Procedures to optimise pasteurisation and storage of colostrum

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Report on procedures to optimise pasteurisation and storage of colostrum

Calves have no natural immunity against disease (Barrington and Parish, 2001) and acquire immunity via the ingestion of colostral immunoglobulins (IgG) (i.e., passive immune transfer) within the first hours after birth (Godden et al., 2019). Failure to achieve passive immunity transfer (FPT) is associated with adverse outcomes for calves and older animals. Calves with FPT are more susceptible to diarrhoea (1.5 times) and respiratory disease (1.8 times) and twice as likely to die (Raboisson et al., 2016). For older animals, FPT has been associated with decreased growth rate, reduced first and second lactation milk production and an increased tendency for culling during the first lactation (DeNise et al., 1989; Faber et al., 2005). The reported prevalence of FPT in calves in several major dairy producing countries is relatively high, varying between 16 and 41% (e.g., Cuttance et al., 2017; Elsohaby et al., 2019; Lora et al., 2018; Todd et al., 2018; Urie et al., 2018).

Colostrum quality is one of the key determinants of successful immune transfer and is characterised by concentrations of two critical components: IgG, and infectious agents (Godden et al, 2019). Good quality colostrum is indicated by concentrations of IgG ≥ 50 g/L (equivalent to ≥ 22% Brix), bacterial counts ≤ 1 x 10^5 colony-forming units (cfu)/mL and coliform counts ≤ 1 x 10^4 cfu/mL.

Pasteurisation is an important tool to help improve the quality of colostrum. It does so in two ways: directly, by eliminating or reducing bacterial/other pathogen contamination; and indirectly, by increasing the efficiency of absorption of IgG (Godden et al., 2019). Feeding appropriately heat-treated colostrum (60°C for 60 min (60/60)) has been shown to improve calf health. In a large field trial in the USA, calves fed pasteurised colostrum had a reduced risk for diarrhoea (Godden et al., 2012). Feeding 60/60 pasteurised colostrum followed by pasteurised milk (heated to 63°C for 30 min) has been shown to reduce morbidity and mortality (Armengol and Fraile, 2016). Calves fed colostrum heated to 60°C for 30 min showed increased growth rates to weaning and less diarrhoea and pneumonia (Rafiei et al., 2019).

Colostrum contains other bioactive constituents including leukocytes, growth factors, hormones, nonspecific antimicrobial factors, and nutrients. These, and IgG levels, may be impacted by inappropriate pasteurisation and storage techniques. The credible scientific literature that has reported on procedures for optimising colostrum pasteurisation and storage was reviewed and is reported herewith. In particular, this report considered the effects of temperature and duration, preservation of antibodies and other constituents, kill rates of bacteria and other common pathogens, antibody absorption and short-term storage.

1. Methods for pasteurization

The main aim of pasteurisation is to minimise pathogen contamination whilst protecting the levels of IgG and other important constituents.

a. Effects of temperature and duration on antibody preservation, kill rates of bacteria and kill rates of common pathogens

Several studies have shown that heating colostrum to **60°C for 60 min (60/60)** maintains IgG levels and fluidity and reduces bacterial and coliform counts well below the accepted threshold levels for good quality colostrum (Godden et al., 2019, Hesami et al., 2021). Further, 60/60 heating eliminates or significantly reduces key pathogens including *Escherichia coli, Salmonella enteritidis and Mycoplasma bovis*, and significantly reduces contamination by *Mycobacterium avium subsp paratuberculosis.* There may be a small loss of IgG from heating very high-quality batches of colostrum at 60/60 but the concentrations remain well within acceptable levels (Donahue et al., 2012).

Heating at **60°C for a shorter duration (30 min) (60/30)** does not affect colostrum IgG concentrations and the reductions in pathogen microorganisms are reported to be similar to those for the 60/60 protocol. For example, the reductions in total bacterial count and individual organisms (environmental streptococci, Streptococcus agalactiae, Staphylococcus aureus, coliform counts, non-coliform counts) were similar for 60/60 and 60/30 (Elizondo-Salazar et al., 2010).

Other organisms are less well controlled with 60/30 than 60/60. The 60/30 procedure was effective in eliminating *M. bovis, Listeria monocytogenes, E. coli O157:H7,* and *S. enteritidis* from colostrum, but a 60 min duration was required to completely control *M avium subsp paratuberculosis* (Godden et al., 2006). Hesami et al. (2020) also reported that 60/60 pasteurisation achieved much greater control of pathogens (*E. coli K99, Rotavirus, Coronavirus* and *Cryptosporidium parvum*) than 60/30.

Heating at **60°C for longer than 60 min i.e., 90 min**, does not provide any additional control of pathogenic organisms (Elizondo-Salazar et al.,2010; Hesami et al., 2020), and may have the disadvantage of reducing IgG concentrations (Hesami et al., 2020), although McMartin et al. (2006) observed no effect of heating at 60°C for up to 120 min on IgG concentrations.

One potential advantage of pasteurisation at a higher temperature i.e., **63°C for 30 min** (63/30) is the inactivation of bovine leucosis virus (BLV) (Sandoval-Monzón et al., 2021). However, heating colostrum above 61°C significantly decreases IgG levels and increases colostrum viscosity (McMartin et al., 2006, Elizondo-Salazar et al., 2010). Note, some authors have reported that heating to 63°C for 30 min does not affect serum IgG levels in calves (see Robbers et al., 2021). Furthermore, an alternative method is available to inactivate BLV in colostrum via freezing/thawing - due to loss of lymphocyte viability (Kanno et al., 2014; Roberts et al., 1983). As appropriate freezing/thawing of colostrum is not likely to affect colostrum quality (Morrill et al., 2015), using a 60/60 heating protocol together with freezing should inactivate BLV whilst preserving IgG viability. There appears to be no published reports on the effects of pasteurisation on clostridia.

b. Preservation of other constituents

In additional to IgG, fresh colostrum contains many other biologically active factors, both nutritive and nonnutritive. Heat treatment (60/60) has no adverse effects on colostrum dry matter (%), true protein (%), crude fat (%), solids-not-fat (%) and other solids (%) (see Godden et al., 2015)

Non-nutritive constituents include those with a potential influence on calf immune responses (e.g., leucocytes, cytokines, oligosaccharides), nonspecific antimicrobial factors (e.g., lactoferrin), proteins (e.g., growth factors such as insulin-like growth factor (IGF) I and II, fibrinogen, trypsin inhibitors) and metabolites.

The scientific evaluation of the effects of the recommended (60/60) colostrum pasteurization procedure on constituents other than IgG and pathogens is in its infancy. As with freezing, heat treatment kills most/all colostral leucocytes (Godden et al., 2019). The absence of leucocytes in colostrum on practical calf health outcomes during development (and later life) remain to be fully elucidated Godden et al., 2019).

The 60/60 heating protocol reduces IGF-I in colostrum (Mann et al., 2021a) but not in calf serum (Mann et al., 2021b). Further, 60/60 heating decreases the abundance of other proteins (mainly involved with immunity, enzyme function,

and transport-related processes (e.g., Tacoma et al., 2017). In addition, Xu et al. (2021) reported that pasteurisation reduced the concentration of a very small proportion of metabolites in colostrum but no changes in the metabolite profiles of calves fed the heat-treated colostrum were detected. Gelsinger and Heinrichs (2017) suggested that the immune response of young calves is not inhibited by heat treatment of colostrum, although more research on the practical health outcomes of changes in colostrum constituents following pasteurized is required.

c. Antibody absorption in pasteurised and unpasteurised colostrum

The apparent level of absorption of IgG (and serum IgG levels) is higher in calves fed pasteurized colostrum compared to animals consuming raw colostrum (Elizondo-Salazar and Heinrichs, 2009). High levels of bacteria in colostrum may restrict IgG absorption (e.g., Gelsinger et al., 2014; James et al., 1981). Gelsinger et al. (2015) provided convincing evidence that lower bacterial populations in heat-treated colostrum is a key factor underpinning improved IgG absorption. It has been hypothesised that high levels of bacteria in colostrum may bind to free IgG in the gut lumen, and/or directly block uptake and transport of IgG molecules across intestinal epithelial cells, thereby restricting antibody transfer (James et al., 1981; Godden et al 2019). Note, factors other than improved IgG absorption may contribute to the better health outcomes of calves consuming pasteurised colostrum. For example, there may be enhanced gastrointestinal tract colonisation by beneficial organisms (*Bifidobacterium*) as well as reduced colonisation by pathogens (*E coli*) (Malmuthuge et al., 2015).

d. Short term storage options – justification and effects on quality

Bacterial levels in unpasteurised colostrum begin to increase within hours of storage at ambient temperatures or under refrigeration at 4°C (Cummins et al., 2016). Depending on initial bacterial counts, under refrigeration the concentrations can exceed acceptable thresholds within 24 h to 48 h (Stewart et al., 2005; Cummins et al., 2016). Appropriate pasteurisation (e.g., 60/60) immediately reduces bacterial counts in fresh colostrum to very low levels, and if refrigerated in a clean covered container, the shelf life of heat-treated colostrum is at least eight days (Bey et al., 2007; Godden et al 2019). Adding a preservative (potassium sorbate) to pasteurised colostrum does not provide any further benefits for bacterial control under refrigerated conditions (Bey et al., 2007).

