorption of colostral immunoglobulins, especially IgG, across the intestinal epithelium during the first 24 h of life to establish a protective serum IgG concentration (Bush and Staley, 1980). The literature provides abundant information on the major factors affecting serum IgG levels in calves. The two most important of these are the age of the calf at which colostrum is first fed and the mass of IgG ingested, which is determined by the volume of colostrum fed and the colostral IgG concentration (Stott et al., 1979a,b,c; Besser and Gay, 1985; Mohammed et al., 1991).

Colostrum not only provides passive immunity for the newborn calf, but it can also have profound effects on development of the neonatal intestine, since it contains a number of bioactive and growth-promoting substances such as peptide hormones, growth factors, cytokines, steroid hormones, thyroxine, nucleotides, polyamines, and enzymes (Koldovsky, 1989). It has been demonstrated that villous circumference, area, height, and height to crypt depth ratio in the duodenum are higher for calves fed colostrum compared with colostrum-deprived calves (Buhler et al., 1998; Blattler et al., 2001). Calves fed colostrum also have higher plasma xylose concentrations after oral administration of xylose compared with calves fed milk replacer, suggesting enhanced absorptive capabilities in colostrum-fed animals (Hammon and Blum, 1997; Kuhne et al., 2000).

Colostrum is also the first source of nutrients for the calf after birth. It contains proteins, essential and nonessential amino acids, fatty acids, lactose, vitamins, and minerals. Except for lactose, colostrum contains nutrients in higher concentrations than does whole milk (Koldovsky,

1989; Kehoe et al., 2007). Contents of energy, protein, fat and some minerals are well known to be markedly higher in colostrum than in mature milk (Davis and Drackley, 1998).

Despite the important nutritional and immune benefits explained before, colostrum feeding may also offer the calf the first opportunity for exposure to infectious pathogens (Swan et al., 2007), since collection, handling, and storage of colostrum introduces risks of microbial contamination (Stewart et al., 2005). In a study carried out to describe the bacteria most frequently isolated from colostrum fed to calves in commercial dairy herds, Fecteau et al. (2002) found that *Staphylococcus* spp., gram-negative rods, coliforms, and *Streptococcus uberis* were among the most frequently isolated bacteria. Other disease-causing pathogens that can be transferred to newborns via colostral secretions, either by direct shedding from the mammary gland or from post-harvest contamination, may include *Mycobacterium avium* subsp. *paratuberculosis* (Sweeney et al., 1992; Streeter et al., 1995; Grant et al., 1996), *Listeria monocytogenes* (Doyle et al., 1987), *Campylobacter jejuni* (Lovett et al., 1983), *Salmonella* spp. (Spier et al., 1991), and *Escherichia coli* (Steele, 1997).

Pasteurization has been suggested as one possible control measure to reduce or eliminate transfer of colostrum-borne pathogens to dairy calves (Godden et al., 2006). However, early studies showed that this process can reduce IgG concentrations (Meylan et al., 1996; Godden et al., 2003) and increase viscosity (McMartin et al., 2006). Large-scale field studies are needed to describe whether the practice of feeding heat-treated colostrum can be successfully adopted on commercial dairy farms without interfering with passive transfer in calves and to describe and quantify any short- or long-term health or performance benefits in calves fed heat-treated colostrum. With this in mind, the first objective of this study was to describe the effect of heat treatment of colostrum at 60°C for 30 min on colostrum characteristics including standard plate

count (**SPC**, cfu/mL), coagulase-negative staphylococci count (**CNS**, cfu/mL), environmental streptococci count (**ES**, cfu/mL), coliform count (**CC**, cfu/mL), gram-negative noncoliform count (**NC**, cfu/mL), *Streptococcus agalactiae* count (**SAG**, cfu/mL), *Staphylococcus aureus* count (**SA**, cfu/mL), IgG_1 and IgG_2 concentrations (g/L), and viscosity (Pa·s). The second objective was to describe the effect of feeding heat-treated (vs. unheated) colostrum on passive transfer of colostral immune parameters, health, and growth characteristics to 8 wk of age.

4.3 MATERIALS AND METHODS

4.3.1 Colostrum Management

First milking colostrum with an immunoglobulin concentration > 50 g/L (measured by colostrometer; Biogenics, Mapleton, OR) was collected from Holstein cows and frozen at -20°C until a total of 170 L was gathered. Once collected, colostrum was thawed at 4°C, pooled and mixed for 20 min in a commercial batch pasteurizer (Girton Manufacturing Co, Millville, PA) to create a unique batch. A subsample was taken and stored at -20°C for later analysis. Half of the colostrum was transferred into new, clean 1.89 kg plastic containers and frozen at -20°C until needed for feeding (**unheated colostrum**). The remaining half of the colostrum was placed into 3 stainless steel containers (28 L each). All 3 containers were placed into a steam vat pasteurizer (Girton Manufacturing Co, Millville, PA). Temperature for the water and the colostrum was monitored every 5 min. For the 3 containers, water was heated until colostrum reached the target temperature of 60°C, held for 30 min, and then ice water was used to cool it down (Figure 4.1). A subsample was collected from each of the three containers and pooled as one for later analysis.

Colostrum was then transferred into 1.89 kg plastic containers, and frozen at -20°C until needed for feeding (**heat-treated colostrum**).

4.3.2 Colostrum Sample Analyses

Unheated and heat-treated colostrum samples were thawed at 4°C and examined for SPC, CNS, ES, CC, NC, SAG, and SA according to Jayarao et al. (2004). The colostrum samples were mixed thoroughly by inverting the tube 20 to 25 times, and 50 μL were placed on selective and non-selective media using an inoculating loop. Plate count agar was used for enumeration of SPC. The numbers of ES and SAG in colostrum samples were estimated using modified Edward's agar supplemented with colistin sulfate and oxolinic acid (Sawant et al., 2002). MacConkey's agar no. 3 (Oxoid, Hampshire, England) was used to determine CC and NC. Baird Parker's agar (Difco, LePont de Claix, France) was used to determine CNS and presence of SA. Plates for enumeration of SPC were incubated at 32°C for 48 h. Plates for enumeration of CNS, ES, CC, SAG, and NC were incubated at 37°C for 48 h. IgG_1 and IgG_2 concentrations were determined in all samples by immunoprecipitation using single radial immunodiffusion (**RID**; VWRD, Pullman, WA) as described by (Hadorn and Blum, 1997). A monocular comparator (VMRD, Pullman, WA) was used to read the precipitin rings. Viscosity was measured at 39°C with a digital viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA) using a parallel plate geometry (plate diameter = 50 mm). The gap between plates was set at 0.50 mm to allow good contact between the sample and the plates. Shear rate was set at 1.0 rpm. The unit used for viscosity was the Pa·s which is equivalent to $kg \cdot m^{-1} \cdot s^{-1}$.

Colostrum samples were also analyzed for ash, DM (AOAC, 1990), CP (Leco FP-528 Nitrogen Combustion Analyzer; Leco, St. Joseph, MI), and crude fat (AOAC, 2000) using a Tecator Soxtec System HT 1043 Extraction unit (Tecator, Foss NA, Eden Praire, MN). Colostrum samples were sent to the Agricultural Analytical Services Laboratory at the Pennsylvania State University to be analyzed for Ca, P, Mg, Na, K, Zn, Fe, Cu, S, and Mn. Samples were also sent to the Diagnostic Center for Population and Animal Health of the Michigan State University to be analyzed for fat soluble vitamins. Compositional analyses and characteristics of colostrum samples before and after heat treatment are presented in Table 4.1. It can be noted that thermal treatment did not have major compositional or characteristic changes in colostrum and the compositional analysis is in accordance with the values reported by Kehoe et al. (2007).

4.3.3 Calf Treatment Allocation, Sample Collection and Records

Protocols used for this study were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Holstein heifer calves from the Pennsylvania State University dairy herd were separated from their dams within 20 to 30 min of birth, before suckling occurred, placed in a 1.0- x 1.0-m holding pen until colostrum was fed, and then housed in 1.2- × 2.4-m individual pens in a naturally and mechanically ventilated barn bedded with wood shavings. Calves remained in the individual pens until 8 wk of age, and calf to calf contact was eliminated by pen arrangement. A total of 40 heifer calves weighing ≥ 32 kg at birth were systematically enrolled into 1 of 2 treatment groups receiving either unheated or heat-treated colostrum for the first feeding. Information for each dam and calf was recorded, including cow

ID, date and time of calving, calving ease, parity, calf identification number, treatment allocation, and age at feeding. Birth weight, heart girth, hip height, and withers height were also recorded for every calf.

Before feeding colostrum, a jugular blood sample was collected from each calf into 8.5-mL serum and 7-mL K_3-EDTA Vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ). For the first feeding, 3.8 L of colostrum were bottle fed between 1.5 and 2 h of life according to randomized treatment allocation. Colostrum was warmed to approximately 38°C using a hot water bath heated to approximately 52°C. In order to ensure that all calves received an equal amount of colostrum, an esophageal feeder was used in calves with reduced appetite. For the second and third feeding, pasteurized whole milk at 5% of birth BW was fed. For the remaining time of the trial, calves were fed a milk replacer containing 20% CP (all milk protein) and 20% fat (North American Nutrition Company, Inc., Lewisburg, OH) at 10% of birth BW, 5% fed in the morning and 5% fed in the afternoon, until wk 5. Then milk replacer was reduced to only morning feeding until weaning at 6 wk of age. Blood samples were also collected from every calf at 4, 8, 12, 16, 20, 24, and 48 h and at wk 1 to 8 of age. A subsample from each blood sample was collected for measurement of packed cell volume by micro-hematocrit centrifugation (Quigley et al., 2006). Health scores were assigned daily for each calf to evaluate scours, respiration and general appearance (Lesmeister and Heinrichs, 2004). Electrolyte therapy was initiated when an animal had a fecal score > 3 or was visibly dehydrated and continued until signs abated. Growth measures including heart girth, hip height, BW, and withers height were taken weekly 4 h after the morning feeding for all animals. Fresh calf starter feed (East Gate Feed & Grain, LLC, Reedsville, PA) was offered beginning at 4 d of age and refusals were recorded once a week. A sample of calf starter was collected twice monthly for the duration of

the trial. All collected samples were stored (-20°C) and sent to Cumberland Valley Analytical Services (Hagerstown, MD) for analysis (Table 4.2). Clean water was available all the time starting from d 2.

4.3.4 Blood Sample Analysis in Calves

All precolostral (0 to 1 h) and postcolostral blood samples collected into serum (red top) Vacutainer tubes were refrigerated overnight, centrifuged, and the serum separated from the clot within 24 h of collection (Johnson et al., 2007). Serum total protein (**STP**) concentrations (g/L) were determined using a commercially available hand-held refractometer (VET 360, Reichert Inc., Depew, NY). Sera were then stored at -20°C until analyzed. Serum IgG concentrations (g/L) were determined using a commercially available RID kit (VWR, Pullman, WA) (Hadorn and Blum, 1997). Apparent efficiency of absorption (%) of IgG, a calculated measure that estimates what proportion of the total IgG mass fed is actually absorbed into the calf's circulation, was calculated using the accepted equation described by Quigley, III and Drewry (1998) assuming a plasma volume of 9.5% of birth weight.

Blood samples from wk 1 to 4 collected into 7-mL K_3-EDTA Vacutainer tubes were used for lymphocyte (CD8, CD4) and natural killer cell identification using flow cytometric analysis as explained by Pelan-Mattocks et al. (2001).

4.3.5 Statistical Analysis

Descriptive statistics were generated to define calf and dam characteristics for the 2 treatment groups. Blood and growth observations were analyzed using repeated measures

analysis and the MIXED procedure of SAS 9.1 (SAS Institute., 2006). Calf was used as the random effect. The statistical model used for analysis was:

$$Y_{ijk} = \mu + T_i + W_j + (TW)_{ij} + calf_k + e_{ijk}$$

where:

Y_{ijk} = dependent variables,

μ = overall mean,

T_i = fixed effect of treatment i, where i= unheated or heat-treated,

W_j = repeated measure of time j,

$(TW)_{ij}$ = effect of treatment by time interaction,

$Calf_l$ = random effect of calf l,

e_{ijk} = residual.

AR(1) structure was used in the model. Initial measurements for growth and blood parameters were offered as additional covariates into each model. However, none of these terms was significant and none interacted with the variable describing colostrum treatment group, so they were subsequently removed from the final models. Final significance was declared at $P < 0.05$ for all models.

4.4 RESULTS AND DISCUSSION

Composition of calf starter used throughout the study and milk replacer used for the trial is presented in Table 4.2. Descriptive statistics generated to explain calf and dam characteristics for the 2 treatment groups are shown in Table 4.3. No statistical differences were found in any of the parameters studied. Mean birth weight was 43.3 and 41.4 kg for calves in the untreated and

heat-treated colostrum groups, respectively. Age of calves at first feeding ranged from 90 to 120 min for both treatment groups. The median parity of dam was 3.0 and 2.5 for the unheated and heat-treated colostrum group, respectively. Calving score for all dams in the study was never higher than 3 on a scale from 1 to 5.

