REVIEW: Heat Treating Bovine Colostrum

J. A. Elizondo-Salazar, PAS, and A. J. Heinrichs, PAS
Department of Dairy and Animal Science, The Pennsylvania State University, University Park 16802

ABSTRACT

The syndesmochorial placenta of the bovine prevents transmission of Ig in utero and calves are born agammaglobulinemic. Consequently, ingestion and absorption of adequate amounts of colostral Ig are 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 Ig concentration of the colostrum ingested. In addition, because 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 reduced or eliminated important bacterial pathogens; however, this process reduced Ig concentration and increased viscosity. More recent studies using lower temperatures have shown no reduction in colostral Ig concentration or fluidity and have concluded that heat-treated colostrum can be successfully fed on commercial dairy farms without interfering with passive transfer of immunity in calves. The objective of this paper is to 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.

Key words: colostrum, pasteurization, immunoglobulin G, bacterial contamination

INTRODUCTION

Colostrum feeding is a critical step in raising healthy calves as a result of the physiology and metabolism of the bovine species. The syndesmochorial placenta of the bovine forms a syncytium between the maternal endometrium and the fetal trophoderm, separating the maternal and fetal blood supplies and preventing transmission of Ig 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 Ig are essential for establishing immunity until the calf’s own immune system becomes completely functional (Robison et al., 1988; Weaver et al., 2000). The transfer of Ig from the dam to the neonate, termed passive transfer, is important in protecting 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 United States 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 (2007) dairy studies reported that mortality rate among preweaned dairy heifers on US farms averaged 7.9%, and a great proportion of these deaths could be attributed to FPT. Measuring total serum protein in calves within the first 3 d 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 protein (National Animal Health Monitoring System, 2007).

The small intestine of the newborn calf possesses the capacity to absorb intact large molecules such as Ig and other proteins (Stott and Menefee, 1978; Larson et al., 1980; Hopkins and Quigley, 1997; Morin et al., 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 reflects a combination of exhaustion of pinocytotic capability and enterocyte replacement.
by a more mature population of gut epithelial cells (Broughton and Leece, 1970). In calves, closure occurs at approximately 24 h postpartum (Stott et al., 1979a). For this reason, the absorption of sufficient Ig to provide passive immunity to the calf must happen before closure occurs.

The cornerstones of a successful colostrum management program are age of calf at first feeding, volume of colostrum administered, and Ig concentration of the colostrum ingested (Stott et al., 1979a,b,c). 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 Ig molecules (James et al., 1981).

Several bacterial pathogens can be transmitted in colostrum and milk. 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 using preservatives 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 bovine colostrum (McMartin et al., 2006). The adoption of commercial on-farm pasteurization systems for the purpose of pasteurizing nonsealable 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 also been done using the same times and temperatures recommended for milk (Godden et al., 2003). However, limited laboratory and field studies have investigated the practice of heat treating colostrum and reported results vary with respect to effect of heating on colostral Ig concentrations and rates of FPT. 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 paper 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.

**REVIEW AND DISCUSSION**

**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 Ig (Foley and Otterby, 1978; Oyeniyi and Hunter, 1978; Barrington et al., 2001; Madsen et al., 2004), which provides 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 development of the neonatal intestine because it contains several bioactive and growth-promoting substances such as peptide hormones, growth factors, cytokines, steroid hormones, thyroxine, nucleotides, polyamines, enzymes, lactoferrin, lysozymes, insulin, cytokines, IGF-1, and IGF-2 (Koldovský, 1989; Pakkanen and Aalto, 1997). Villous circumference, area, height, and height-to-crypt depth ratio in the duodenum have been shown to be greater for calves fed colostrum compared with colostrum-deprived calves (Buhler et al., 1998; Blattler et al., 2001). Calves fed colostrum also have greater plasma xylose concentrations after oral administration of xylose compared with calves fed milk replacer, suggesting enhanced absorptive capabilities in colostrum-fed animals (Hammond 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. Except for casein and lactose, colostrum contains nutrients in greater concentrations than mature milk (Foley and Otterby, 1978; Koldovský, 1989; Blum and Hammond, 2000). Concentration of energy, protein, fat, and some minerals are markedly higher in colostrum than in mature milk (Davis and Drackley, 1998). It is important to emphasize that the concentrations of proteins and peptides diminishes quickly after the onset of lactation (Hadorn and Blum, 1997; Madsen et al., 2004). Likewise, the concentration of Ig is significantly reduced in subsequent milkings (Oyeniyi and Hunter, 1978; Stott et al., 1981; Davis and Drackley, 1998).