Summary

Bacterial contamination of fresh colostrum is common on dairy farms and often exceeds the accepted thresholds (e.g., Godden et al., 2012; Hesami et al 2020). Pasteurisation of colostrum immediately reduces bacterial levels to very low levels (and well below thresholds). The weight of scientific evidence strongly suggests that the optimal pasteurisation heat treatment is 60°C for 60 min, which preserves antibody concentrations, maintains colostrum fluidity and controls/inactivates bacteria and most common pathogens. Feeding pasteurised colostrum enhances antibody absorption and has been reported to reduce the risk of poor health in calves.

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Colostrum and milk pasteurization improve health status and decrease mortality in neonatal calves receiving appropriate colostrum ingestion

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ABSTRACT

The objective of the study was to evaluate if on-farm heat treatment of colostrum and bulk tank milk can improve calf health status and morbidity and mortality rates during the first 21 d of life in neonatal Holstein calves receiving appropriate colostrum ingestion. A total of 587 calves were randomly assigned to 2 groups of males and females over 18 mo. The nonpasteurized group (n = 287, 143 males and 144 females) was fed frozen (−20°C) colostrum (6–8 L during the first 12 h of life) that was previously reheated up to 40°C. They were also fed refrigerated (4°C) raw milk from the bulk tank that was also reheated up to 40°C (1.8 L every 12 h). The pasteurized group (n = 300, 150 males and 150 females) was also fed colostrum and milk, but both were pasteurized before freezing. Blood samples were drawn from all calves to obtain serum at 2 to 5 d of life. Serum total protein (g/dL) was determined using a commercially available refractometer. Colostrum and milk underwent routine bacteriological analysis to determine total plate counts (cfu/mL) and total coliform counts (cfu/mL). All the calves underwent clinical examination every 24 h during the first 21 d of life. Every day, calves were clinically diagnosed either as being healthy or suffering from respiratory disease, neonatal calf diarrhea, or suffering other diseases. On-farm heat treatment for colostrum and milk reduced total plate counts and total coliform counts between 1 and 2 $\log_{10}$. Pasteurization of colostrum and milk significantly decreased the morbidity and mortality (5.2 and 2.8%) in comparison with calves receiving nonpasteurized colostrum and milk (15.0 and 6.5%), respectively, during the first 21 d of life, even in animals receiving appropriate colostrum ingestion.
Key words: on-farm heat treatment, colostrum, milk, health status

INTRODUCTION

Passive immunity is the only source of early immunity for calves due to the inability of bovine placenta to transmit maternal immunoglobulins to the fetus (Richter and Götze, 1993; Baintner, 2007). Thus, it is compulsory to acquire these natural defenses by colostrum ingestion. In this sense, the immune status of calves during the preweaning period depends directly on the quality and quantity of colostrum ingested during the first hours of life (Heinrichs and Elizondo-Salazar, 2008). The gold standard method accepted to evaluate passive transfer is a direct measurement of IgG concentration through radial immunodiffusion. Failure of passive transfer appears if the calf serum IgG concentration is less than 10 mg/mL when sampled between 24 and 48 h of age (Weaver et al., 2000; Godden, 2008), because a value below 10 mg/mL is a risk factor for developing diseases during the neonatal period (Godden, 2008). Unfortunately, radial immunodiffusion is not useful as an on-farm method. Evaluation of serum total proteins (**STP**) by refractometry is widely used by veterinarians and producers to evaluate adequate passive transfer of immunoglobulin in calves (Tyler et al., 1996); this is because the correlation between STP and IgG in blood is very good the first days of life, considering that IgG is the most abundant protein ingested through colostrum (Calloway et al., 2002). Thus, a value of STP between 5.0 and 6.0 g/dL is considered to prevent failure of passive transfer after colostrum intake (Donovan et al., 1998; Windeyer et al., 2014). Moreover, a cut-off for STP of 5.2 g/dL is accepted to guarantee the equivalent threshold value of 10 mg/mL of IgG in calf serum (Tyler et al., 1996; Calloway et al., 2002; Windeyer et al., 2014).

An appropriate colostrum ingestion program begins feeding the calf within 4 to 6 h after birth and has to ensure at least 4 to 5 L of colostrum intake during the first 8 h of life. This protocol allows high blood levels of circulating maternal immunoglobulins in 48-h-old calves until their immune system becomes fully functional, at 3 to 6 wk of age (Heinrichs and Elizondo-Salazar, 2008). In any case, failure of passive transfer

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can occur and the incidence of respiratory or digestive disease may increase in these animals (Virtala et al., 1999; Godden et al., 2012; Pardon et al., 2015). As a consequence, cost for treatment of bovine respiratory disease (**BRD**) and neonatal calf diarrhea (**NCD**) and death rates due to both diseases may increase during the first 21 d of life (Meganck et al., 2014; Windeyer et al., 2014). Additionally, management of milk, buckets and sucklers, as well as timing at feeding and quantity of milk are also important factors to ensure a good health status of calves and good production parameters in the first 21 d of life (Wells et al., 1996). A good colostrum feeding protocol should also avoid bacterial contamination (Meganck et al., 2014) through a strict hygiene management of buckets and sucklers, because bacteria in colostrum may interfere with passive absorption of colostral antibodies into the calf's circulation (James et al., 1981). Moreover, it has been described that colostrum and milk can contain pathogens such as *Mycobacterium avium* ssp. *paratuberculosis* (Streeter et al., 1995; Sweeney, 1996), *Mycoplasma* spp. (Butler et al., 2000; Stabel et al., 2004), *Escherichia coli*, *and Salmonella* spp. (Smith et al., 1989; Spier et al., 1991; Stabel et al., 2004). Fortunately, pasteurization is a good way to decrease the bacterial load in colostrum, but heat treatment must avoid colostrum denaturalization and an increase in its viscosity. Previous studies observed that the conventional pasteurizing protocol for milk may alter colostrum characteristics (Meylan et al., 1996; Godden et al., 2003). It has been established that heating colostrum at 60°C for 30 to 60 min can be a good treatment to maintain colostrum quality (McMartin et al., 2006; Heinrichs and Elizondo-Salazar, 2008) and significantly reduce important pathogens that can contaminate it, such as *Listeria monocytogenes*, *E. coli* O157:H7, *Salmonella enteritidis*, and *M. avium* ssp. *paratuberculosis* (Stabel et al., 2004; Godden et al., 2006).

Animals fed on-farm pasteurized colostrum and waste milk showed higher ADG, a lower prevalence of pneumonia and diarrhea, and a lower mortality rate when compared with calves fed with nonpasteurized colostrum and waste milk (Jamaluddin et al., 1996; Godden et al., 2005). However, the experimental design used in these studies did not allow determination of the effect of colostrum and milk pasteurization on the health status and mortality of calves when high levels of colostrum ingestion were guaranteed. The objective of the present study was to evaluate if on-farm pasteurization of raw colostrum and bulk tank milk could improve the health status, reduce neonatal illness, and decrease death rates during the first 21 d of life in calves receiving appropriate colostrum ingestion monitored through a refractometer.