4.4.1 Effect of Heat Treatment on Colostrum IgG Concentration, Viscosity, and Bacterial Counts

The effect of treatment on colostral IgG concentration, viscosity, and bacterial load are presented in Table 4.4. There was no significant difference ($P > 0.05$) in the least square means colostral IgG concentration for unheated and heat-heated colostrum. The IgG_1, IgG_2, and total IgG concentrations in untreated colostrum were 73.33, 3.09, and 76.43 g/L, respectively, whereas in the colostrum treated by heat the concentration was 71.44, 2.98, and 73.42 g/L. Heat-treatment did not significantly affect coagulation or fluid characteristics of colostrum. Early studies with colostrum pasteurization using the same times and temperatures recommended for milk demonstrated that heating resulted in denaturation of 12 to 30% of colostral IgG and increases in viscosity (Meylan et al., 1996; Green et al., 2003); however, it has been demonstrated that possible problems with viscosity and IgG denaturation can be avoided by using a lower temperature (Godden et al., 2006; Johnson et al., 2007).

The results of the present study showed that heat treatment resulted in a significant reduction of SPC, CC, NC, and CNS. Previous laboratory studies have also reported success in reducing or eliminating pathogens when colostrum has been heat-treated (Godden et al., 2006; McMartin et al., 2006; Johnson et al., 2007; Trujillo et al., 2007).

In agreement with another published study Johnson et al. (2007), heat-treating colostrum in this study reduced bacterial load, maintained viscosity, and maintained immunoglobulin concentrations.

4.4.2 Effect of Feeding Heat-Treated Colostrum on Serum Total Protein and IgG Concentration in Calves

The measurement of STP by refractometer as an estimate of serum immunoglobulin concentration is the simplest test to give an indication of adequate passive transfer. A value of 50 g/L at 24 h of age has been established as the cutoff point assessment of passive transfer status (Donovan et al., 1998). Even though there is some concern regarding the effects of age and hydration status since they could increase STP concentrations and create the potential for misclassification of calves that have failure passive of immunity (Besser and Gay, 1985; Tayler et al., 1999; Weaver et al., 2000), some researchers indicate that this measurement is suitable for herd monitoring and appears to provide a reasonably accurate assessment of passive transfer status (Tyler et al., 1996). Tyler et al. (1996) compared the performance of commonly used tests for passive transfer, demonstrating that a STP concentration of 52 g/L was equivalent to an IgG concentration of 10 g/L.

In the present study, STP concentration increased after first feeding in both treatment groups due to absorption of colostral IgG, as expected (Figure 4.2). Calves receiving heat-treated colostrum showed greater ($P < 0.05$) STP concentration at 8, 12, 16, and 20 h (Table 5). Johnson et al. (2007) reported values higher than the ones obtained in this experiment, and 24-h serum concentrations in their study were greater for calves fed heat-treated colostrum when compared with calves fed unheated colostrum. It must be noted that hydration status, feeding time before

blood sampling, and amount and type of liquid feed fed to calves will affect STP measurement (Besser and Gay, 1985; Weaver et al., 2000).

Serum IgG concentrations at birth were below detectable concentrations of the assay and did not produce rings on RID plates, therefore they were assumed to be zero (Table 4.6). However, 4 h after birth calves fed heat-treated colostrum had significantly higher ($P < 0.01$) serum IgG concentrations (Figure 4.3). Peak serum IgG concentrations were reached between 24 and 48 h after birth. It has been postulated that serum IgG concentrations of 10 g/L at 24 to 48 h are sufficient to reduce the risk of infectious disease in most environments (Radostis et al., 2007). Despite the treatment used, calves' serum IgG concentrations in this experiment doubled that value. Dairy producers usually feed a fixed volume of colostrum per calf, and more than 23% of United States dairy operations that hand-fed their calves still feed ≤ 2 L of colostrum during the first 24 h of life (National Animal Health Monitoring System, 2007). A problem with this approach is that only a small proportion of first milking colostrum from Holstein cows contains a sufficiently high concentration of immunoglobulins (Besser and Gay, 1994; Kehoe et al., 2007) and higher volumes of colostrum are required to achieve a serum IgG concentration of 10 g/L at 24 h of age (Pritchett et al., 1991). For this reason, this experiment included feeding calves colostrum artificially to ensure that each calf received an ample amount of selected colostrum early in the absorptive period. Even though the efficiency of immunoglobulin absorption declines somewhat as larger amounts are fed, feeding large volumes of colostrum results in higher serum IgG concentrations in the calf (Stott et al., 1979b; Bush and Staley, 1980; Besser and Gay, 1994). Even so, in one study, calves fed 4 L of high immunoglobulin colostrum at the first feeding absorbed IgG_1 as efficiently as calves fed 2 L of high immunoglobulin colostrum, which indicates that it is advantageous to feed a high volume of colostrum with a high

immunoglobulin concentration (Morin et al., 1997). In agreement with the present study, administration of 4 L of colostrum at one feeding by esophageal feeder caused no signs of discomfort and no evidence of clinical gastrointestinal disease in the calves (Morin et al., 1997). Calves fed heat-treated colostrum had nearly 20% greater ($P < 0.01$) serum total IgG concentration at 24 and 48 h than calves fed unheated colostrum (23.4 vs. 19.6 g/L, and 23.9 vs. 20.2 g/L, respectively).

In this trial, calves were given on average a total IgG mass of 283 g and none of the 40 calves experienced failure of passive transfer, regardless of treatment. Concentrations of IgG and STP at 24 h of age have been shown to be positively correlated (Tyler et al., 1996; Quigley, III et al., 2002; Foster et al., 2006), meaning that the higher the serum IgG concentration, the higher the value for STP and vice versa.. The relationship between circulating serum total IgG and STP in calves fed unheated or heat-treated colostrum is depicted in Figure 4.4. Calves in both treatment groups were administered the same mass of protein and IgG from colostrum; however, absorption of IgG varied among treatment groups. There was a significant difference ($P < 0.01$) between treatment groups as indicated by the difference between the regression lines. Calves fed heat-treated colostrum had greater serum total IgG concentration at 24 h than calves fed the unheated colostrum at the same STP concentration.

The regression equations obtained in this study for the different treatment groups are:

Unheated: Serum total IgG (g/L) = 0.734 x STP (g/L) - 19.4 ($R^2 = 0.462$)

Heat-treated: Serum total IgG (g/L) = 1.01 x STP (g/L) - 32.3 ($R^2 = 0.593$)

Both treatments: Serum total IgG concentrations (g/L) = 0.985 x STP (g/L) - 32.2 ($R^2 = 0.548$)

When the cutoff value of 50 g/L of STP proposed by Donovan et al. (1998) is used in this study, estimates of passive transfer by measuring serum total protein seriously underestimates the adequacy of passive transfer, especially in calves fed heat-treated colostrum. For instance, in one study, Tyler et al. (1996) demonstrated that a serum protein concentration of 52 g/L, measured by refractometry, was equivalent to an IgG concentration of 10 g/L; while in the present study, when STP is near 52 g/L, serum IgG concentration is well above 10 g/L. This may mean that refractometry is not such an accurate means to estimate serum total IgG concentrations when calves are fed high volumes of colostrum or colostrum treated by heat.

Due to the fact that the calves' immune system may take weeks to months to mature and become protective (Robinson et al., 1988; Erhard et al., 1999), and since the half-life of colostral-derived IgG in the neonatal system is between 11.5 and 26 days (Sasaki et al., 1976, 1977), it was intended to monitor serum IgG concentrations through the duration of the experiment. It can be noted in Table 4.7 and Figure 4.5 that the concentration of maternal immunoglobulins start to decline as these proteins are gradually distributed and catabolized; however, the differential in serum total IgG concentration between the two treatment groups was maintained up to 5 wk of age. Erhard et al. (1999) stated that from about day 11 to day 28 of life, there is an overlap between maternal IgG with slowly increasing endogenous IgG production, meaning that the newborn calf starts becoming immunocompetent before passive maternal immunity wanes.

4.4.3 Effect of Feeding Heat-Treated Colostrum on Apparent Efficiency of IgG Absorption

The ability of the newborn calf to absorb colostral immunoglobulin depends on many factors, the most important of which are the age at first feeding (Stott et al., 1979a) and the mass of IgG consumed (Stott et al., 1979c). According to Quigley, III and Drewry (1998) for better understanding of the nature of IgG absorption and the management required to provide adequate passive immunity, it is necessary to calculate AEA, which measures the efficiency with which IgG are absorbed. Reports on AEA are remarkably variable and apparent efficiency of IgG absorption from maternal colostrum, calculated as grams of IgG in the blood at 24 h divided by the grams of IgG intake has been reported from 6 to 88%; however, most values are between 20 and 35% (Quigley, III and Drewry, 1998). This variability is probably due to the physiological and experimental differences (including IgG analysis and estimated serum volume) that exist among studies. In this trial, AEA was significantly greater for calves fed heat-treated colostrum (Table 4.8 and Figure 4.6). The AEA for total IgG from 4 to 48 h of age ranged from 16.3 to 28.5% for calves fed unheated colostrum and from 19.7 to 34% for calves fed heat-treated colostrum. The AEA for total IgG at 24-h of age was very similar to that reported by Johnson et al. (2007), when using 9.9% of body weight to estimate serum volume in calves.

Maternal IgG and other constituents of colostrum are transported across the neonatal intestinal epithelium within the first 24 h of life (Kacskovics, 2004), travel through the lymphatics, and enter blood circulation via the thoracic duct (Balfour and Comline, 1962; Besser and Gay, 1994; Radostis et al., 2007). There is some controversy on how IgG and other proteins are taken up in the small intestine. For many years, non-selective pinocytosis has been pointed out as the mechanism for IgG transport across the intestinal epithelium (Besser and Gay, 1985). On the other hand, recent evidence has pointed toward a role for the neonatal Fc receptor (**FcRn**)

in these processes. The FcRn binds IgG in a pH-dependent manner and was first described as an IgG transporter in the neonatal gut of rodents (Rodewald, 1976). The bovine FcRn has been characterized, and its expression has been found in multiple tissues, including the mammary gland, small intestine, kidney and liver (Kacskovics et al., 2000). In dispute of that, other research (Baintner, 2002) pointed out that the transmission process is independent of FcRn, although further research is required to clarify the precise mechanism. According to Baintner (2002), the apical canalicular system on the luminal side of the enterocyte takes up colostral proteins, including IgG, non-selectively. The transport vacuole is formed in the supranuclear region of the cell and passes to the basolateral side. The colostral proteins are secreted into the dilated intercellular spaces and carried away by the lymph. This sort of transmission differs from other forms of antibody transmission in several ways: (1) high amount of colostral protein is absorbed during the first 24 to 48 h; (2) with the exception of precipitated casein, all kinds of colostral proteins, including IgA and IgM, are absorbed; proteins smaller than serum albumin are cleared from the circulation by the kidneys (physiological proteinuria); (3) due to the large size of the transport vacuole this sort of transmission can be examined by the usual histological techniques (Baintner, 2007).

Only one published study has reported that feeding heat-treated colostrum resulted in greater serum IgG concentrations in calves (Johnson et al., 2007). They observed that calves receiving heat-treated colostrum were able to absorb a greater proportion of the total mass of IgG presented to the small intestine and this may be explained by the phenomenon of lactogenic immunity provided by colostrum (Corley et al., 1977; Saif and Smith, 1985; Acres, 1985). The authors explained that antibodies in colostrum have been shown to bind pathogens present in the gut before absorption can occur. Then by reducing the number of pathogens in heat-treated

colostrum, and as a result, the number of pathogens in the gut, more antibodies are potentially free for absorption. Another potential explanation for an increase in AEA of IgG is lack of bacterial interference at the receptors that are responsible for IgG absorption. Bacteria can bind the nonspecific receptors on neonatal enterocytes, thus reducing the number of receptors available for IgG uptake (James and Polan, 1978; James et al., 1981; Staley and Bush, 1985), even if the receptors are different, exfoliation of the microvillous membrane may eliminate immunoglobulin binding sites (Staley and Bush, 1985). It is not clear whether this hypothesis is true or not. There is some evidence that suggests that high bacterial load in colostrum does not affect IgG absorption (Chapter 4). For this reason, enhancement of IgG absorption by treating colostrum with heat should be explained by other means. In the present study, dairy heifer calves fed a high volume of heat-treated colostrum with high IgG concentration were able to absorb more IgG than calves fed the same quantity and quality of unheated colostrum. A possible explanation could be that thermal treatment of colostrum denatures some proteins that other wise would interfere or compete for receptors on neonatal enterocytes, thus reducing the number of receptors available for IgG uptake; however, this has to be further investigated. It would also be very important to investigate if the same effect could be seen by feeding calves similar volumes of heat-treated colostrum with low IgG concentrations or lower volumes with varying IgG concentrations.