**Ig and Their Importance**

Colostrum contains large quantities of Ig (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 Ig from the serum to the mammary gland begins several weeks before parturition and reaches a peak 1 to 3 d before parturition in the cow (Sasaki et al., 1976; Barrington et al., 1997b). There are 3 major types of Ig in bovine colostrum: G, M, and A (Butler, 1969; Pakkanen and Aalto, 1997; Kehoe et al., 2007). Distribution of Ig classes in colostrum is very variable among cows (Stott et al., 1981; Petrie, 1984). The IgG, IgA, and IgM typically account for 85, 5, and 7% of the total of Ig in colostrum, respectively (Sasaki et al., 1976; Larson et al., 1980). Bovine IgG can be divided into 2 subclasses: IgG1 and IgG2 (Butler, 1969), but IgG1 constitutes more than 90% of total IgG (Pakkanen and Aalto, 1997; Barrington et al., 2001). Although IgG1 and IgG2 are present at approximately equal concentrations in ruminant blood, only the IgG1 subclass is transported in large
amounts from maternal plasma across alveolar epithelial cells into mammary secretions (Larson et al., 1980; Bain-ner, 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 (FcRn) for IgG 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). Despite the important physiological roles of the other classes of Ig, the predominant quantity of IgG makes measurement of total IgG or IgG the obvious change in providing protection to the calf. Insufficient serum Ig 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 Ig often are able to inactivate pathogenic invasions earlier than calves with lower serum Ig that must mount an immune response for defense. Therefore, calves having adequate serum Ig will continue to grow normally and not be deterred as would calves with insufficient Ig (Robison et al., 1988).

IgG Absorption in the Neonatal Dairy Calf

As has been stated, newborn calves obtain maternal antibodies solely from colostrum (Stott et al., 1976, 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). Nonselective pinocytosis has been suggested as the transport mechanism for IgG transport across intestinal epithelium (Besser and Gay, 1985). However, recent evidence has pointed toward a role for the neonatal FeRn in these processes. The FeRn is composed of 2 subunits, β2-microglobulin and an integral membrane polypeptide homologous to the MHC class I pro-teins (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 FeRn 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 human and mouse FeRn, 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 bovine FeRn influence serum IgG concentration in newborn calves (Laegreid et al., 2002; Clawson et al., 2004).

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, E. coli, Salmonella spp., L. 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 Mycobacterium paratuberculosis as calves

Increased neonatal mortality and morbidity are well-accepted consequences of FPT. Virtala et al. (1999) showed that low postcolostral serum IgG concentrations is a significant risk factor for development of pneumonia in heifer calves. 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 6 mo of age, showed a clear association between serum total protein values (<50 g/L) were and mortality. Calves with low serum Ig often are able to inactivate pathogenic invasions earlier than calves with lower serum Ig that must mount an immune response for defense. Therefore, calves having adequate serum Ig will continue to grow normally and not be deterred as would calves with insufficient Ig (Robison et al., 1988). Illnesses often associated with such invasions detract from the normal growth and development of the calf. Calves with adequate serum Ig often are able to inactivate pathogenic invasions earlier than calves with lower serum Ig that must mount an immune response for defense. Therefore, calves having adequate serum Ig will continue to grow normally and not be deterred as would calves with insufficient Ig (Robison et al., 1988).
but often do not develop clinical signs until 2 to 5 yr of age (Stabel, 2001). An important aspect is that *Mycobacterium paratuberculosis* is shed in 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 used 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 noninfected dams. However, this recommendation generates considerable expense for producers, who must dump colostrum because it may be a source of *Mycobacterium paratuberculosis* or other pathogens and purchase colostrum or milk replacer from outside sources. 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 first pasteurized it before feeding it to calves. A higher percentage of large operations (6.4%) pasteurized colostrum compared with 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 *Mycobacterium paratuberculosis* in waste milk (Stabel, 2001). On-farm pasteurization of waste milk held at 65°C for 10 min also destroyed common mastitic *Mycoplasma* organisms such as *M. bovis, M. california*, 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 *Mycobacterium paratuberculosis, Salmonella* spp., and *Mycoplasma* spp. in raw milk and *Mycobacterium paratuberculosis* in colostrum. They showed that a commercial HTST unit (71.7°C for 15 s) was effective in the destruction of *Mycobacterium 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 *Mycobacterium paratuberculosis* present. However, recovery of *Mycobacterium paratuberculosis* from colostrum was reduced by 2 logaux after 10 min and achieved a nadir of <3 cfu/mL of milk after 30 min. Increasing the temperature range (68.3 to 70.8°C) completely abrogated recovery of viable *Mycobacterium paratuberculosis* from colostrum. Stabel et al. (2004) concluded that HTST pasteurization is effective for the destruction of *Mycobacterium paratuberculosis, Salmonella* spp., and *Mycoplasma* spp. in raw milk and effectively destroys *Mycobacterium paratuberculosis* in colostrum, providing dairy producers with an alternative to purchasing commercial replacement products, resulting in reduced costs and reduced calf morbidity (Stabel, 2001).