MATERIALS AND METHODS

Animals and Farm

The study was carried out in a dairy housing an average of 330 lactating Holstein cows with an average production of 11,100 kg of milk (3.6% fat and 3.3% protein) by cow. This farm was located in Lleida (northeast Spain). Cows were milked 3 times daily (at 0400, 1200, and 2000 h). Milk from each cow was sampled and analyzed for milk quality (fat, protein, and lactose concentration) and SCC by technicians of the Central Laboratory for Milk Recording (ALLIC, Catalonia, Cabrils, Spain) once a month. This farm is positive for bovine herpesvirus type I (**BoHV-1**) and bovine viral diarrhea virus (**BVDV**), and these viruses can play an important role as respiratory pathogens in calves. Cows and heifers were vaccinated against BoHV-1, BVDV, bovine respiratory syncytial virus, and influenza virus (Hiprabovis IBR Marker live and Hiprabovis BVD balance, Hipra, Girona, Spain) every 6 mo. Vaccination was carried out by veterinarians and a single-use needle was used per each cow. Additionally, the farm was under an official eradication program of BoHV-1 using glycoprotein E (**gE**)-deleted marked vaccine. Moreover, individual serology was carried out once a year to detect infected (gE-positive) animals. Control of BVDV is also very strict, with vaccination and identification of persistently infected animals through PCR. Finally, bulk tank milk sampling was carried out every 4 mo by PCR for BVDV and ELISA gE for BoHV-1 detection. Cows were moved 3 wk before calving to a facility where parturition in groups occurred (5–10 cows). Newborn calves were removed from the pen immediately after calving to avoid suckling. First milking of colostrum was obtained between 30 to 90 min after calving. Calves were not fed with colostrum coming from their dam but were fed frozen colostrum, whether heat treated or not, from a single cow, because mixing colostrum from different cows was not allowed. Colostrum bags were stored frozen an average of 6 d before calves received it. Only colostrum with a specific gravity $\geq$1,065 was considered suitable for feeding calves (Fleenor and Stott, 1980).

A total of 587 female and male calves were randomly assigned to 2 groups considering ear tag number as allocating criteria (even and odd numbers) for 18 mo. As inclusion criteria, animals had to be singletons born from a normal parturition and with STP values $\geq$5.8 g/dL. This value was chosen taking previous studies into account. Although a cut-off for STP of 5.2 g/dL is accepted by some authors as good enough to guarantee the equivalent threshold value of 10 mg/mL of IgG in calf serum (Tyler et al., 1996; Calloway et al.,

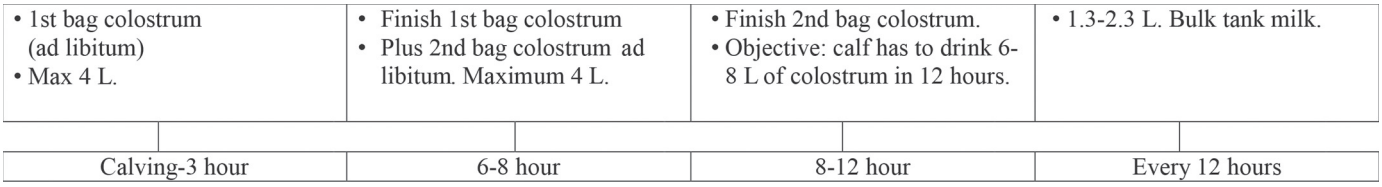

Figure 1. Colostrum and milk feeding protocol of the study farm.

2002; Windeyer et al., 2014), others observed slight improvements in mortality and BRD morbidity rates during the calf life with STP values of 6.0 (Donovan et al., 1998) and 5.7 g/dL, respectively (Windeyer et al., 2014). Thus, we considered an STP value of 5.8 g/dL to undoubtedly be associated with appropriate colostrum ingestion. Blood samples were drawn from all calves at 2 to 5 d of life and serum was obtained. Serum total protein (g/dL) was determined using a commercially available refractometer (AtagoMastersur/NE, Atago U.S.A. Inc., Bellevue, WA) at room temperature (20°C). If the value obtained was higher than 7.5 g/dL, hematocrit was checked to determine if dehydration could cause a misreading. Dehydrated animals were excluded from the study

Calves were housed individually, straw bedded, and following calving time order. If an animal died, straw of the house was removed, walls and floor were cleaned with high pressure water and soap, disinfected with Kenocox (CID Lines, Ark Animal Care Ltd., Newbridge, Ireland), and the house was not used to allocate another newborn calf at least during a 15-d wash-out period. Feeding of the animals as well as colostrum and milk management was always carried out by the same employee except for one day a week (Saturday) and two 15-d periods of vacation when these duties were carried out by the farmer. Colostrum and milk were always provided with nipple buckets, cleaned after every use, and disinfected with chlorhexidine between feedings. The feeding protocol guaranteed an ingestion of 6 to 8 L of colostrum by the calf during the first 12 h of life (Figure 1). Afterward, calves were fed an average of 1.8 L (1.3–2.3 L) of milk from the bulk tank (pasteurized or not) every 12 h (0800 and 2000 h).Calves assigned to the nonpasteurized group (**NP**; even numbers; n = 287, 143 males and 144 females) were fed frozen (−20°C) colostrum that was previously reheated to 40°C. They were then fed refrigerated (4°C) raw milk from the bulk tank that was also reheated to 40°C. Calves assigned to the pasteurized group (**P**; odd numbers; n = 300, 150 males and 150 females) were under a similar feeding protocol, but both colostrum and milk had been pasteurized before freezing (Figure 2). All calves were allowed fresh water and dry food ad libitum from d 2 onwards.

Colostrum and Milk Management

Colostrum immunoglobulin concentration was measured on-farm at 21°C with a colostrometer (Biogenics, Florence, OR) through specific gravity. Only colostrum with density ≥1,065 was used. Colostrum was stored in 4-L aluminum bags identified with the cow number (Perfect Udder 4, Dairy Tech Inc., Greeley, CO). Colostrum of the P group was heat-treated at 60°C for 60 min using a commercial on-farm batch pasteurization system (DT Silver, Dairy Tech Inc.). The temperature was subsequently lowered to 37°C and bags were frozen at −20°C. Afterward, colostrum was heated to 40°C before feeding the calves (Figure 2). Bulk tank milk for the P group was heat-treated at 63°C for 30 min using a commercial on-farm batch pasteurization system (Urban MilkShuttle Pasteur, Urban Gmbh & Co, Wüsting, Germany) and then cooled down to 40°C before feeding the calves (Figure 2). Timing and temperatures used for pasteurization were strictly followed according to the manufacturer's instructions and have been used in other studies (Johnson et al., 2007). Paired colostrum and milk samples, assigned to the NP and P groups, were collected just before feeding the calves at 40°C. They underwent routine bacteriological analysis to determine total plate counts (**TPC**; cfu/mL) and total coliform counts (**TCC**; cfu/mL) as previously described by Jayarao et al. (2004).

Health Monitoring of Calves

All calves underwent a clinical examination every 24 h during the first 21 d of life. For each animal, rectal temperature, breathing rate, consistency of the feces, attitude, and navel were checked. Calves that showed clinical signs of disease were allocated to 3 different categories. The first group included calves that were clinically diagnosed with BRD. Calves were included in this category when at least 2 of the following clinical signs were observed: inducible cough on tracheal mas-

sage, abnormal sounds on respiratory tract auscultation, high rectal temperature (>39.5°C) with increased respiratory rate, serous nasal discharge, coughing, and hyporexia or anorexia (Virtala et al., 1999). The second group included calves with NCD, which was clinically diagnosed using a score from 1 to 4 based on consistency of the feces up to d 2 of life (1 = solid; 2 = semisolid; 3 = liquid feces; 4 = watery). Animals with a score of 3 or 4 were considered to have NCD (Larson et al., 1977). The third group included calves suffering from nonrespiratory, nondigestive diseases, and they were assigned to this group when any of the following clinical signs were observed: trauma, congenital disease, hypothermia (<37.5°C; Berchtold, 2009), neurological signs, weakness, reluctance to stand, difficulty in suckling or absence of suckling reflex, swollen joints, cloudy eyes or omphalitis, and fever (>39.5°C) in the absence of respiratory or digestive signs.

Clinical examination and establishment of curative treatment for sick animals was carried out by the same veterinarians (the 2 authors of the present study) for both experimental groups to avoid any bias. A necropsy was performed on dead animals and causes of death were divided into 3 groups based on the main system affected. Thus, a respiratory cause (BRD) was established if the lung was consolidated, fibrin was noted on pleural surfaces, or emphysema, atelectasis, or tracheitis was observed. A digestive cause (NCD) was established if lesions (enteritis and fluid content) were noted in the digestive tract. Cause of death was established as other if lesions were observed in systems other than respiratory or digestive.