4.4.4 Effect of Feeding Heat-Treated Colostrum on Body Weight, Intake, and Growth Measures

Least square means of BW at birth were similar among treatments (Table 4.9). While the effects of heat treatment of colostrum on serum IgG concentrations in neonates have been

reported, no studies have evaluated long term effects on BW and feed intake, and it is not known if thermal treatment of colostrum may denature hormones and growth factors, which in turn may reduce growth or gastrointestinal development, making this practice unsuitable for the dairy industry. In this experiment, overall weight and feed intake were not affected at any time during the duration of the experiment (Table 9). Least square means of weekly BW (Figure 4.7) clearly show that feeding heat-treated colostrum did not have any negative effect on growth. The BW in this study are in agreement with other reports (Quigley et al., 2006; Khan et al., 2007a,b). Calf starter intake was similar across both treatments and increased in a curvilinear fashion until the end of the experiment as would be expected (Figure 4.8). Similar to present results, a slow increase in solid feed consumption with age during the preweaning period, has been demonstrated by other workers (Lesmeister and Heinrichs, 2005; Khan et al., 2007a; Khan et al., 2007b).

Least square means for heart girth, hip height, and withers height are presented in Table 10. There were no significant differences ($P > 0.05$) for heart girth, hip height, and withers height for any group, and similar values were reported by Lesmeister and Heinrichs (2005).

4.4.5 Effect of Feeding Heat-Treated Colostrum on Health and Lymphocyte Counts

Fecal, respiratory, and general appearance scores are given in Table 4.11. Researchers have wondered about the effect of feeding heat-treated colostrum on calf health (Godden et al., 2003). There was no difference among treatment groups on fecal, respiratory, or appearance scores pre- or post-weaning. It is important to note that since serum IgG concentrations for both treatment groups were about 20 g/L, all calves had adequate passive immunity and reduced the

risk of suffering from infectious disease. No death or diseases related to treatment effect were observed during the duration of the experiment. In agreement with this study, Johnson et al. (2007) reported no effect on health for calves receiving heat-treated colostrum.

T lymphocytes arise in the bone marrow and migrate to the thymus gland to mature (Kindt et al., 2007). There are 2 well-defined subpopulations of T lymphocytes: helper and cytotoxic cells, which can be distinguished from one another by the presence of cell surface proteins or cluster of differentiation (**CD**) markers, either CD4 or CD8, for helper and cytotoxic cells, respectively (Ellis et al., 1997). The body also contains a small population of large, granular lymphocytes called natural killer cells that constitute a major component of the innate immune system and display cytotoxic activity against a wide range of tumor cells and against cells infected with some but not all viruses (Kindt et al., 2007). Overall, no significant differences were found among treatment groups at any given time for any of the different lymphocytes (Table 4.12). However, in wk 4 there was a tendency (P = 0.07) for CD4 to be higher in calves fed unheated colostrum. The reasons for this difference are unknown and it may be an artifact of sample size. Johnson et al. (2007) when evaluating the use of heat-treated colostrum versus raw colostrum in neonatal calves at 24 h of age found no differences in total peripheral white blood cell count, neutrophil counts or percentages, total lymphocyte counts or percentages, or counts or percentages of CD4, CD8, CD14, and B lymphocytes.

4.5 CONCLUSIONS

Based on the current study, batch heat treatment of high quality colostrum at 60°C for 30 min resulted in reduced bacteria concentrations while preserving IgG concentration and

viscosity. Apparent efficiency of absorption of IgG was significantly greater for calves fed heat-treated (vs. unheated) colostrum. Serum IgG concentrations were significantly higher for calves fed heat-treated colostrum. Calves fed heat-treated colostrum showed no negative effects on health or growth parameters. Further studies are needed to clarify the precise mechanisms behind the increased IgG absorption and to investigate if feeding different volumes of heat-treated colostrum with different IgG concentrations would have a similar increase in IgG absorption if fed to calves.

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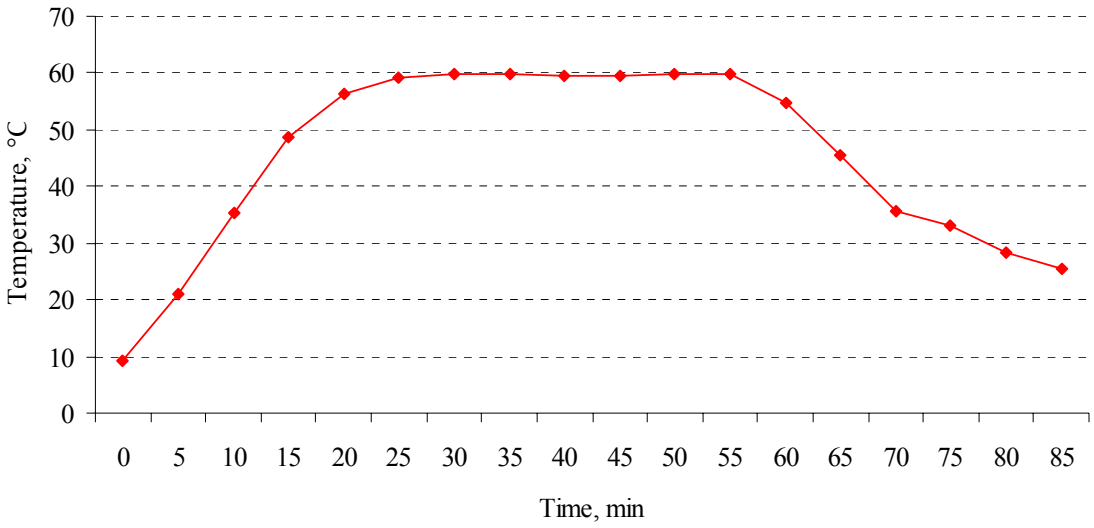

Figure **4.1**: Temperature changes during heat treatment of bovine colostrum in a steam vat pasteurizer.

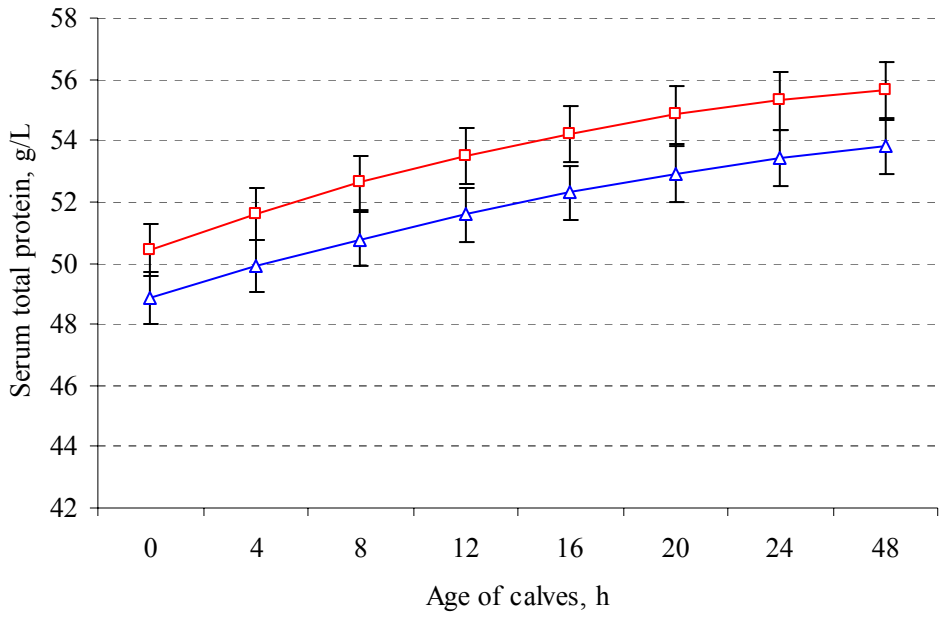

Figure **4.2**: Serum total protein in heifer calves receiving unheated (Δ) or heat-treated (□) colostrum.

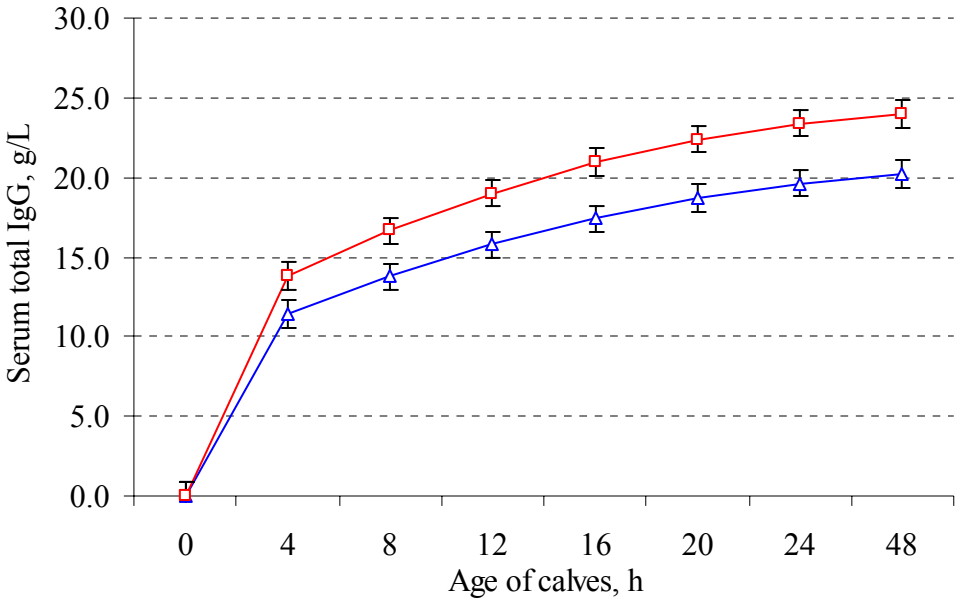

Figure **4.3**: Serum total IgG in heifer calves receiving unheated (Δ) or heat-treated (□) colostrum.

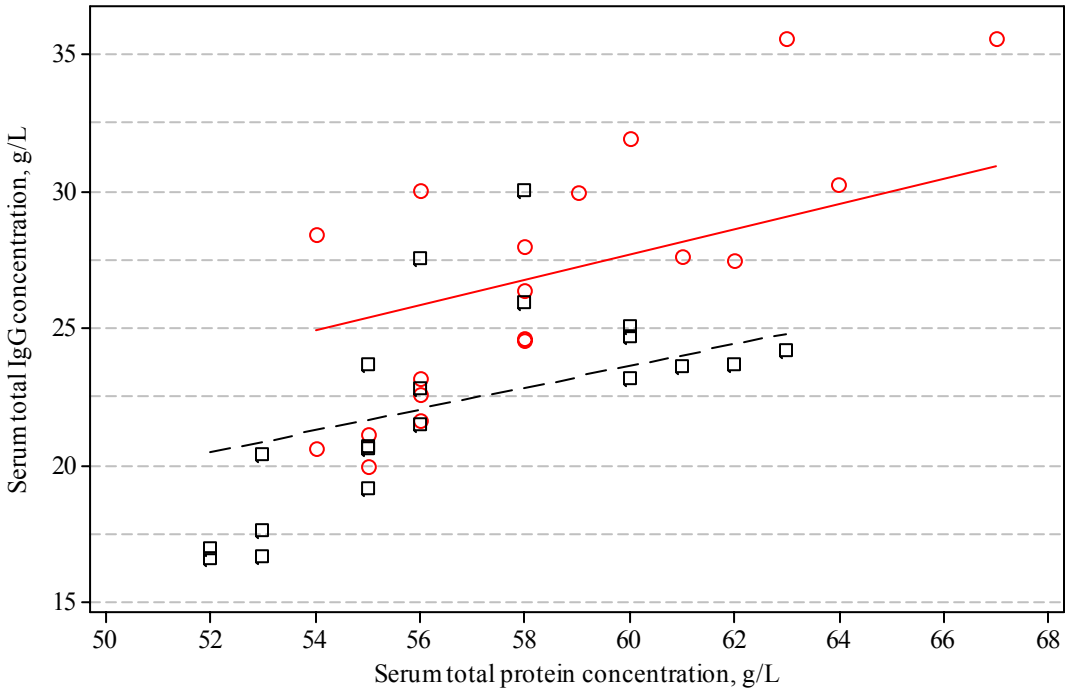

Figure **4.4**: Regression of serum IgG and serum total protein concentrations at 24 h of age in heifer calves receiving unheated (□) (Serum total IgG (g/L) = 0.734 x STP (g/L) - 19.4; R^2 = 0.462) or heat-treated colostrum (○) (Serum total IgG (g/L) = 1.01 x STP (g/L) - 32.3; R^2 = 0.593).