**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 2 common methods of pasteurizing: batch pasteurization and HTST continuous flow pasteurization.

Batch pasteurization is accomplished when a batch (usually a vat or tank) of milk is heated to 63°C (145°F) for 30 min (Jay, 2000). Thereafter, the milk 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 because very large batches may take several hours to reach the desired temperature; this generates 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, milk is circulated through a network of heated coils, rapidly heated to 72°C (161°F) and held there for 15 s (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 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. 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 Ig 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 colostrometer before heat treatment and the amount fed was adjusted to ensure successful passive transfer of immunity.

A field study reported by 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 vs. pasteurized colostrum. Results showed that pasteurization (63°C for 30 min) reduced colostrum IgG concentration, with the percentage reduction averaging 58.5 and 23.6% for 95- and 57-L batches, respectively. Pasteurization of 57-L batches produced lower levels of IgG denaturation, and colostrum
consistency was normal or mildly thickened. When pasteurized colostrum was fed, serum IgG concentrations were higher for calves fed fresh colostrum and for calves with a shorter time interval (<6 h) between first and second colostrum feedings. After controlling for 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 Ig activity. Presenting 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, they suggested that colostrum could be heated to 60°C for up to 120 min without changing viscosity or IgG concentration. They later sought to confirm this finding by heating 50-mL aliquots from 30 unique batches of colostrum in an RVA for 120 min at 60 and 63°C. The second study showed that heating colostrum to 63°C resulted in an estimated 34% decrease in IgG concentration and 33% increase in viscosity. However, there was no difference in IgG concentration between preheat-treated (73.4 ± 26.5 g/L) and postheat-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/L) suffered greater losses of IgG and greater viscosity changes when heated to 63°C than did moderate-quality colostrum (<73.0 g/L). However, the effect of colostrum quality was minor if high-quality colostrum was heated to only 60°C. The results of a bovine viral diarrhea virus 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 because of a high proportion of missing results, owing to the congealing of many samples after heat treatment.

The results of this study indicate 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. Godden et al. (2006) inoculated 30-L batches of first-milking bovine colostrum with Mycoplasma bovis (10^8 cfu/mL), L. monocytogenes (10^6 cfu/mL), E. coli O157:H7 (10^6 cfu/mL), Salmonella enteritidis (10^6 cfu/mL), and Mycobacterium paratuberculosis (10^3 cfu/mL). Colostrum batches were heat treated at 60°C for 120 min in a commercial on-farm batch pasteurizer system. Subsamples 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 mean log$_2$ bovine viral diarrhea virus type 1 serum neutralization titer (pre = 12.3; post = 12.0). Viable Mycoplasma bovis, L. monocytogenes, E. coli O157:H7, and Salmonella 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 Mycobacterium paratuberculosis 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 Mycobacterium paratuberculosis 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 Mycobacterium paratuberculosis culture results.

A field study using HTST pasteurization (72°C for 15 s) reported that total colostral IgG mass received by 150 calves fed pasteurized colostrum (mean = 203.1 g) and pasteurized waste milk was significantly lower than for 150 calves fed unpasteurized colostrum (mean = 212.8 g) and nonpasteurized milk (Jamaluddin et al., 1996). Yet there was no 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 concentration 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.

Most recently, in a study designed to describe the effect of feeding heat-treated (60°C for 60 min) colostrum vs. 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, β-carotene, or vitamin A.
E-to-cholesterol ratio, or on serum bovine viral diarrhea virus type 1 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 authors considered the latter results inconclusive because it has yet to be determined if neonatal calves can absorb nondam colostral leukocytes and if passive absorption of colostral cells is affected by cellular immune fractions or functions of these cell fractions are affected by heat treating colostrum.