Statistical Analysis

All statistical analyses were carried out using the SAS system V.9.1.3 (SAS institute Inc., Cary, NC). For all analyses, the individual calf was used as the experimental unit. The significance level was set at 0.05 with statistical tendencies reported when $P < 0.10$. The treatment variable was the pasteurization process (NP and P) and the outcome variables were its effect on TPC and TPP in colostrum and on the health of the calves (disease or death). The variables included in the statistical analyses were classified as categorical [pasteurization status (NP/P), illness (yes/no), death (yes/no) and cause of death (BRD, NCD, and other), or continuous (STP, TPC, and TPP)]. Shapiro-Wilk

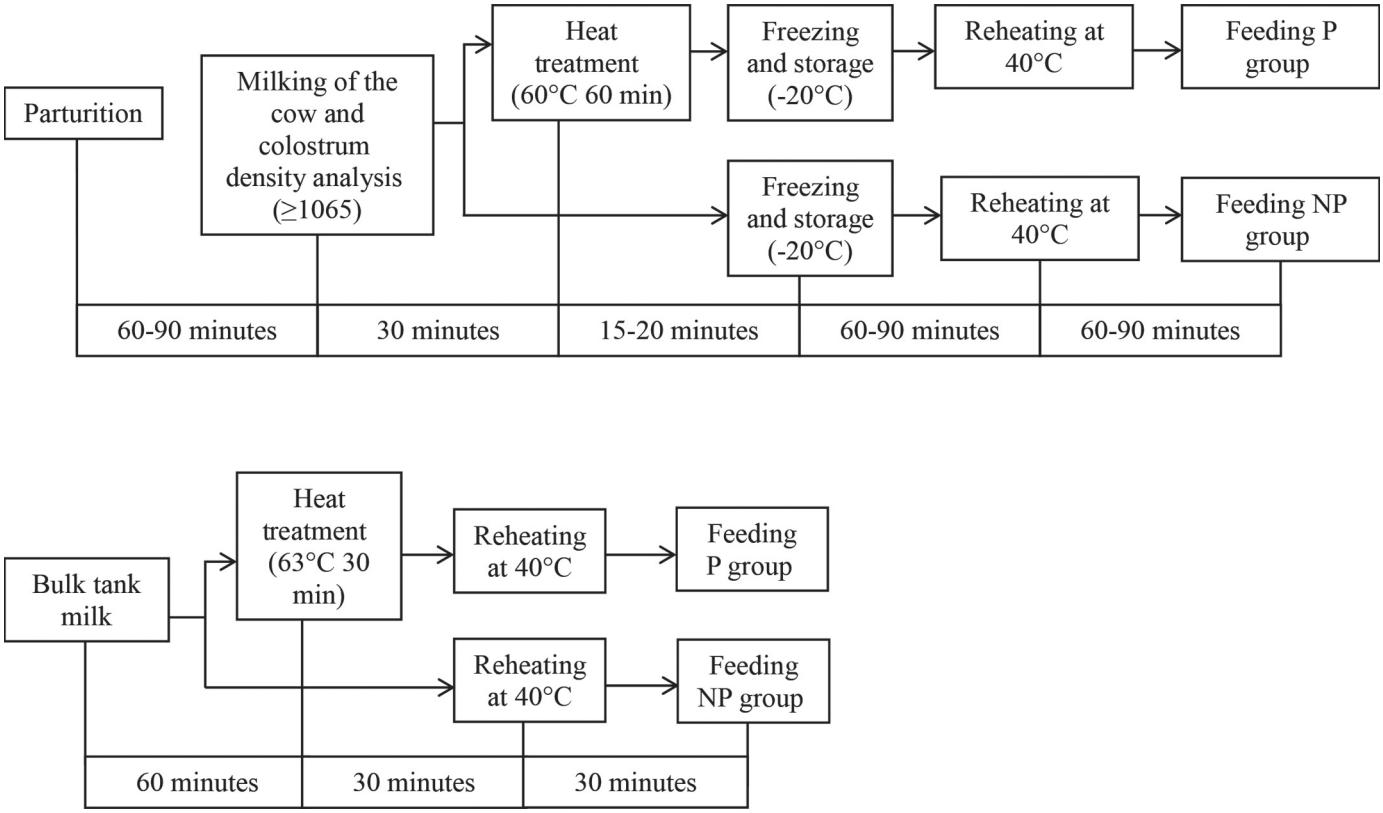

Figure 2. Processing of colostrum and bulk tank milk for calves fed pasteurized colostrum and milk (P group) and calves fed nonpasteurized colostrum and milk (NP group).

Table 1. Effect of heat treatment of colostrum and milk on STP levels, mortality and morbidity rates, and cause of illness and death in 21-d-old Holstein calves fed with raw (NP group) and pasteurized (P group) colostrum and milk

Parameter	Treatment group		*P*-value
	NP group (n = 287)	P group (n = 300)	
STP[1] (g/dL)	7.27[a] (5.8–9.2)	7.34[a] (5.8–9.0)	0.12
Mortality (%)	6.5[a]	2.8[b]	<0.001
Distribution of mortality (%)			
BRD[2]	36.6	38.9	0.80
NCD[3]	43.9	33.3	0.50
Other	19.5	27.8	0.55
Morbidity	15.0[a]	5.2[b]	<0.0001
Distribution of morbidity			
BRD	40.9	43.3	0.82
NCD	39.1	35.7	0.70
Other	20.0	21.0	0.90

[a,b]Values within a row with different superscript letters are significantly different.

[1]STP = serum total protein. Reported values reflect mean and range in parentheses.

[2]BRD = bovine respiratory disease.

[3]NCD = neonatal calf diarrhea.

and Levene tests were used to evaluate the normality of the distribution of the continuous variables and the homogeneity of variances, respectively. To study the association between pasteurization status with the continuous non-normally distributed variables (TPC and TPP), the Wilcoxon test (with the Mann-Whitney U-test to compare each pair of values) was used, whereas an ANOVA test (with Student's *t*-test to compare each pair of values) was used to analyze the association between continuous normally distributed variables (STP). Contingency tables (Chi-square or Fisher exact tests) were used to assess the association between categorical variables. Finally, conditional logistic regression was used to estimate the univariate odds ratios (**OR**) and 95% confidence intervals for death and illness with pasteurization. A multivariate logistic regression analysis was also carried out to decipher the effect of STP and pasteurization and its interaction on death and illness.

RESULTS

Descriptive Statistics of the Results

Average serum total protein was 7.27 and 7.34 g/dL for the NP and P groups, respectively. In both cases, the lowest value observed was 5.8 g/dL and the maximum value observed was 9.2 and 9 g/dL for the NP and P groups, respectively. For the NP and P groups, the coefficient of variation was 7.8% (Table 1). Regarding bacterial load, TPC and TCC were always higher in colostrum than in milk (Table 2).

Morbidity was 15 and 5.2% for the NP and P groups, respectively, and the prevalence of BRD, NCD, and other causes was 40.9, 39.1, and 20% for the NP group and 43.3, 35.7, and 21% for the P group, respectively (Table 1). Mortality was 6.5 and 2.8% for the NP and P groups, respectively, and the cause of death associated with BRD, NCD, and other causes was 36.6, 43.9, and 19.5% for the NP group and 38.9, 33.3, and 27.8% for the P group, respectively (Table 1).

Effect of Heat Treatment on STP and Bacterial Counts

Mean TPC and TCC were significantly lower in pasteurized colostrum and milk ($P < 0.001$ in all cases) than in nonpasteurized ones (Table 2). No significant difference ($P = 0.12$) was observed for STP between

Table 2. Colostrum and milk bacteriology parameters before and after heat treatment[1,2]

Parameter[3] (log10 cfu/mL)	Treatment group		*P*-value
	Raw	Heat-treated	
Colostrum			
TPC	7.43 ± 0.42[a]	6.55 ± 0.41[b]	<0.001
TCC	6.15 ± 0.34[a]	5.90 ± 0.28[b]	<0.001
Milk			
TPC	6.60 ± 0.64[a]	4.80 ± 0.39[b]	<0.001
TCC	4.88 ± 1.10[a]	2.53 ± 0.33[b]	<0.001

[a,b]Values within a row with different superscript letters are significantly different.

[1]Reported values reflect mean ± SE.

[2]Heat treatment for colostrum: 60°C for 60 min; Heat treatment for milk: 63°C for 30 min.

[3]TPC = total plate count; TCC = total coliform count.

the NP (7.27 ± 0.57 g/dL) and P (7.34 ± 0.54g/dL) groups (Table 1).

Effect of Heat Treatment of Colostrum and Milk on the Health Status of Animals

Calves from the NP group were at greater risk of illness (OR = 3.8; CI = 2.5–5.8) and death (OR = 2.5; CI = 1.39–4.3) than calves from P group during the first 21 d of life. Thus, pasteurization of colostrum with a density ≥1,065 and milk significantly decreased the morbidity ($P < 0.0001$) and mortality ($P < 0.001$) in comparison with calves receiving nonpasteurized colostrum and milk (Table 2). However, the distribution of the cause of illness or death was not significantly different ($P > 0.05$) between the NP and P groups during the first 21 d of life (Table 1). The proportion of diseased or dead calves were not significantly affected by STP in either the NP ($P > 0.05$) or in the P groups ($P > 0.05$) in the range of values studied. Pasteurization was the only significant variable in a multivariate logistic regression analysis on death ($P = 0.002$) and illness in calves ($P < 0.0001$) without observing a significant interaction ($P > 0.05$) with STP.