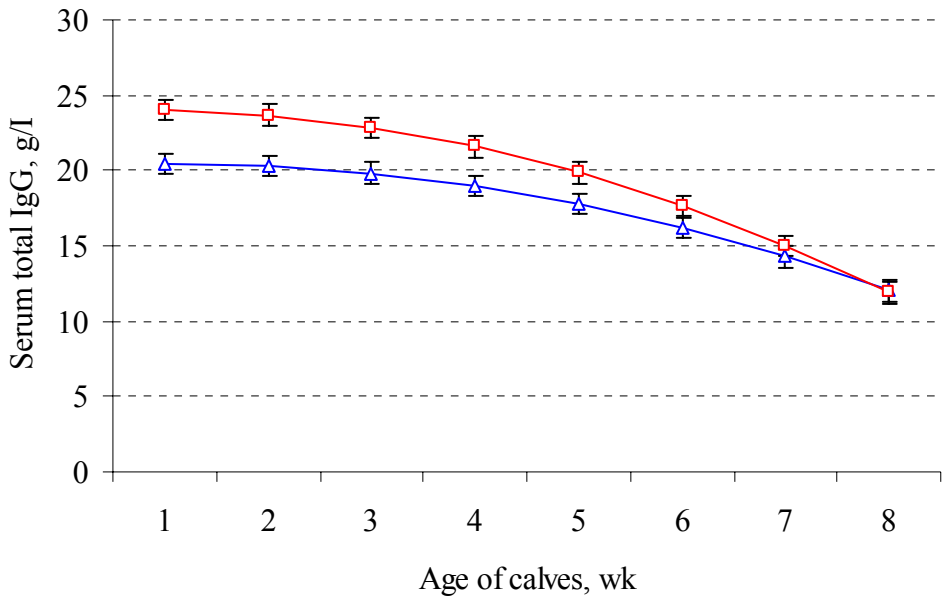

Figure **4.5**: Serum total IgG in heifer calves receiving unheated (Δ) or heat-treated (□) colostrum.

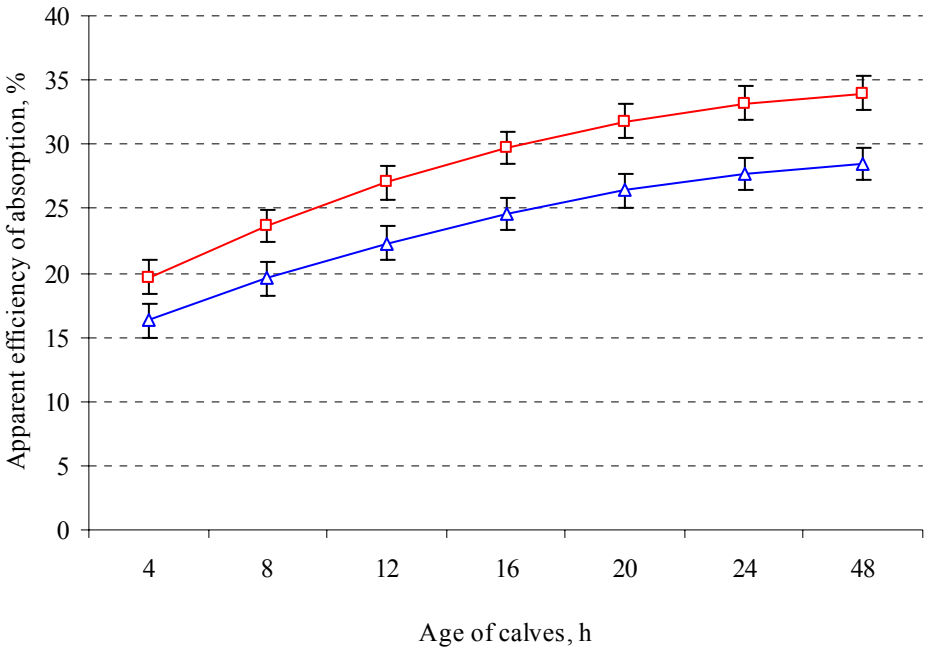

Figure **4.6**: Apparent efficiency of absorption in heifer calves receiving unheated (Δ) or heat-treated (□) colostrum.

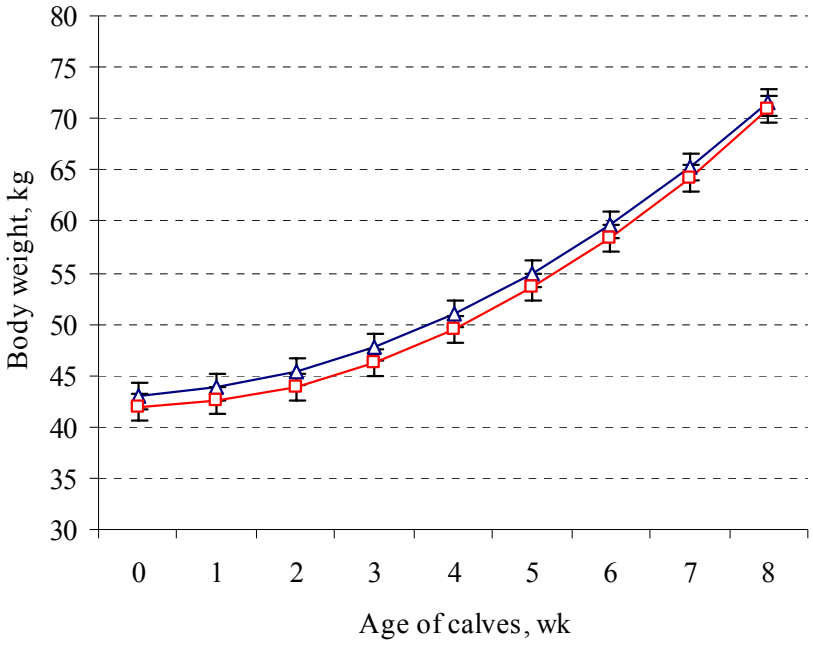

Figure **4.7**: Weekly BW of heifer calves receiving unheated (Δ) or heat-treated (□) colostrum.

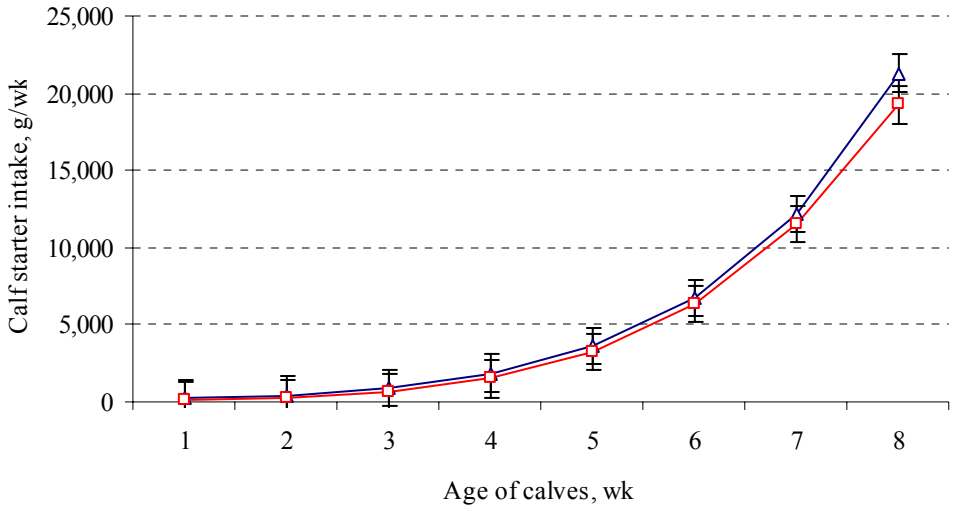

Figure **4.8**: Starter intake in heifer calves receiving unheated (Δ) or heat-treated (□) colostrum.

Table **4.1**: Compositional analysis and characteristics of colostrum samples before and after heat treatment.

Item	Unheated	Heat-treated
Density	1.063	1.063
pH	6.10	6.09
DM, %	26.29	25.51
Fat, %	6.27	6.33
Protein, %	14.20	15.75
Lactose, %	3.50	3.10
Total solids, %	33.51	30.94
Ash, %	4.27	4.36
Ca, mg/kg	0.21	0.22
P, mg/kg	0.18	0.19
Mg, mg/kg	0.04	0.04
Na, mg/kg	0.06	0.07
K, mg/kg	0.13	0.13
Zn, mg/kg	20.93	22.25
Fe, mg/kg	< 6.00	< 6.00
Cu, mg/kg	0.40	0.90
S, mg/kg	0.17	0.18
Mn, mg/kg	< 1.00	< 1.00
Retinol, µg/g	4.97	3.40
Tocopherol, µg/g	< 0.10	< 0.10
Vitamin E, µg/g	17.00	9.73

Table **4.2**: Compositional analysis of calf starter and milk replacer[1] used in the study.

Item	Calf starter	SEM	Milk replacer[2]
CP, %	21.26	0.35	20.10
Fat, %	6.35	0.99	20.10
ADF, %	7.30	0.17	0.09
NDF, %	17.90	0.52	0.10
Ash, %	7.84	0.24	6.79
NFC, %[3]	48.21	0.70	37.18
Ca, %	1.12	0.06	0.81
P, %	0.66	0.02	0.60
Mg, %	0.46	0.02	0.09
K, %	1.37	0.04	1.62
Na, %	0.26	0.02	0.58
Mn, mg/kg	176.25	8.94	0.04
Zn, mg/kg	235.12	8.36	0.05
Cu, mg/kg	52.25	2.63	0.01
Vitamin A, IU/kg[4]	75,950.00	---	76,550.00
Vitamin D, IU/kg[4]	16,275.00	---	16,400.00
Vitamin E, IU/kg[4]	325.50	---	328.10

[1]All values are expressed on a DM basis.
[2]Based on manufacturer's analysis.
[3]Calculated.
[4]Based on manufacturer's analysis.

Table **4.3**: Description of calf parameters for heifer calves receiving unheated or heat-treated colostrum.

Parameter[1]	Unheated	Heat-treated	*P* value
Mean birth weight, kg	43.3 (1.2)	41.4 (1.1)	0.26
	(32.3 to 55.3)	(32.7 to 48.4)	
Mean packed cell volume at birth	41.8 (1.7)	41.3 (1.9)	0.86
	(21.0 to 57.0)	(31.0 to 56.0)	
Mean packed cell volume at 24 h of age	34.5(1.5)	34.1 (1.6)	0.86
	(17.0 to 48.0)	(22.0 to 45.0)	
Mean packed cell volume at 48 h of age	34.8 (1.4)	33.1 (1.4)	0.90
	(19.0 to 48.0)	(21.0 to 44.0)	
Mean age at first feeding, min	103.6 (2.2)	106.2 (1.8)	0.35
	(90 to 119)	(91 to 120)	
Median parity of dam	3.0	2.5	0.60
	(1 to 4)	(1 to 5)	
Median calving ease score	1	1	0.57
	(1 to 3)	(1 to 3)	
Mean days treated with electrolytes	6.0 (0.9)	5.5 (0.9)	0.71
	(2 to 13)	(1 to 14)	

[1]Values reflect mean (SE) with range in parentheses in the row below.

Table **4.4**: Colostral IgG levels, viscosity, and bacteriology of unheated or heat-treated colostrum.

Variable	Unheated	Heat-treated	SEM
IgG, g/L	76.43	73.42	1.65
IgG_1, g/L	73.33	71.44	1.14
IgG_2, g/L	3.09	2.98	0.15
Viscosity, Pa·s	0.66	1.09	0.60
SPC[1], log_{10}cfu/mL	5.48[b]	3.07[a]	0.71
ES[2], log_{10}cfu/mL	5.50[b]	2.92[a]	1.00
CNS[3], log_{10}cfu/mL	2.21[b]	0.00[a]	0.20
CC[4], log_{10}cfu/mL	2.93[b]	0.00[a]	0.57
NCC[5], log_{10}cfu/mL	3.55[b]	1.38[a]	0.69

[ab] $P < 0.05$, comparing LS means for unheated or heat-treated colostrum.
[1] Standard plate count.
[2] Environmental streptococci count.
[3] Coagulase negative staphylococci count.
[4] Coliform count.
[5] Non-coliform count.

Table **4.5**: Serum total protein concentration (g/L) in heifer calves receiving unheated or heat-treated colostrum.

Age, h	Unheated	Heat-treated	SEM	*P* value
0	48.9	50.4	1.1	0.18
4	49.9	51.6	1.0	0.09
8	50.8	52.6	0.9	0.05
12	51.6	53.5	0.9	0.04
16	52.3	54.2	0.9	0.04
20	52.9	54.8	1.0	0.05
24	53.4	55.3	1.0	0.07
48	53.8	55.6	1.0	0.08

Table **4.6**: Serum IgG concentrations (g/L) in heifer calves receiving unheated or heat-treated colostrum.

Age	IgG_1			IgG_2			Total IgG		
h	Unheated	Heated	SEM	Unheated	Heated	SEM	Unheated	Heated	SEM
0	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00
4	10.8	13.1*	0.80	0.6	0.7	0.05	11.4	13.8*	0.84
8	13.1	15.8*	0.79	0.7	0.8*	0.04	13.8	16.6*	0.82
12	15.1	18.1*	0.81	0.7	0.9**	0.04	15.8	19.0*	0.84
16	16.7	20.0*	0.85	0.8	0.9**	0.04	17.4	20.9*	0.88
20	17.9	21.4*	0.87	0.8	1.0**	0.04	18.7	22.4**	0.90
24	18.8	22.4**	0.88	0.9	1.1**	0.04	19.6	23.4**	0.92
48	19.3	22.9**	0.87	0.9	1.1**	0.04	20.2	23.9**	0.91

* $P < 0.05$, comparing treatment LS means at each time point.
** $P < 0.01$, comparing treatment LS means at each time point.