The IgG Molecule Structure

The stability of bovine IgG 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 disulfide interactions (Indyk et al., 2008). deWit and Klarenbeek (1984), reporting on effects of heat treatment on structure and solubility of the Ig fraction of whey, showed that Ig are among the most heat-stable whey proteins, which is attributed to their 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 thermostable among the Ig (Mainier et al., 1997).

According to Price (2000), for a protein to display its biological activity it must adopt its correct 3-dimensional structure. For this reason, changes in secondary or tertiary structures may be responsible for changes in biological activity upon heating (Li et al., 2005). However, it is important to notice that at the low temperatures used in the animal sciences, some unfolding of the 3-dimensional structure may occur, but this unfolding is reversible and the native structure can be regained (Goto and Hamaguchi, 1982a,b; Price, 2000). Another important aspect is that denaturation of a multidomain 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. Mainier et al. (1997) concluded that low-temperature, long-time pasteurization at 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 Ig (compared with IgM and IgA), and heat treatment for 40 min at that temperature did not reduce its activity. Lindstron et al. (1994) reported on the thermally induced unfolding of bovine milk Ig using differential scanning calorimetry in the temperature range 25 to 100°C and demonstrated that thermal unfolding of Ig at pH 6.6 took place at 80.9°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 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, this research indicates that a substantial proportion of IgG antibody activity is retained after commercial processing, with the exception of severe thermal treatment processes such as those encountered during production of canned evaporated milk and ultra-high-temperature-sterilized milk (Li-Chan et al., 1995). Thus, it seems that higher temperatures than the ones used in the animal science studies (and in practice on farms) are needed to greatly reduce colostral IgG antigen-binding activity.

The reported differences in IgG concentrations after pasteurization in some publications could be attributed to the experimental techniques used to determine IgG concentrations. 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 occur 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 immunoactivity during heating (Dominguez et al., 1997). Other more specific immunological methods are designed to estimate the antigen-binding activity of specific IgG against bacterial lipopolysaccharides after heat treatment (Dominguez et al., 1997). In contrast, in the radial immunodiffusion (RID) analysis used in most animal science trials, the tested antigen (IgG) is allowed to diffuse in anti-IgG antibody-containing agar and reacts with the antibody by its antigenic determinants, the constant regions of IgG, to form complexes. Then IgG concentration is determined by measuring the diameter of the precipitation ring (Cao et al., 2007). Other studies (McMartin et al., 2006; Johnson et al., 2007) used turbidimetric immunoassay (TIA) and demonstrate clear reduction in IgG levels when heat treating at temperatures of 62 and 63°C. However, TIA uses the same reaction of anti-IgG antibodies with the Fc region of IgG; TIA differs in the method of quantifying this reaction and the speed of the assay (Ettel et al., 1997). Sensitivity of RID is between 10 and 50 μg antibody/mL, whereas ELISA sensitivity is...
between 0.0001 to 0.01 μg antibody/mL (Kindt et al., 2007).

The data relating to active IgG molecules is therefore not consistent, and it appears that the form and function of the molecule may change at different temperatures. It appears that IgG may be more heat tolerant than RID measures might suggest, and further research is needed on this topic.

**Hypothesis on Why Feeding Heat-Treated Colostrum Alters IgG Absorption**

Johnson et al. (2007) have described increased efficiency of IgG absorption and as a result, higher serum IgG concentrations in calves fed heat-treated vs. raw colostrum. However, what causes the increase in IgG absorption remains to be elucidated. 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 Ig 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). Another possible explanation could be that thermal treatment of colostrum denatures some proteins that would otherwise interfere or compete for receptors on neonatal enterocytes, thus reducing the number of receptors available for IgG uptake. There may be more possible explanations to this phenomenon, and further research is needed to establish the actual mechanisms behind the increase in IgG absorption.

**IMPLICATIONS**

Early studies on pasteurization of bovine colostrum, using the same times and temperatures recommended for milk, reduced or eliminated important bacterial pathogens; however, this process reduced Ig concentration and increased viscosity. More recent studies using lower temperatures have shown no reduction in colostral Ig concentration or fluidity. Although IgG molecules have been shown to be very heat tolerant, temperatures above some critical point may result in lower biologically active levels in colostrum, and the detection of IgG may be influenced by analytical procedures. Although there are many fundamental studies describing effects of heat treatment on colostrum, there is still a need for more research on IgG absorption in the calf.

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