DISCUSSION

Colostrum with density ≥1,065 and milk pasteurization improve health status and decrease mortality during the first 21 d of life in neonatal calves receiving appropriate colostrum ingestion (STP ≥5,8 g/dL).

Effect of Heat Treatment on STP and Bacterial Counts

Measurement of STP by refractometer is a good descriptive marker to estimate the amount of immunoglobulins in neonatal calf serum with the goal of assessing the calf passive immunity transfer after colostrum ingestion (Tyler et al., 1996; Donovan et al., 1998). It has been described that STP values above 5.2 g/dL are linked with appropriate passive immunity transfer (Tyler et al., 1996; Calloway et al., 2002). Previous studies have concluded that animals fed colostrum heat treated at 60°C for 30 to 60 min had significantly greater STP when compared with calves fed with raw colostrum (Johnson et al., 2007; Gelsinger et al., 2014); thus, colostrum pasteurization seems to be a good tool to increase the STP in calves. However, we did not detect significant differences in STP between NP and P calves in the current study. In our case, it must be taken into account that we are only including a calf population fed high-quality colostrum (density ≥1,065) and showing high STP values (cut-off ≥5.8 g/dL). Thus, it seems that the pasteurization process is unable to increase the STP value in calves receiving appropriate colostrum ingestion, as suggested in previous studies (Gelsinger et al., 2014). What our study did find, as expected, is that heat treatment of raw colostrum and milk can reduce the number of pathogens as previously described (Godden et al., 2006; McMartin et al., 2006; Johnson et al., 2007). The exposure of calves to pathogenic bacteria via colostrum and milk could cause diseases, such as diarrhea or septicemia, and facilitate the transmission of microorganisms, such as *Mycoplasma* spp., *M. avium* ssp. *paratuberculosis*, fecal coliforms, and *Salmonella* spp. (Jayarao et al., 2004; Godden, 2008). Fortunately, feeding pasteurized colostrum and milk can be helpful to reduce fecal-oral transmission of pathogens (Godden et al., 2003; Jayarao et al., 2004; McGuirk and Collins, 2004).

Effect of Heat Treatment on the Health Status of Animals

Although neonatal calf diseases are multifactorial (Lorenz et al., 2011; Al Mawly et al., 2015), a proper quantity of good-quality colostrum and milk intake is essential to control them (Meganck et al., 2014). Other factors that could alter the prevalence of neonatal calf diseases are individual farm management practices and the preventive medicine programs applied to each farm. The current study, designed to test the potential benefit of pasteurization when calves are fed high-quality colostrum, was carried out within the same farm to reduce interfarm variability (Mac Farlane et al., 2015). With this design, the internal validity of the current is sound because potential confounding factors have been greatly minimized. The current study was carried out on a conventional Spanish dairy farm using Holstein cows and standard operation procedures. Even with these factors in mind, the external validity of our study could be limited mainly because it was carried out in a single farm. For this reason, it would be advisable to have similar studies carried out in other farms with differences in terms of management and facilities.

In our study, we observed a similar reduction in terms of morbidity (9.8%) and mortality (3.7%) in calves fed pasteurized colostrum and milk in comparison with animals receiving nonpasteurized colostrum and milk during the first 21 d of life. As reported by Godden et al. (2005), dairy calves fed pasteurized nonsaleable milk have lower morbidity and mortality rates (11.6 and 2.2%, respectively) than calves fed milk replacer (32.1 and 12.1%, respectively). In the same study, calves fed with milk replacer had a higher risk for treatment during summer and winter months (OR = 3.99) as well as death during winter (OR = 29.81) than calves fed

pasteurized nonsaleable milk. Godden et al. (2012) also reported a significant increase in risk for treatment of NCD in calves fed fresh colostrum (OR = 1.32) compared with calves fed colostrum heat-treated at 60°C for 60 min (16.5%) during the preweaning period.

Finally, the cause of illness or death was not significantly different between the NP and P groups during the first 21 d of life in our study, but the difference in mortality between the NP and P groups was 8.3 and 10.6% for NCD and other causes, respectively (Table 1). Obviously, this difference is biologically relevant although a significant difference was not observed. A plausible explanation for this apparent contradiction could be that our study has a low statistical potency to detect differences in causes of illness or death and consequently increase the probability to have a type 2 error. Additional studies are necessary to tackle this question.

Curiously, the clinical improvement observed in our study cannot be explained by a significant increase of STP in calves receiving pasteurized colostrum and milk versus calves receiving raw feed. Our study paves the way to carry out additional studies to decipher the mechanisms involved in improving health status in calves receiving pasteurized colostrum and milk. Several research lines can be explored, such as whether pasteurization can have a positive effect on the calf gut microbiota or on the absorption of cellular immunity. It must be taken into account that the positive effect of colostrum is relevant not only during the neonatal phase of life but also during the whole productive life of animals. (Robison et al., 1988; DeNise et al., 1989; Wells et al., 1996; Donovan et al., 1998; Weaver et al., 2000; Faber et al., 2005).

CONCLUSIONS

Pasteurization of colostrum (density ≥1,065) and milk significantly improves calf health status and reduces morbidity and mortality during the first 3 wk of life, even in calves with STP values ≥5.8 g/dL.

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Heat-Treatment of Bovine Colostrum. II: Effects of Heating Duration on Pathogen Viability and Immunoglobulin G

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ABSTRACT

Batches (30-L) of first-milking bovine colostrum, inoculated 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), were heat-treated at 60°C for 120 min in a commercial on-farm batch pasteurizer system. Duplicate 50-mL 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 (mg/mL) and antibody activity [$\log_2$(bovine viral diarrhea virus type 1 serum neutralization titer)]. Four replicate batches of colostrum were run for each of the 5 pathogens studied. There was no effect of heating moderate- to high-quality colostrum at 60°C for at least 120 min on mean IgG concentration (pre = 60.5 mg/mL; post = 59.1 mg/mL). 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. Average bacteria counts showed that *Map* was not detected when batches were heated at 60°C for 60 min. Although the authors believe that heat-treating colostrum at 60°C for 60 min should be sufficient to eliminate *Map* from colostrum in most situations, further research is needed to determine whether these findings may be replicated, given that variability was observed in *Map* culture results.

Key words: colostrum, pasteurization, pathogen, immunoglobulin

INTRODUCTION

First-milking colostrum is an important source of nutrients and an immediate source of passively absorbed maternal antibodies, which are critical in the protection of the newborn calf against infectious diseases in the first weeks and months of life (Davis and Drackley, 1998). However, colostrum can also represent one of the earliest potential exposures of dairy calves to infectious agents, including *Mycoplasma* spp. (Walz et al., 1997), *Mycobacterium avium* subsp. *paratuberculosis* (**Map**; Streeter et al., 1995), *Escherichia coli* (Clark et al., 1989; Steele et al., 1997), *Salmonella* spp. (McEwen et al., 1988; Giles et al., 1989; Steele et al., 1997), *Listeria monocytogenes* (Farber et al., 1988; Steele et al., 1997), and *Campylobacter* spp. (Lovett et al., 1983; Steele et al., 1997). For example, *Map* has been recovered from the colostrum of 22.2% of clinically normal but *Map*-infected cows (36% of heavy fecal shedders; 16% of light fecal shedders; Streeter et al., 1995). Some of these pathogens can act directly to produce costly short-term consequences (e.g., neonatal enteritis or septicemia), whereas others may cause longer term losses (e.g. Johne's disease caused by *Map*). It has also been suggested that the presence of bacteria in the small intestine at the time of colostrum arrival could indirectly harm calf health by interfering with systemic absorption of Ig molecules (James and Polan, 1978; James et al., 1981; Staley and Bush, 1985), thus contributing to the failure of passive transfer. In one observational study of commercial dairies, Poulsen et al. (2002) reported that 82% of colostrum samples collected exceeded the industry goal of 100,000 cfu/mL total plate count, suggesting that the feeding of contaminated colostrum is a common occurrence on commercial dairy farms (McGuirk and Collins, 2004).