Table **4.7**: Serum IgG concentrations (g/L) in heifer calves receiving unheated or heat-treated colostrum.

Age	IgG_1			IgG_2			Total IgG		
wk	Unheated	Heated	SEM	Unheated	Heated	SEM	Unheated	Heated	SEM
1	19.5	22.9**	0.84	1.0	1.1**	0.04	20.4	24.0**	0.87
2	19.3	22.5**	0.79	1.0	1.2*	0.05	20.3	23.7**	0.82
3	18.7	21.7**	0.72	1.1	1.2	0.06	19.8	22.9**	0.75
4	17.8	20.4*	0.65	1.1	1.2	0.07	19.0	21.6*	0.68
5	16.6	18.6*	0.59	1.1	1.2	0.08	17.8	19.8*	0.64
6	15.0	16.5	0.59	1.2	1.2	0.10	16.2	17.7	0.66
7	13.0	13.8	0.69	1.2	1.1	0.15	14.3	15.0	0.78
8	10.7	10.7	0.87	1.3	1.1	0.19	12.0	11.9	0.99

* $P < 0.05$, comparing treatment LS means at each time point.
** $P < 0.01$, comparing treatment LS means at each time point.

Table **4.8**: Apparent efficiency of absorption (AEA, %)[1] of IgG in heifer calves receiving unheated or heat-treated colostrum.

Age	AEA of IgG_1			AEA of IgG_2			AEA of total IgG		
h	Unheated	Heated	SEM	Unheated	Heated	SEM	Unheated	Heated	SEM
4	16.1	19.2	1.24	21.0	35.0**	2.14	16.3	19.7	1.26
8	19.4	23.2*	1.27	22.9	39.5**	1.92	19.5	23.7*	1.27
12	22.2	26.5*	1.34	24.8	43.5**	1.85	22.3	27.0*	1.33
16	24.5	29.2*	1.41	26.7	47.1**	1.85	24.6	29.7*	1.40
20	26.3	31.3*	1.46	28.5	50.1**	1.87	26.4	31.8*	1.46
24	27.6	32.6*	1.48	30.3	52.7**	1.87	27.7	33.2*	1.48
48	28.4	33.4*	1.47	32.0	54.9**	1.88	28.5	34.0*	1.47

[1] AEA = ((birth weight × 0.095 × serum IgG)/total IgG fed) × 100.

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

** $P < 0.01$, comparing treatment LS means at each time point.

Table **4.9**: Starter intake (kg/wk) and body weight (kg) in heifer calves receiving unheated or heat-treated colostrum.

Age	Intake			Weight		
wk	Unheated	Heat-treated	SEM	Unheated	Heat-treated	SEM
Birth	-	-	-	43.04	41.82	1.03
1	0.207	0.097	1.350	43.81	42.46	1.04
2	0.446	0.263	1.260	45.39	43.95	1.07
3	0.927	0.657	1.260	47.77	46.30	1.14
4	1.861	1.518	1.260	50.95	49.50	1.22
5	3.607	3.234	1.230	54.93	53.55	1.31
6	6.750	6.356	1.170	59.70	58.46	1.43
7	12.198	11.521	1.100	65.28	64.23	1.57
8	21.286	19.266	1.100	71.65	70.84	1.77

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

Table **4.10**: Least square means for structural growth measurements of Holstein heifer calves receiving unheated or heat-treated colostrum.

Age	Heart girth, cm			Hip height, cm			Withers height, cm		
wk	Unheated	Heated	SEM	Unheated	Heated	SEM	Unheated	Heated	SEM
Birth	84.53	81.89*	0.69	81.02	80.33	0.66	76.01	76.30	0.64
1	86.03	84.25*	0.53	82.04	81.41	0.64	77.07	77.07	0.58
2	87.62	86.57	0.58	83.10	82.56	0.64	78.17	78.17	0.58
3	89.31	88.86	0.66	84.18	83.78	0.66	79.32	79.25	0.61
4	91.08	91.12	0.74	85.29	85.06	0.69	80.50	80.43	0.64
5	92.94	93.35	0.79	86.43	86.40	0.71	81.73	81.70	0.64
6	94.90	95.55	0.81	87.59	87.81	0.69	82.99	83.07	0.66
7	96.94	97.72	0.81	88.79	89.29	0.71	84.30	84.54	0.71
8	99.08	99.86	0.94	90.01	90.83	0.74	85.65	86.11	0.79

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

Table **4.11**: Least squares of health parameters in heifer calves receiving unheated or heat-treated colostrum.

	Age, wk							
Scores[1]	1	2	3	4	5	6	7	8
Fecal								
Unheated	1.93	2.27	2.21	1.58	1.25	1.26	1.37	1.32
Heat-treated	1.78	2.38	2.26	1.57	1.31	1.27	1.32	1.26
SEM	0.09	0.10	0.14	0.09	0.07	0.08	0.09	0.08
Respiratory								
Unheated	1.01	1.00	1.02	1.00	1.00	1.02	1.07	1.08
Heat-treated	1.00	1.00	1.00	1.01	1.00	1.00	1.01	1.03
SEM	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02
Appearance								
Unheated	1.02	1.00	1.00	1.00	1.00	1.00	1.02	1.01
Heat-treated	1.00	1.00	1.00	1.00	1.01	1.02	1.00	1.00
SEM	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00

[1]Scores on a scale of 1 to 5 as described by Lesmeister and Heinrichs (2004). Lower numbers indicate more normal calf health.

Table **4.12**: Lymphocyte counts (%) in heifer calves receiving unheated or heat-treated colostrum.

Age	T helper cells (CD8)			T cytotoxic cells (CD4)			Natural killer cells (NK)		
wk	Unheated	Heated	SEM	Unheated	Heated	SEM	Unheated	Heated	SEM
1	6.12	6.41	0.79	10.10	9.18	1.39	30.81	31.04	3.55
2	7.70	8.12	0.79	11.74	12.18	1.32	34.40	34.21	3.71
3	7.79	8.59	0.80	13.19	12.63	1.23	35.78	36.26	3.65
4	6.39	7.72	2.30	14.48	10.55[a]	1.36	34.95	37.21	4.00

[a]$P = 0.07$, comparing treatment LS means at each time point.

Chapter 5

FEEDING HEAT-TREATED COLOSTRUM AND UNHEATED COLOSTRUM WITH TWO DIFFERENT BACTERIAL CONCENTRATIONS TO NEONATAL DAIRY BULLS: EFFECTS ON BLOOD PARAMETERS

5.1 ABSTRACT

The objective of this study was to determine the effects of feeding heat-treated colostrum and unheated colostrum of different bacterial counts on passive transfer of immunity in neonatal bull calves. First milking colostrum with > 50 g IgG/L (measured by colostrometer) was collected from Holstein cows and frozen at -20°C until a total of 126 L were collected. One third of the colostrum was transferred into 1.89 L plastic containers and frozen at -20°C until needed for feeding (unheated-low bacteria). Another third was heat-treated at 60°C for 30 min, transferred into 1.89 L plastic containers, and then frozen at -20°C until needed for feeding (heat-treated). The final third of colostrum was also transferred into new 1.89 kg plastic containers and stored at room temperature for bacteria to grow during 24 h (unheated-high bacteria). Colostrum was then placed into a freezer at -20°C until needed for feeding. A total of 30 Holstein bull calves weighing ≥ 30 kg at birth were systematically enrolled into 1 of the 3 treatment groups. Calves were separated from their dams at birth before suckling occurred. Before feeding colostrum, a jugular blood sample was collected from each calf. For the first feeding, 3.8 L of colostrum were fed using an esophageal feeder between 1.5 to 2 h of age. For the second and third feeding, pasteurized whole milk at 5% of birth BW was fed. Blood samples were collected before colostrum feeding and at 24 and 48 h of age. Serum from samples was used to determine IgG concentrations and serum total protein concentration. Batch heat treatment of colostrum at 60°C for 30 min resulted in lower colostrum bacteria concentration while maintaining colostral

IgG concentration and viscosity. Calves fed heat-treated colostrum had significantly greater serum total protein and IgG concentrations at 24 h, plus greater apparent efficiency of IgG absorption (total protein = 62.5 g/L; IgG = 26.7 g/L; apparent efficiency of absorption = 43.9%) compared with calves fed unheated-low bacteria colostrum (total protein = 57.0 g/L; IgG = 20.2 g/L; apparent efficiency of absorption = 35.4%) or unheated-high bacteria colostrum (total protein = 56.2 g/L; IgG = 20.1 g/L; apparent efficiency of absorption = 32.4%). High bacteria load in colostrum did not interfere with IgG absorption.

5.2 INTRODUCTION

Calves are immunocompetent, but immunologically naïve and hypogammaglobulinemic at birth (Besser and Gay, 1994). Protective concentrations of autogenous immunoglobulins are reached until the calf is weeks or even months of age (Robinson et al., 1988). Until then, humoral immunity is provided by passive immunity derived from colostral immunoglobulins ingested and absorbed during the first 24 h of life (McCoy et al., 1970; Stott et al., 1979a). Adequate passive transfer of immunity is critical for the well being and survival of calves (Nocek et al., 1984; Hancock, 1985; DeNise et al., 1989). The concept of failure of passive transfer is attributable to the fact that immunoglobulins are such a large constituent of colostrum and they have been the most thoroughly studied component of colostrum (Barrington and Parish, 2001). It has been stated that serum IgG concentrations of 10 g/L at 24 to 48 h are sufficient to reduce the risk of infectious disease in most environments (Radostis et al., 2007). However, the risk of a calf contracting infectious disease is with no doubt a complex equation in which serum immunoglobulin concentrations are just a single factor (Barrington and Parish, 2001).

Colostrum has been pointed out as a potential method of transmission of infectious diseases to newborn calves and heat treatment of colostrum has been suggested as a control measure to eliminate or reduce the transfer of colostrum-borne pathogens to dairy calves (Godden et al., 2006). Recent studies on heat treatment of colostrum have shown great advantage in reducing the number of bacteria (Godden et al., 2006; Johnson et al., 2007), and it has also been shown that feeding heat-treated colostrum to neonatal dairy calves increases IgG absorption and, as a result, serum IgG concentration (Johnson et al., 2007). The mechanism for this is unknown; however, Johnson et al. (2007) hypothesized that since antibodies in colostrum can bind pathogens present in the gut before absorption can occur, by reducing the number of

pathogens in heat-treated colostrum, and as a result, the number of pathogens in the gut, more antibodies are potentially free for absorption (James et al., 1981). The authors also added that bacteria can bind the nonspecific receptors on neonatal enterocytes, thus decreasing the number of receptors available for IgG uptake, then again by reducing the number of pathogens in colostrum there are more receptors available for IgG binding (James and Polan, 1978; James et al., 1981; Staley and Bush, 1985).

On this basis, it was hypothesized that feeding colostrum with high bacterial load decreases IgG absorption and thus serum IgG concentration in neonatal calves. The objective of this study was to determine the effects of feeding heat-treated colostrum and unheated colostrum with two different bacterial concentrations on passive transfer of immunity in neonatal dairy bull calves.

5.3 MATERIALS AND METHODS

5.3.1 Colostrum Management

First milking colostrum with IgG concentrations > 50 g/L as measured by colostrometer (Biogenics. Mapleton, OR) was collected from Holstein cows and frozen immediately at -20°C to inhibit bacterial growth. Once 126 L were collected, colostrum was thawed at 4°C, pooled and mixed for 20 min in a commercial batch pasteurizer (Girton Manufacturing Co, Millville, PA) to create a unique batch. A subsample was taken into a sterile 15-mL screw-cap centrifuge tube and stored at -20°C for later analysis. One third of the colostrum was transferred into new, clean 1.89 L plastic containers and frozen at -20°C until needed for feeding (**unheated-low bacteria colostrum**). Another third was divided into two 21-L batches and each batch was placed into

stainless steel containers. The 2 containers were placed into a steam vat pasteurizer (Girton Manufacturing Co, Millville, PA). Temperature for the water and the colostrum was monitored every 5 min. Water was heated until colostrum reached the target temperature of 60°C, held for 30 min, and then ice water was used to cool it down (Figure 5.1). Heat-treated colostrum was transferred into new 1.89 L plastic containers, and frozen at -20°C until needed for feeding (**heat-treated colostrum**). Prior to freezing, a subsample was collected from the two containers and pooled as one for later analysis. The final third of colostrum was also transferred into new 1.89 L plastic containers and stored at room temperature during 24 h for bacteria to grow (**unheated-high bacteria colostrum**). A subsample was taken from all containers and pooled as one for further analysis. Colostrum was then placed into a freezer at -20°C until needed for feeding.