On-farm pasteurization of colostrum has been suggested as one possible control measure to reduce or eliminate transfer of colostrum-borne pathogens to dairy calves. However, early research on pasteurizing colostrum at 72°C for 15 s using a commercial HTST

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continuous-flow pasteurization method reported 25 to 30% reduction in IgG concentration (mg/mL) plus unacceptable feeding characteristics (Green et al., 2003; Stabel et al., 2004). Batch pasteurization of colostrum at conventional times and temperatures commonly used for pasteurizing milk (63°C × 30 min) produced milder increases in viscosity but still resulted in 25 to 30% reduction in IgG concentration (Godden et al., 2003; Green et al., 2003). In light of this knowledge, the goal was set to develop a longer and lower-temperature method for heat-treating colostrum that would preserve important colostral antibodies and prevent viscosity changes while still achieving good pathogen kill. In an earlier study, we learned that 50-mL aliquots of bovine colostrum could be heat-treated in a Rapid Visco Analyzer (Newport Scientific, Warriewood, Australia) at 60°C for as long as 120 min without affecting either colostrum viscosity or IgG concentration (McMartin et al. 2006). The objectives of this study were to describe the effects of heat-treatment on IgG concentration and activity, and to describe the duration of heating necessary to eliminate important pathogens when heating large volumes of bovine colostrum to 60°C in a commercial on-farm batch pasteurization system.

MATERIALS AND METHODS

Inoculation and Heat Treatment of Colostrum with Mycoplasma bovis, L. monocytogenes, E. coli O157:H7, and Salmonella enteritidis

First-milking colostrum was previously collected from Holstein cows on one commercial dairy farm and stored at –20°C for 2 to 16 wk prior to use in this study. Thawed colostrum at 20°C was pooled to create a unique 30-L batch, transferred into a commercial on-farm batch pasteurization system (DairyTech Inc., Windsor, CO), and then inoculated with the following 4 pathogens to reach the final indicated concentrations: *M. bovis* (10^8 cfu/mL), *L. monocytogenes* (10^6 cfu/mL), *E. coli* O157:H7 (10^6 cfu/mL), and *S. enteritidis* (10^6 cfu/mL). The batch of colostrum was then heated over a 30-min period to reach the target temperature of 60°C, held at 60°C for 120 min, and then cooled over a 15-min period to reach 38°C. The colostrum was agitated continuously throughout the heat-treatment process. Duplicate 50-mL samples were aseptically collected from the batch immediately prior to inoculation, immediately postinoculation, on reaching the target temperature of 60°C, and thereafter at 15-min intervals throughout the entire heat-treatment process until the colostrum was cooled to 38°C. These samples were aseptically collected into sterile 50-mL centrifuge tubes (Corning Inc., Corning, NY) and then placed immediately on ice until completion of the heat-treatment process. Four unique replicate batches of colostrum were run containing the aforementioned pathogens.

Chilled colostrum samples were submitted immediately for microbial culture for *M. bovis, L. monocytogenes, S. enteritidis,* and *E. coli* O157:H7. Duplicate samples intended for measurement of IgG concentration and activity were frozen at –20°C for 2 to 4 wk and then submitted for testing at the Veterinary Diagnostic Laboratory at the University of Minnesota (St. Paul, MN).

Inoculation and Heat Treatment of Colostrum with Map

As was described for previous batches, thawed bovine colostrum at 20°C was pooled to create a unique 30-L batch, transferred into a commercial on-farm batch pasteurization system (DairyTech Inc.), and then inoculated with *Map* to reach a final concentration of 10^3 cfu/mL. The heat-treatment and sampling procedures were the same as described for the other 4 pathogens. All samples were stored at –20°C and then transported to USDA-ARS, National Animal Disease Center (Bacterial Diseases of Livestock Research Unit, Ames, IA), where they were tested for *Map* by bacterial culture and by PCR.

As for the other pathogens, 4 unique replicate batches of colostrum were run for *Map*. However, one difference in methodology for heat-treatment of the 4 *Map*-inoculated colostrum batches should be noted. For the first 2 batches of *Map*-inoculated colostrum, early results for *Map* culture showed that both the positive and negative control samples were contaminated when cultured on slants of Herrold's egg yolk medium (**HEYM**), precluding the authors' ability to demonstrate the presence of *Map* in the positive control samples. Nonetheless, the authors believe that it is still valid to report the results from these first 2 batches because *Map* was clearly cultured (and confirmed with PCR) from the third, fourth, and fifth samples (collected at 0, 15, and 30 min at 60°C, respectively), after heat-treatment had effectively removed all other contaminants from the colostrum. However, in an effort to remove these contaminants prior to inoculating the batch with *Map,* the third and fourth batches of colostrum were pre-heat-treated at 60°C for 60 min and then cooled to 20°C before inoculating the batch with *Map*. After inoculation, the 120-min heat-treatment process at 60°C, and the associated sample collection process, was then completed in full as described above. The authors were comfortable in taking this approach for the last 2 *Map*-inoculated batches because previous research has

shown that the pre-heat-treatment process does not change the fluid characteristics of the colostrum in any way that should alter the results of heat-treating *Map* in this pre-heat-treated colostrum (McMartin et al., 2006).

Culture of L. monocytogenes, E. coli O157:H7, S. enteritidis, and M. bovis from Colostrum

The FDA Bacteriological Analytical Manual methods were used to confirm the presence or absence of viable bacteria except that the media for *Salmonella* consisted of tryptic soy agar (40 g), yeast extract (6 g), ferric ammonium citrate (0.8 g), sodium thiosulfate (6.8 g), and sodium pyruvate (1 g) in 1 L of distilled water (V. Burgula and S. Tatini, unpublished data). Serial 10-fold dilutions of the samples made in 0.1% peptone water were inoculated in appropriate media using the spread-plate technique. The plates were incubated at 37°C for 24 to 48 h and examined for the presence or absence of the pathogens. For *M. bovis,* serial 10-fold dilutions of colostrum were made in sterile *Mycoplasma* broth and 200 μL of each dilution was plated on 3 sterile *Mycoplasma* agar plates. The inoculated plates were incubated at 37°C in 10% carbon dioxide for 6 d prior to examination for the presence of bacterial growth.

Culture of Map from Colostrum

For the culture of *Map,* approximately 10 mL of each colostrum sample was transferred to a sterile 15-mL conical polypropylene tube and centrifuged at 739 × *g* for 30 min at 4°C. After centrifugation, the whey was decanted and the pellet and cream were resuspended in 2 mL of 0.75% hexadecylpyridinium chloride (Sigma Chemical Co., St. Louis, MO) prepared in 50% brain heart infusion broth (Becton-Dickinson, Franklin Lakes, NJ). Samples were then incubated at 39°C for 5 h followed by centrifugation at 1,160 × *g* for 20 min at 4°C. Pellets were resuspended in 1 mL of antibiotic brew (100 μg/mL of nalidixic acid, 100 μg/mL of vancomycin, and 50 μg/mL of amphotericin B; Sigma Chemical Co.) and incubated for 24 h. Samples were diluted 1:10 in 0.15 *M* PBS (pH 7.4), and 200 μL of the 1:10 dilution was inoculated onto 2 agar slants of HEYM containing mycobactin J (2 mg/L; Allied Monitor, Fayetteville, MO), nalidixic acid (50 μg/mL), and vancomycin (50 μg/mL). Samples were also inoculated onto 2 slants of commercially prepared HEYM. During incubation at 39°C for 12 wk, tubes were monitored for bacterial growth at 4, 8, and 12 wk. Colony growth was confirmed by PCR as described below.

Extraction of Map DNA Using PCR Analysis

A 500-μL aliquot of each colostrum sample (pre- and postinoculation and heating) was transferred to a sterile 1.5-mL microcentrifuge tube and vortexed briefly. Samples were centrifuged at 18,237 × *g* for 15 min and the supernatant was decanted. Proteinase K (1 mg/mL; Sigma Chemical Co.) was added to each sample and incubated in a 50°C shaker water bath overnight. Following incubation, 22 μL of Tris-EDTA-NaCl buffer (50 m*M* Tris, 100 m*M* EDTA, and 150 m*M* NaCl; pH 8.0) and 10 μL of 0.4 *M* NaOH was added to each sample and the tubes were vortexed. The tubes were then placed in a heating block (100°C) in water and boiled for 30 min. After cooling, samples were subjected to a phenol-chloroform-isoamyl alcohol (25:24:1; Amresco, Solon, OH) extraction and overnight precipitation in 100% ethanol; DNA pellets were resuspended in sterile water and stored at –20°C.

To validate *Map* growth on HEYM agar after 12 wk of culture, suspect colonies were scraped with a sterile loop and placed into 250 μL of 1× Tris-EDTA buffer in a 1.5-mL microcentrifuge tube. Tubes were placed in a heating block (100°C) in water and boiled for 10 min. After cooling to room temperature, 4 μL of RNase was added to each tube and samples were stored at –20°C until PCR was performed.

The DNA was amplified using a nested PCR protocol previously described for milk samples (Stabel and Lambertz, 2004). For the first amplification reaction, forward and reverse primers (5′-GTTCGGGGCCGTCGC TTAGG-3′ and 5′-GAGGTCGATCGCCCACGTGA-3′) were used to amplify a 400-bp region of the insertion element IS900. A second amplification reaction further amplified the product of the first reaction, using internal forward and reverse primers (5′-GCTTAGGCTTC GAATTGCC-3′ and 5′-CTCCGTAACCGTCATTGTCC-3′) and resulted in a final product of 194 bp. After amplification, DNA was electrophoresed in a 4% agarose gel (Reliant Gel Systems, FMC Bioproducts, Rockland, ME), containing ethidium bromide in Tris-borate-EDTA buffer (89 m*M* Tris, 89 m*M* boric acid, and 2 m*M* EDTA), and bands were visualized using a UV transilluminator (Bio-Rad, Hercules, CA). A positive control DNA sample (*Map* DNA) was included in each PCR run and on each gel for assay verification. A negative control consisting of buffer only was run each time to verify lack of cross-contamination of samples.

Measurement of IgG Concentration and Activity in Colostrum

Analysis of colostrum samples for total IgG concentration (mg/mL) was completed using a turbidometric-immunoassay (instrument: Olympus AU400e; Olym-

pus America Inc., Melville, NY; reagents: Midland Bioproducts Corp., Boone, IA). The turbidometric-immunoassay is a highly sensitive automated lateral-flow immunoassay that directly measures turbidity of the antigen–antibody complex, producing accurate IgG measures as compared with the more time-consuming and labor-intensive radial immunodiffusion method (Etzel et al., 1997; McVicker et al., 2002). A paired *t*-test was used to contrast the colostrum IgG concentration (mg/mL) measured at each of the 15-min interval sampling points against the original IgG concentration measured in sample number 2 (postinoculation sample at 20°C). Statistical significance was established at $P < 0.05$.

A microtitration serum neutralization (**SN**) assay was used to evaluate antibody activity in colostrum samples. Frozen samples of colostrum were thawed at room temperature and then centrifuged at 28,800 × *g* for 2 h. Clear whey was used as the starting material for the detection of antibodies against bovine viral diarrhea virus type 1 (**BVDV-1**) using the SN test. Serial 2-fold dilutions of whey were made in modified Eagle's medium with Earle's salts, 150 IU/mL of penicillin, 150 μg/mL of streptomycin, 50 μg/mL of neomycin, and 1 μg/mL of fungizone. Each dilution (25 μL) was placed in wells of a 96-well, flat-bottomed microtiter plate followed by the addition of an equal volume of virus suspension containing approximately 300 $TCID_{50}$ (i.e., 50% tissue culture infective dose) of BVDV-1 (Singer strain). The whey–virus mixture was incubated at 37°C for 60 min. A suspension of bovine turbinate cells (5×10^5 cells/mL) was then added to all wells at 50 μL per well. A drop of mineral oil was also placed in all wells to minimize liquid evaporation. The plates were incubated at 37°C in a 5% CO_2 atmosphere for 7 d. Each set of plates had cell controls and a virus back-titration control in addition to individual whey controls. Antibody titers were expressed as the reciprocal of the highest dilution that prevented the development of viral cytopathic effects. After BVDV SN titers were log transformed, a 2-tailed paired *t*-test was used to contrast the colostrum BVDV SN titers [$\log_2$(titer)] at each of the 15-min sampling points to the original BVDV SN titer measured in sample number 2 (postinoculation sample at 20°C). Statistical significance was established at $P < 0.05$.

RESULTS

Culture of Bacterial Pathogens from Colostrums

For each of the 5 pathogens studied, the average results of bacterial recovery from the 4 replicate batches of heat-treated colostrum are presented in Table 1. For all 4 replicate batches, *M. bovis* was consistently not detected in colostrum samples collected upon reaching the target temperature of 60°C, indicating the organism had not survived the 30-min heat-up phase (Table 1). For all 4 replicate batches, *E. coli* O157:H7 was consistently not detected in colostrum samples collected after 15 min of heating at 60°C, and *L. monocytogenes* and *S. enteritidis* were consistently not detected in colostrum samples collected after 30 min of heating at 60°C (Table 1).

Upon averaging the culture results from the 4 replicate batches of colostrum inoculated with *Map,* the organism was not detected after 60 min of heating at 60°C (Table 1). However, these results differed slightly from the results obtained for the other 4 pathogens, in that the duration of heat-treatment at 60°C required before *Map* could no longer be detected was not consistent across all 4 replicate batches. In particular, the third batch showed no detectable growth after heat-treatment for 60 min, but barely visible pinprick colonies (indicative of heat-treated *Map* and counted as less than 1 cfu/mL) were detected in samples collected after heat-treatment for 75 and 90 min (Table 1). These pinprick colonies were confirmed as *Map* using PCR analysis.

IgG Concentration and Activity in Colostrum

Immunoglobulin G concentration results were available for 5 batches of colostrum heat-treated at 60°C for 120 min. The mean IgG concentration was not different between raw colostrum (60.5 mg/mL; SD = 4.3; range = 54.8 to 65.8) and colostrum heated at 60°C for 120 min (59.1 mg/mL; SD = 1.9; range = 57.3 to 61.8; $P > 0.05$; Figure 1). The mean percent reduction in IgG concentration for these 5 batches of moderate- to high-quality colostrum, after heating for 120 min, was 2.2% (SD = 4.2; −4.4 to 6.1).

Readers should be aware that IgG concentrations were measured for the additional 3 batches, but because IgG measurements for these latter 3 batches were not available beyond heating for 90 min in duration, they were not included in the final analysis or in Table 1. Nonetheless, even when they were included in the analysis (up to 90 min), there was still no significant effect of heating on IgG concentration. However, it is worth noting that for 2 of these 3 batches that were of very high quality (>75 mg/mL of IgG), a larger numerical percent reduction in IgG concentration was observed than occurred for the other batches. After heating at 60°C for 90 min, the IgG concentration in batch M6 had dropped from 76.6 to 70.8 mg/mL (7.5% reduction)

Table 1. Recovery of *Mycoplasma bovis, Escherichia coli* O157:H7, *Salmonella enteritidis, Listeria monocytogenes,* and *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) from colostrum during heat-treatment at 60°C for 120 min

	Duration (min) at 60°C (temperature, °C)											
Bacterial species (inoculation dose, cfu/mL)[1]	–30 (20)[2]	–30 (20)[3]	0 (60)	15 (60)	30 (60)	45 (60)	60 (60)	75 (60)	90 (60)	105 (60)	120 (60)	120 (38)
M. bovis (1×10^8 cfu/mL)	ND	G	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
E. coli O157:H7 (1×10^6 cfu/mL)	ND	G	G	ND	ND	ND	ND	ND	ND	ND	ND	ND
S. enteritidis (1×10^6 cfu/mL)	ND	G	G	G	ND	ND	ND	ND	ND	ND	ND	ND
L. monocytogenes (1×10^6 cfu/mL)	ND	G	G	G	ND	ND	ND	ND	ND	ND	ND	ND
M. paratuberculosis (1×10^3 cfu/mL)	ND	G	G	G	G	G	ND	ND	ND	ND	ND	ND
Map batch 1	C	C	G	G	G	G	G	ND	ND	ND	ND	ND
Map batch 2	C	C	G	ND	G	ND	ND	ND	ND	ND	ND	ND
Map batch 3	ND	G	G	G	ND	G	ND	G	G	ND	ND	ND
Map batch 4	ND	G	G	G	ND	G	ND	ND	ND	ND	ND	ND

[1]Results are averaged from 4 replicate runs. For *Map,* average values and individual values are presented. C = contaminated; G = growth detected; ND = growth not detected.

[2]Preinoculation sample (negative control); –30 represents a time point 30 min before the target temperature was reached.

[3]Postinoculation sample (positive control); –30 represents a time point 30 min before the target temperature was reached.

and the IgG concentration in batch M25 had dropped from 79.1 to 69.8 mg/mL (11.7% reduction).