5.3.2 Colostrum Sample Analyses

Samples of all colostrum were thawed at 4°C and examined for standard plate count (**SPC**), coagulase-negative staphylococci count (**CNS**), environmental streptococci count (**ES**), coliform count (**CC**), gram-negative noncoliform count (**NC**), *Streptococcus agalactiae* count (**SAG**), and *Staphylococcus aureus* count (**SA**) according to Jayarao et al. (2004). The colostrum samples were mixed thoroughly by inverting the tube 20 to 25 times; 50 μL were placed on selective and non-selective media using an inoculating loop. Plate count agar was used for enumeration of SPC. The numbers of ES and SAG in colostrum samples were estimated using modified Edward's agar supplemented with colistin sulfate and oxolinic acid (Sawant et al., 2002). MacConkey's agar no. 3 (Oxoid, Hampshire, England) was used to determine CC and

NC. Baird Parker's agar (Difco, LePont de Claix, France) was used to determine the number of CNS and presence of SA. Plates for enumeration of SPC were incubated at 32°C for 48 h. Plates for enumeration of CNS, ES, CC, SAG, and NC were incubated at 37°C for 48 h. IgG_1 and IgG_2 concentrations were determined in all samples by immunoprecipitation using single radial immunodiffusion (**RID**; VWRD, Pullman, WA). Serum samples (3 μL) were applied to serial RID plates containing agarose gel with anti bovine IgG. The plates were left undisturbed for 20 h at room temperature after adding samples. The resulting ring diameters were measured with a monocular comparator (VMRD, Pullman, WA), and the IgG content of the samples was calculated by regression analysis. A standard curve was generated with reference sera supplied by the manufacturer.

Colostrum samples were also analyzed for ash, DM (AOAC, 1990), CP (Leco FP-528 Nitrogen Combustion Analyzer; Leco, St. Joseph, MI), and crude fat (AOAC, 2000) using a Tecator Soxtec System HT 1043 Extraction unit (Tecator, Foss NA, Eden Praire, MN). Colostrum samples were sent to the Agricultural Analytical Services Laboratory at the Pennsylvania State University to be analyzed for Ca, P, Mg, Na, K, Zn, Fe, Cu, S, and Mn. Samples were also sent to the Diagnostic Center for Population and Animal Heatlth to be analyzed for fat soluble vitamins. Compositional analyses and characteristics of colostrum samples before and after heat treatment are presented in Table 5.1. It can be noted that thermal treatment did not have major compositional or characteristics changes in colostrum and the compositional analysis is in accordance with the values reported by Kehoe et al. (2007). An important aspect is the reduction in pH for the unheated-high bacterial colostrum which is a reflection of the increase in the concentration of environmental streptococci count.

5.3.3 Calf Treatment Allocation, Sample Collection, and Records

Protocols used for this study were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Holstein bull calves from the Pennsylvania State University dairy herd were separated from their dams 20 to 30 min after birth, before suckling occurred and placed into 1.0- × 1.0-m holding pens until fed colostrum and then were housed in 1.0- × 2.6-m individual calf condos naturally ventilated and bedded with straw. A total of 30 bull calves weighing ≥ 30 kg at birth were systematically enrolled into 1 of the 3 treatment groups receiving unheated-low bacteria, unheated-high bacteria, or heat-treated colostrum for the first feeding. Information for each dam and calf was recorded, including cow ID, date and time of calving, calving ease, calf identification number, treatment allocation, and age at feeding. Before feeding colostrum, a jugular blood sample was collected from each calf into 8.5-mL serum EDTA Vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ). For the first feeding, 3.8 L of colostrum were fed using an esophageal tube feeder between 1.5 and 2 h of life according to randomized treatment allocation. The use of an esophageal feeder was chosen since colostrum feedings of 2 L or more are difficult to accomplish by bottle feeding and many calves will not drink 3.8 L during the period of best immunoglobulin transfer (Besser and Gay, 1994). Colostrum was warmed to approximately 38°C using a hot water bath heated to approximately 52°C. For the second and third feeding, pasteurized whole milk at 5% of birth BW was fed. For the remaining feedings, calves were fed a milk replacer containing 20% CP (all milk protein) and 20% fat (North American Nutrition Company, Inc., Lewisburg, OH) at 10% of birth BW, 5% fed in the morning and 5% fed in the afternoon., until 2 wk of age. Blood samples were also collected from every calf at 24 and 48 h of age. A subsample from each blood sample was

collected for measurement of packed cell volume by micro-hematocrit centrifugation (Quigley et al., 2006).

5.3.4 Blood Sample Analysis in Calves

All precolostral (0 to 1 h) and postcolostral blood samples collected into serum (red top) Vacutainer tubes were refrigerated overnight, centrifuged, and the serum separated from the clot within 24 h of collection (Johnson et al., 2007). Serum total protein concentrations (**STP**; g/L) were determined using a commercially available hand-held refractometer method (VET 360, Reichert Inc., Depew, NY). Sera were then stored at -20°C until analyzed. Serum IgG (g/L) concentrations were determined using a commercially available RID kit (VWR, Pullman, WA) as described by (Hadorn and Blum, 1997). Apparent efficiency of absorption (**AEA**, %) of IgG, a calculated measure that estimates what proportion of the total IgG mass fed is actually absorbed into the calf's circulation, was calculated using the accepted equation described by Quigley, III and Drewry (1998), assuming a plasma volume of 9.5% of birth weight.

5.3.5 Statistical Analysis

Descriptive statistics were generated to describe calf and dam characteristics for the two treatment groups. Blood observations were analyzed using repeated measures analysis and the MIXED procedure of SAS 9.1 (SAS Institute., 2006). Calf was used as the random effect. The statistical model used for analysis was:

$$Y_{ijk} = \mu + T_i + W_j + (TW)_{ij} + calf_k + e_{ijk}$$

where:

Y_{ijk} = dependent variables,

μ = overall mean,

T_i = fixed effect of treatment i, where i= unheated-low bacteria, unheated-high bacteria or heat-treated,

W_j = repeated measure of time j,

$(TW)_{ij}$ = effect of treatment by time interaction,

$Calf_l$ = random effect of calf l,

e_{ijk} = residual.

AR(1) structure was used in the model. Initial measurements for blood parameters were offered as additional covariates into each model. However, none of these terms was significant and none interacted with the variable describing colostrum treatment group, so they were subsequently removed from the final models. Final significance was declared at $P < 0.05$ for all models.

5.4 RESULTS AND DISCUSSION

Descriptive statistics generated to describe calf and dam characteristics for the 3 treatment groups are shown in Table 5.2. No statistical differences were found in any of the parameters studied. Mean birth weight was 48.9, 45.0, and 42.3 kg for the unheated-low bacteria, unheated-high bacteria, and heat-treated groups, respectively. Age of calves at first feeding ranged from 90 to 120 min for all treatment groups. Median parity of dam was close to 2.5 for all

groups; however, parity ranged from 1 to 9. Calving score for all the dams in the study was never higher than 3 on a scale from 1 to 5.

5.4.1 Effect of Heat Treatment on Colostrum IgG concentration, and Bacterial Counts

There was no difference in the least square means colostral IgG concentration for unheated and heat-heated colostrum. Total IgG concentration for calves fed unheated-low bacteria, unheated-high bacteria, and heat-treated colostrum was 69.6, 69.6, and 66.0 g/L, respectively (Table 5.3). Early studies pasteurizing colostrum using the same methods and temperatures typically used to pasteurize milk (Meylan et al., 1996; Green et al., 2003), yielded unacceptable results with reduction in colostral IgG and increases in viscosity. However, more recent research has determined that problems with viscosity and possible problems with IgG denaturation can be avoided by using a lower temperature (Godden et al., 2006; Johnson et al., 2007).

The results of the present study showed that heat treatment resulted in a significant reduction of SPC, CC, NC, and CNS. Other laboratory studies have also reported success in reducing or eliminating pathogens when colostrum has been treated by heat (Godden et al., 2006; McMartin et al., 2006; Johnson et al., 2007; Trujillo et al., 2007).

5.4.2 Effect of Feeding Heat-Treated Colostrum on Serum Total Protein and IgG Concentration in Calves

The measurement of STP by refractometer as an estimate of serum immunoglobulin concentration provides rapid and inexpensive test results, and as such is a useful tool for

monitoring passive transfer status (Tyler et al., 1998). Calves are defined as having failure of passive transfer if calf STP is less than 50 g/L at 24 h of age (Donovan et al., 1998).

Serum total protein increased after first feeding in all treatment groups due to absorption of colostral IgG, as expected. When measured at 0 h (precolostal sample), there were no differences between treatment groups in STP (Table 5.4). However, 24- and 48-h serum concentrations of total protein were significantly greater ($P < 0.01$) for calves fed the heat-treated colostrum (Figure 5.2). Nonetheless, there were no significant differences ($P > 0.05$) for STP between calves fed unheated-low bacteria or unheated-high bacteria colostrum. Serum total protein concentrations for all treatment groups at 24 h of age were within normal ranges. Johnson et al. (2007) reported values very similar to the ones obtained in this experiment and 24-h serum concentrations in their study were also greater for calves fed heat-treated colostrum when compared with calves fed unheated colostrum.

Serum IgG concentrations at birth were below detectable concentrations of the assay and did not produce rings on RID plates, therefore they were assumed to be zero (Table 5.5). However, 24 and 48 h after birth calves fed heat-treated colostrum had significantly higher ($P < 0.01$) serum IgG concentrations than the other 2 treatment groups (Figure 5.3). Serum total IgG concentrations at 24-h were 20.2, 20.1, and 26.7 g/L for calves fed unheated-low bacteria, unheated-high bacteria, and heat-treated colostrum, respectively. Calves are defined as having failure of passive transfer if serum IgG concentration is less than 10 g/L when sampled between 24 and 48 h of age (Weaver et al., 2000; Radostis et al., 2007). Despite the treatment used, serum IgG concentrations obtained in this experiment doubled that value. It has been suggested that calves should consume at least 100 g of IgG soon after birth to ensure adequate serum IgG and protection against disease (Davis and Drackley, 1998). Whether adequate serum IgG is actually

obtained also depends on the efficiency of absorption (Morin et al., 1997). Dairy producers usually feed a fixed volume of colostrum per calf, and more than 23% of US dairy operations that hand-fed their calves still feed ≤ 2 L of colostrum during the first 24 h of life (National Animal Health Monitoring System, 2007). The problem with this is that only a small proportion of first milking colostrum from Holstein cows contains a sufficiently high concentration of immunoglobulin (Besser and Gay, 1994; Kehoe et al., 2007) and higher volumes of colostrum are required to achieve this mass intake (Pritchett et al., 1991). For this reason, this experiment included feeding calves colostrum artificially with an oral feeder to ensure that each calf received an ample amount of selected colostrum early in the absorptive period. Even though the efficiency of immunoglobulin absorption declines somewhat as larger amounts are fed, feeding large volumes of colostrum results in higher serum IgG concentrations in the calf (Stott et al., 1979b; Bush and Staley, 1980; Besser and Gay, 1994). Even so, in a study calves fed 4 L of high immunoglobulin colostrum at the first feeding absorbed IgG_1 as efficiently as calves fed 2 L of high immunoglobulin colostrum, which indicates that it is advantageous to feed a high volume of colostrum with a high immunoglobulin concentration (Morin et al., 1997). In agreement with the present study, administration of 4 L of colostrum at one feeding by esophageal feeder caused no signs of discomfort and no evidence of clinical gastrointestinal disease in the calves (Morin et al., 1997).

In the present trial, calves received an average mass of total IgG equal to 254 g and none of the 30 calves experienced failure of passive transfer, regardless of treatment. Concentrations of total protein and IgG in serum at 24 h have been shown to be positively correlated (Stott and Fellah, 1983; Tyler et al., 1996; Quigley, III et al., 2002; Foster et al., 2006), which means the higher the serum IgG concentration, the higher the value for STP and vice versa. The

relationship between circulating serum total IgG and STP in calves in the different treatment groups is depicted in Figure 5.4. Calves in all treatment groups were administered the same mass of protein and IgG from colostrum; however, absorption of IgG varied among treatment groups. There was a significant difference ($P < 0.01$) between treatment groups as indicated by the difference between regression lines. Calves fed heat-treated colostrum had greater serum total IgG concentration at 24 h than calves fed the unheated-low bacteria or unheated-high bacteria colostrum at the same STP concentration. However, there were no differences between calves fed unheated-low bacteria or unheated-high bacteria colostrum.

The regression equations obtained in this study for the different treatment groups are:

Unheated-low bacteria: Serum total IgG (g/L) = 0.559 x STP (g/L) - 11.0 ($R^2 = 0.563$)

Unheated-high bacteria: Serum total IgG (g/L) = 0.548 x STP (g/L) - 9.86 ($R^2 = 0.548$)

Heat-treated: Serum total IgG (g/L) = 0.704 x STP (g/L) - 16.7 ($R^2 = 0.518$)

All treatments: Serum total IgG (g/L) = 0.719 x STP (g/L) - 18.9 ($R^2 = 0.656$)

When the cutoff value of 50 g/L of STP proposed by Donovan et al. (1998) is used in this study, estimates of passive transfer by measuring STP seriously underestimates the adequacy of passive transfer, especially in calves fed heat-treated colostrum. In the present study, when STP is near 52 g/L, serum IgG concentration is above 10 g/L and this is more evident for the calves fed colostrum treated by heat; meanwhile, Tyler et al. (1996) demonstrated that a serum protein concentration of 52 g/L, measured by refractometry, was equivalent to an IgG concentration of 10 g/L. This may mean that refractometry is not such an accurate means to estimate serum total IgG concentrations when calves are fed high volumes of colostrum or heat-treated colostrum.

5.4.3 Effect of Feeding Heat-Treated Colostrum on Apparent Efficiency of IgG Absorption

The colostral IgG concentration, along with the volume of colostrum ingested, determines the mass of IgG presented to the calf for absorption, the most important factor determining the calf's subsequent blood IgG concentration (Bush and Staley, 1980). The ability of the newborn calf to absorb colostral immunoglobulin decreases rapidly following birth (Stott et al., 1979a). Mean AEA for IgG from maternal colostrum, calculated as grams of IgG in the blood at 24 h divided by the grams of IgG intake is remarkably variable, ranging between 6 to 88%; however, most values are between 20 and 35% (Quigley, III and Drewry, 1998). The wide variation in reported AEA could be attributed in part to the value used to estimate the plasma volume of animals and different methodologies used to determine IgG concentrations. It is interesting that AEA was significantly greater ($P < 0.01$) for calves fed heat-treated colostrum (Table 5.6 and Figure 5.5). The AEA for total IgG at 24 h ranged from 32.4 to 43.9% and from 29.5 to 41.0% at 48 h for calves in all treatment groups. The average values at 24 h of age were higher than those reported by Johnson et al. (2007), nonetheless, they also observed a greater AEA for total IgG in calves fed heat-treated colostrum. Overall, heat treatment of colostrum did not appear to have a detrimental effect on calf health. In agreement with this study, Johnson et al. (2007) reported no effect on health for calves receiving heat-treated colostrum. The authors also reported no effect of treatment on 24-h serum concentrations of IgM or IgA.

Only one recent study has reported that feeding heat-treated colostrum increased IgG absorption and as a result serum IgG concentrations in calves (Johnson et al., 2007). They hypothesized that bacteria in colostrum may bind free IgG in the gut lumen or directly block uptake and transport of IgG molecules across intestinal epithelial cells, thus interfering with passive absorption of colostral immunoglobulins (James et al., 1981). Subsequently, by reducing

the number of pathogens in heat-treated colostrum, and as a result, the number of pathogens in the gut, more antibodies are potentially free for absorption (James and Polan, 1978; James et al., 1981; Staley and Bush, 1985). However, there were not any significant differences between AEA or serum IgG concentrations in calves fed unheated-low bacteria or unheated-high bacteria colostrum. This suggests that the type of bacteria and the high bacterial counts present in the unheated-high bacterial colostrum used in this experiment did not interfere with IgG absorption, and that other factors associated with the thermal treatment of colostrum are coming into play. A possible explanation could be that heat treatment denatures some colostral proteins that otherwise would interfere or compete for receptors on neonatal enterocytes, thus reducing the number of receptors available for IgG uptake; however, this has to be further investigated.

In the present study dairy bull calves fed a high volume of heat-treated colostrum with high IgG concentration were able to absorb more IgG than calves fed unheated colostrum. It would be very important to investigate if the same effect could be seen by feeding calves similar volumes of colostrum with low IgG concentrations or lower volumes with varying IgG concentrations.

5.5 CONCLUSIONS

Based on the current study, batch heat treatment of colostrum at 60°C for 30 min reduced bacteria concentrations and preserved IgG concentration. Apparent efficiency of absorption of IgG was significantly greater for calves fed heat-treated (vs. unheated-low bacteria or unheated-high bacteria) colostrum. Serum IgG concentrations were significantly higher for calves fed heat-treated colostrum. High bacterial load in colostrum did not interfere with IgG absorption. While

the precise mechanism for IgG absorption from colostrum is not yet known in the bovine, understanding this better could provide an improvement in practical feeding systems allowing higher blood IgG concentrations at 24 to 48 h of age. In addition it is necessary to know if feeding different volumes of heat-treated colostrum with varying IgG concentrations would have a similar increase in IgG absorption.

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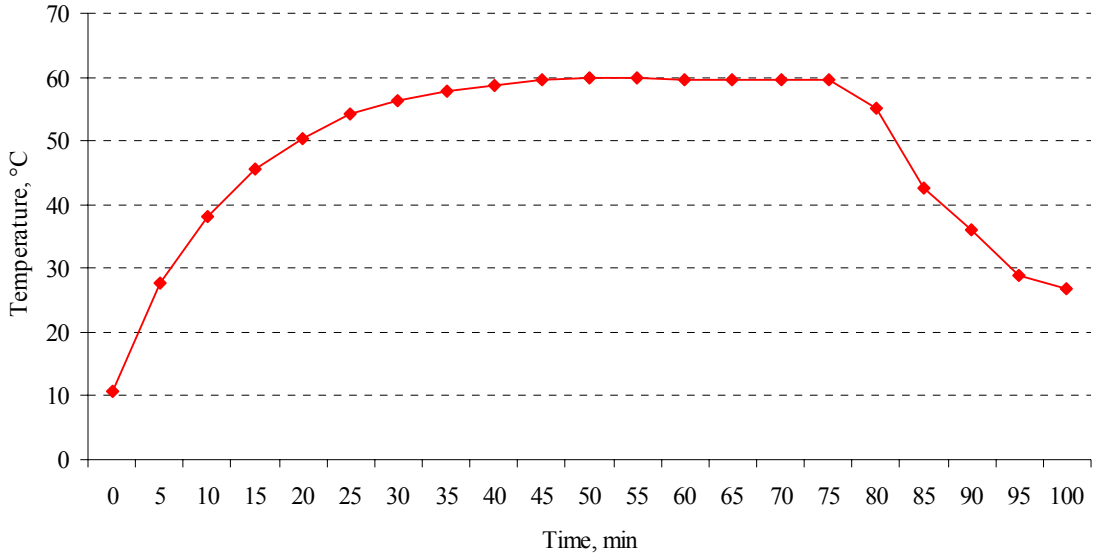

Figure **5.1**: Temperature changes during heat treatment of bovine colostrum in a steam vat pasteurizer.

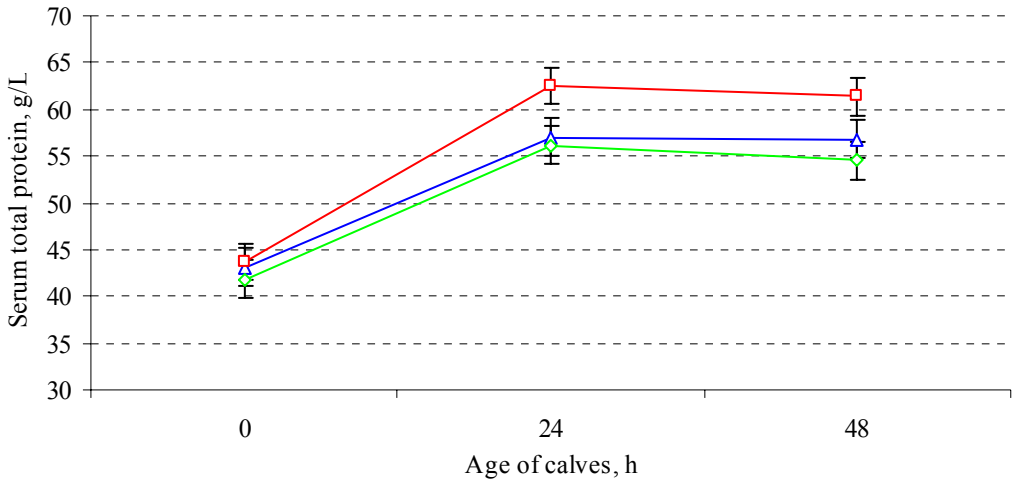

Figure **5.2**: Serum total protein concentration in bull calves fed unheated-low bacteria (Δ), unheated-high bacteria (◊), or heat-treated colostrum (□) ($P < 0.01$).

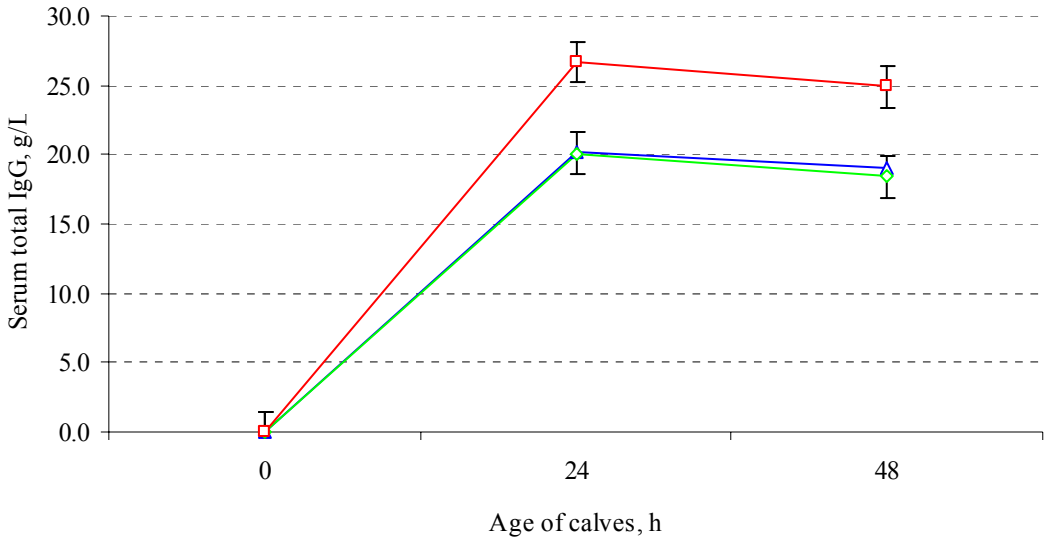

Figure **5.3**: Serum total IgG concentration in bull calves fed unheated-low bacteria (Δ), unheated-high bacteria (◊), or heat-treated colostrum (□) ($P < 0.01$).

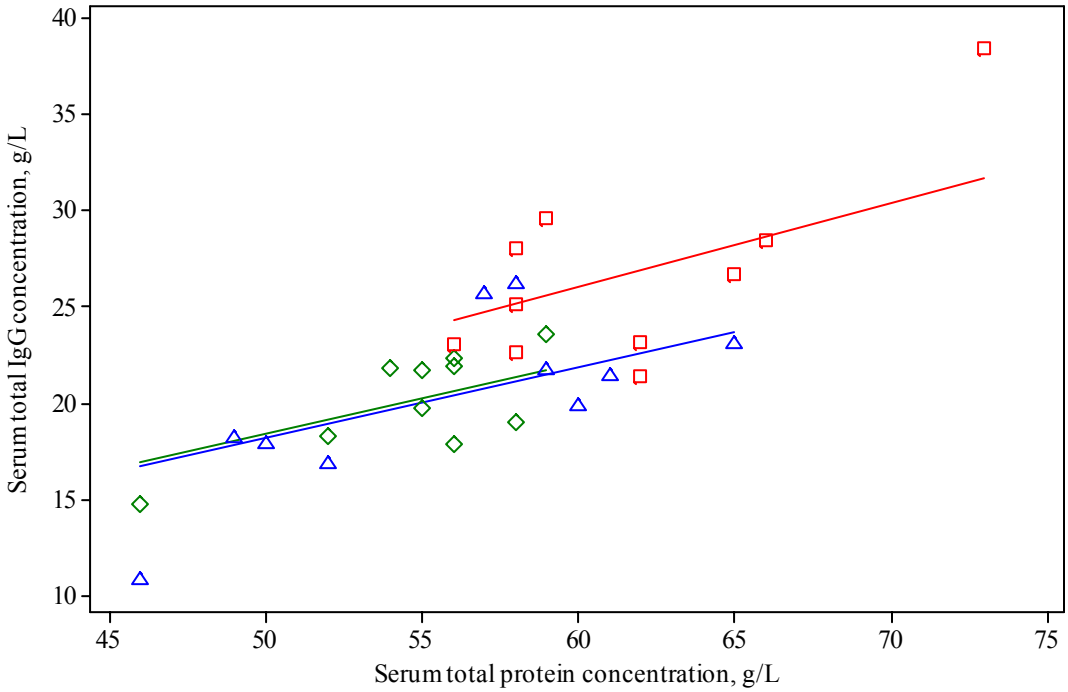

Figure **5.4**: Regression of serum total IgG and serum total protein concentrations at 24 h of age in bull calves fed unheated-low bacteria (Δ) (Serum total IgG (g/L) = 0.559 x STP (g/L) - 11.0;R^2 = 0.563), unheated-high bacteria (◊) (Serum total IgG (g/L) = 0.548 x STP (g/L) - 9.86; R^2 = 0.548), or heat-treated colostrum (□) (Serum total IgG (g/L) = 0.704 x STP (g/L) - 16.7; R^2 = 0.518) ($P < 0.01$).

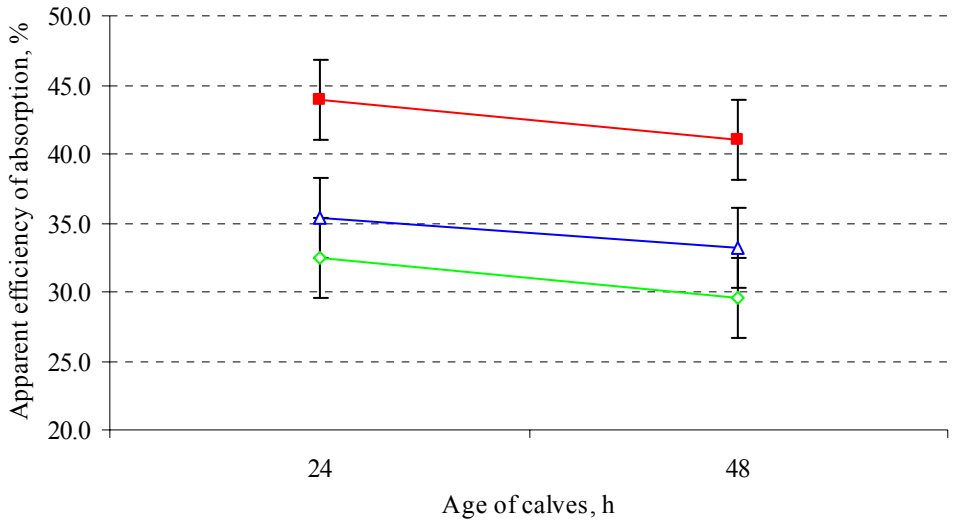

Figure **5.5**: Apparent efficiency of absorption of total IgG in bull calves fed unheated-low bacteria (Δ), unheated-high bacteria (◊), or heat-treated colostrum (□) ($P < 0.01$).

Table **5.1**: Compositional analysis of colostrum used for the different treatments of the study.

	Colostrum treatment		
Item	Unheated-low bacteria	Unheated-high bacteria	Heat-treated
Specific gravity	1.071	1.071	1.072
pH	6.11	4.83	6.11
DM, %	26.29	26.46	26.48
Fat, %	5.69	5.93	5.67
Protein, %	16.79	16.82	16.46
Lactose, %	9.47	8.51	9.51
Total solids, %	27.55	27.84	28.09
Ash, %	4.79	4.77	4.72
Ca, mg/kg	0.26	0.26	0.26
P, mg/kg	0.22	0.22	0.22
Mg, mg/kg	0.05	0.05	0.05
Na, mg/kg	0.07	0.07	0.06
K, mg/kg	0.15	0.17	0.15
Zn, mg/kg	23.96	23.36	23.51
Fe, mg/kg	< 6.00	< 6.00	< 6.00
Cu, mg/kg	0.45	0.60	0.50
S, mg/kg	0.19	0.19	0.19
Mn, mg/kg	< 1.00	< 1.00	< 1.00
Retinol, µg/g	1.95	1.07	1.08
B-Carotene, µg/g	< 0.10	< 0.10	< 0.10
Vitamin E, µg/g	1.55	2.24	2.13

Table **5.2**: Description of calf parameters for the different treatment groups (n = 10 per group).

	Colostrum treatment			
Parameter[1]	Unheated-low bacteria	Unheated-high bacteria	Heat-treated	*P* value
Mean birth weight, kg	48.9 (2.0) (34.6 to 56.7)	45.0 (1.1) (41.5 to 51.2)	42.3 (2.6) (30.0 to 57.1)	0.08
Mean packed cell volume at birth	38.4 (3.0) (30.0 to 58.0)	36.7 (1.1) (31.0 to 41.0)	36.1 (1.4) (28.0 to 42.0)	0.70
Mean packed cell volume at 24 h of age	34.0 (2.7) (25.0 to 52.0)	33.1 (1.0) (27.0 to 38.0)	30.4 (1.8) (21.0 to 39.0)	0.70
Mean packed cell volume at 48 h of age	32.6 (2.9) (24.0 to 53.0)	30.7 (1.0) (24.0 to 36.0)	28.8 (1.5) (20.0 to 36.0)	0.65
Mean age at first feeding, min	109.8 (2.8) (94 to 120)	100.7 (3.9) (90 to 120)	102.4 (2.3) (92 to 117)	0.10
Median parity of dam	2.5 (1 to 9)	2.0 (1 to 5)	2.5 (1 to 4)	0.46
Median calving ease score	1 (1 to 3)	1 (1 to 3)	1 (1 to 3)	0.75

[1]Values reflect mean (SE) with range in parentheses in the row below.

Table **5.3**: IgG concentration (g/L) and bacterial counts in untreated-low bacteria, unheated-high bacteria, and heat-treated colostrum samples.

	Colostrum treatment			
Variable	Unheated-low bacteria	Unheated-high bacteria	Heat-treated	SEM
IgG, g/L	69.55	69.55	65.96	1.86
IgG1, g/L	66.46	66.46	63.28	1.73
IgG2, g/L	3.09	3.09	2.89	0.13
SPC[1], log_{10}cfu/mL[6]	3.97[b]	5.61[c]	2.81[a]	0.81
ES[2], log_{10}cfu/mL	1.86[b]	5.59[c]	0.90[a]	1.43
CNS[3], log_{10}cfu/mL	0.00[a]	0.90[b]	0.00[a]	0.30
CC[4], log_{10}cfu/mL	2.02[b]	3.16[c]	0.00[a]	0.92
NCC[5], log_{10}cfu/mL	3.37[b]	5.39[c]	1.83[a]	1.03

[a-c]$P < 0.05$, comparing LS means for unheated-low bacteria, unheated-high bacteria, or heat-treated colostrum.

[1] Standard plate count.
[2] Environmental streptococci count.
[3] Coagulase negative Staphylococci count.
[4] Coliform count.
[5] Non-coliform count.
[6] Colony forming units/mL (log transformed values).

Table **5.4**: Serum total protein concentration (g/L) in bull calves fed unheated-low bacteria, unheated-high bacteria or heat-treated colostrum.

	Colostrum treatment			
Age, h	Unheated-low bacteria	Unheated-high bacteria	Heat-treated	SEM
0	43.1	41.8	43.7	0.23
24	57.0[b]	56.2[b]	62.5[a]	0.23
48	56.8[b]	54.5[b]	61.4[a]	0.23

[ab]$P < 0.01$, comparing LS means for groups at each time point.

Table **5.5**: Serum IgG concentrations (g/L) in bull calves fed unheated-low bacteria, unheated-high bacteria or heat-treated colostrum.

Age	IgG_1				IgG_2				Total IgG			
h	U-LB[1]	U-HB[2]	HT[3]	SEM	U-LB	U-HB	HT	SEM	U-LB	U-HB	HT	SEM
0	0.0	0.0	0.0	0.00	0.0	0.0	0.0	0.00	0.0	0.0	0.0	0.00
24	19.1[b]	19.1[b]	25.3[a]	1.41	1.1[b]	1.0[b]	1.4[a]	0.08	20.2[b]	20.1[b]	26.7[a]	1.47
48	18.1[b]	17.4[b]	23.6[a]	1.41	1.0[b]	1.0[b]	1.3[a]	0.08	19.1[b]	18.4[b]	24.9[a]	1.47

[ab] $P < 0.01$, comparing LS means for groups at each time point.

[1] Unheated-low bacteria colostrum.

[2] Unheated-high bacteria colostrum.

[3] Heat-treated colostrum.

Table **5.6**: Apparent efficiency of absorption (AEA, %) for IgG in bull calves fed unheated-low bacteria, unheated-high bacteria or heat-treated colostrum.

Age	AEA of IgG_1				AEA of IgG_2				AEA of total IgG			
h	U-LB[1]	U-HB[2]	HT[3]	SEM	U-LB	U-HB	HT	SEM	U-LB	U-HB	HT	SEM
24	34.9^{b}	32.1^{b}	43.4^{a}	2.90	42.1^{b}	38.1^{b}	54.6^{a}	3.60	35.4^{b}	32.4^{b}	43.9^{a}	2.90
48	32.7^{b}	29.4^{b}	40.4^{a}	2.90	38.7^{b}	35.4^{b}	52.2^{a}	3.60	33.2^{b}	29.5^{b}	41.0^{a}	2.90

$^{ab}P < 0.01$, comparing LS means for groups at each time point.

[1] Unheated-low bacteria colostrum.

[2] Unheated-high bacteria colostrum.

[3] Heat-treated colostrum.

Chapter 6

CONCLUSIONS

This dissertation investigated the effect of thermal treatment of bovine colostrum on physical and chemical properties and on neonatal blood and growth parameters.

The first study was designed to identify the optimal temperature and timing, at which heat treatment of bovine colostrum would produce no significant changes in viscosity and IgG concentrations and produce a significant change in bacterial count. Thermal treatment significantly reduced the bacterial load in a variety of bovine colostrum samples, indicating that treating colostrum by heat could serve as an effective method for reducing pathogen exposure to newborn calves. However, heat treatment of 10-mL of bovine colostrum at 60°C and above resulted in significant denaturation of colostral IgG_1 as measured by radial immunodifusion; conversely, colostral IgG_2 concentrations were not reduced when the temperature was held at 60°C for 30 or 60 min. Viscosity was not affected when temperature was held at 60°C for less than 60 min. The findings of this study suggest that heat treatment of bovine colostrum at 60°C for 30 to 60 min may be used as an optimal temperature and timing, at which heat treatment would produce no significant changes in viscosity, a small reduction in measured IgG concentrations, and a significant reduction in bacterial count.

In the second study, the effect of feeding heat-treated colostrum on growth characteristics and blood parameters in neonatal dairy heifer calves was investigated. Batch heat treatment of colostrum at 60°C for 30 min resulted in reduced bacteria concentrations in colostrum while preserving the colostral IgG concentration and viscosity. Apparent efficiency of absorption of IgG was significantly greater ($P < 0.05$) for calves fed heat-treated (vs. unheated) colostrum. The apparent efficiency of absorption for total IgG at 24 h of age was 27.7 and 33.2% for calves

receiving unheated and heat-treated colostrum, respectively. Serum IgG concentrations were significantly higher for calves fed heat-treated colostrum. Calves fed heat-treated colostrum had nearly 20% greater ($P < 0.01$) serum total IgG concentration at 24 and 48 h than calves fed unheated colostrum (23.4 vs. 19.6 g/L, and 23.9 vs. 20.2 g/L, respectively). Calves fed heat-treated colostrum showed no negative effects on health or growth parameters. Further studies are needed to clarify the precise mechanisms behind the increased IgG absorption and to find out if feeding different volumes of heat-treated colostrum with different IgG concentrations would have similar effects on absorption.

The third study investigated the effects of feeding heat-treated colostrum and unheated colostrum of different bacterial counts on passive transfer of immunity in neonatal bull calves. Batch heat treatment of colostrum at 60°C for 30 min reduced bacteria concentrations and preserved IgG concentration and fluidity. Serum IgG concentrations were significantly higher ($P < 0.01$) for calves fed heat-treated colostrum. Serum total IgG concentrations at 24 h after birth were 20.2, 20.1, and 26.7 g/L for calves fed unheated-low bacteria, unheated-high bacteria, and heat-treated colostrum, respectively. Apparent efficiency of absorption of IgG was significantly greater ($P < 0.01$) for calves fed heat-treated (vs. unheated-low bacteria or unheated-high bacteria) colostrum. Apparent efficiency of absorption at 24 h after birth was 35.4, 32.4, and 43.9% for calves receiving unheated-low bacteria, unheated-high bacteria, and heat-treated colostrum, respectively. High bacterial load in colostrum did not interfere with IgG absorption in a significant manner. While the precise mechanism for IgG absorption from colostrum is not yet known in the bovine, understanding this better could allow an improvement in practical feeding systems allowing improved blood IgG concentrations at 24-48 h of age. In addition we need to

know if feeding different volumes of heat-treated colostrum with varying IgG concentrations would have a similar increase in IgG absorption.

VITA

Jorge Alberto Elizondo Salazar

Jorge Alberto Elizondo Salazar was born in San José, Costa Rica. In 1985 while finishing high school he was granted a scholarship in order to continue studies at Kirkwood Community College, in Cedar Rapids, IA. In 1987 he obtained an Associate Degree in Farm and Ranch Technology. After returning to Costa Rica, he attended the University of Costa Rica in which he graduated with a Licentiate in Animal Sciences. After working a few years for some private companies and for the Costa Rican Ministry of Education, he joined the University of Costa Rica in 2000 to work as an instructor. In 2004, after doing some teaching, research, and extension in the Alfredo Volio Mata Experiment Station of the University of Costa Rica, he was granted a Fulbright Scholarship to obtain a Master's degree in Animal Science at the Pennsylvania State University. In 2006 he received his degree and was offered an assistantship in the Department of Dairy and Animal Science to work toward a Ph. D. In 2008 he received his Ph. D. and returned to work at the Alfredo Volio Mata Experiment Station of the University of Costa Rica in which he continued doing research, extension, teaching, and helping producers to improve animal production and welfare.