Serum neutralization titers for anti-BVDV antibodies were available for 3 batches of colostrum heat-treated at 60°C for 120 min. The mean $\log_2$(BVDV SN titer) was not different for raw colostrum samples (12.3; SD = 0.58; range = 12.0 to 13.0) compared with samples heated at 60°C for 120 min (12.0; SD = 0; range = 12.0 to 12.0; $P > 0.05$; Figure 1). Readers should be aware that SN titers for anti-BVDV antibodies were measured for 2 additional batches, but because results for these latter 2 batches were not available beyond heating for 75 min in duration, they were not included in the final analysis or in Table 1. Nonetheless, even when they

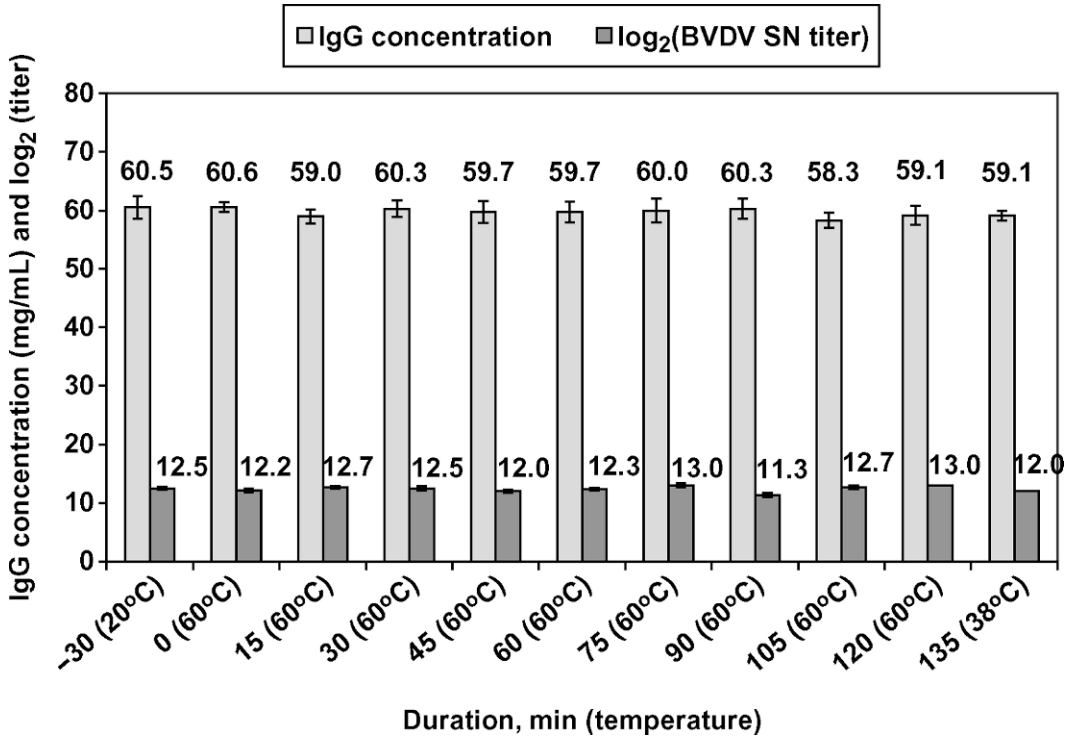

Figure 1. Mean (±SE) IgG concentration (data from 5 batches of colostrum) and bovine viral diarrhea virus type 1 serum neutralization titer (BVDV SN titer; data from 3 batches of colostrum) in 30-L batches of colostrum during heat-treatment in a commercial on-farm batch pasteurizer at 60°C for 120 min.

were included in the analysis (up to 75 min), there was still no reduction observed in antibody activity.

DISCUSSION

Pasteurization of colostrum could serve as an effective and practical method of reducing pathogen exposure to highly susceptible newborn calves, reducing the high rate of preweaning mortality (8.4 to 10.7%) experienced in dairy replacement heifers in the United States (National Animal Health Monitoring System, 1993, 1996, 2002) as well as reducing the risk for transmitting specific economically important pathogens such as *Map*.

Although a considerable number of studies have investigated the effectiveness of pasteurization in destroying important pathogens in milk, far fewer studies have reported on the pasteurization of colostrum. Green et al. (2002) reported that bovine colostrum can be successfully pasteurized using either commercial batch pasteurization (63°C for 30 min) or HTST pasteurization (72°C for 15 s) systems to eliminate important bacterial pathogens including *Salmonella* spp., *L. monocytogenes,* and *E. coli* O157:H7. Stabel et al. (2004) reported that HTST pasteurization of colostrum using a commercial on-farm pasteurizer (at 71.7°C for 15 s) was effective in destroying *Map* inoculated in colostrum at 10^5 cfu/mL. Meylan et al. (1995) reported that simulated batch-pasteurization of 50-mL volumes of colostrum, held at 63°C for 30 min, effectively eliminated *Map* from colostrum samples that were inoculated with 10^2 or 10^3 cfu/mL. However, viable *Map* was recovered from 2 of 6 pasteurized samples that were inoculated with 10^4 cfu/mL.

Past studies have suggested that pasteurization of colostrum using conventional methods can eliminate or significantly reduce exposure to pathogens. However, an important consequence of this procedure is that it results in increased viscosity, adversely affecting feeding and cleaning characteristics. In addition, there is significant denaturation of colostral Ig (Meylan et al., 1995; Godden et al., 2003; Green et al., 2003; Stabel et al., 2004), which in turn will produce lower serum IgG concentrations in newborn calves fed pasteurized colostrum (Godden et al., 2003).

However, this problem may be overcome by using a longer, lower-temperature approach to heat-treat colostrum. In a recent study, 50-mL volumes of bovine colostrum were successfully heat-treated in a Rapid Visco Analyzer at 60°C for as long as 120 min without causing increases in viscosity or reductions in IgG concentration (McMartin et al., 2006). Although these results appeared to be extremely promising, the authors still needed to verify whether the same results could be achieved with large volumes of colostrum that were similarly heat-treated when using a commercial on-farm batch pasteurization system. Furthermore, they needed to describe the duration of heating necessary at 60°C to eliminate important bacterial pathogens.

The results of this study showed that large (30-L) batches of moderate- to high-quality colostrum can be successfully heat-treated at 60°C in a commercial on-farm batch pasteurization system for at least 120 min without reducing the IgG concentration (mg/mL) or activity [$\log_2$(BVDV SN titer)]. Our results agreed with an earlier study (McMartin et al., 2006) in that very high quality colostrum (>75 mg/mL) suffered a greater absolute percent numerical reduction in IgG concentration (mean reduction of 9.6%) than did moderate- to high-quality colostrum (<75 mg/mL; nonsignificant numerical reduction of 2.2%). However, the authors feel that a 10% reduction in IgG concentration is of no practical importance because the end product is still of exceptionally high quality (i.e., a very high quality sample can afford to experience a 10% reduction in IgG concentration and still remain a very high quality sample to feed to calves).

This study also demonstrated that heat-treating large (30-L) batches of colostrum in a commercial on-farm batch pasteurization system at 60°C for 30 min should be adequate to eliminate high concentrations of *L. monocytogenes, E. coli* O157:H7, *S. enteritidis,* and *M. bovis*. If these results can be achieved on commercial dairy farms, then the most immediate benefit from feeding heat-treated colostrum may be to reduce enteritis caused by fecal coliforms, as well as to prevent transmission of other economically important pathogens such as *Salmonella* and *Mycoplasma* spp. Finally, if the hypothesis is true that living bacteria in colostrum may interfere with passive absorption of colostral antibodies, then feeding heat-treated colostrum could help improve passive transfer of IgG in calves (James and Polan, 1978; James et al., 1981; Staley and Bush, 1985).

In addition to the potential short-term benefits described, producers will also be very interested to know whether feeding heat-treated colostrum could serve as a potential control point in a comprehensive Johne's disease control program. Although the results of this research were less consistent for *Map* than for the other 4 pathogens studied, when averaging the results from all 4 replicate batches, the authors' interpretation of the results was that heating at 60°C for 60 min should be sufficient to eliminate *Map* under most conditions, because the results from batch 3 were not repeatable. However, it is important not to dismiss the variability observed in the *Map* culture results. The accuracy (especially sensitivity) of the sampling and culture methods used may be one factor contributing to the variabil-

ity observed in our results. For example, for batch 3 the sample collected after 60 min yielded no detectable growth, even though the samples collected after 75 and 90 min yielded detectable growth (but at very small concentrations). Similar observations (but at earlier time points) were observed in batches 2 and 4. Although the current results suggest that heating at 60°C for 60 min should be sufficient to eliminate *Map* in most situations, these findings should be further investigated to determine whether they can be replicated by additional studies.

One factor readers should consider when interpreting the *Map* culture results from this or any other study is the inoculation dose used. Inoculum concentrations used for all pathogens in this study were selected to meet or exceed concentrations of the organism that might be expected to exist in naturally infected colostrum under field conditions. The inoculation dose for *Map* used in the study (10^3 cfu/mL) was considerably higher than concentrations known to be cultured from milk or colostrum of naturally shedding Johne's-infected cows (5 to 8 cfu/50 mL of milk; Taylor et al., 1981; Sweeney et al., 1992). Thus, and precluding significant fecal contamination of colostrum during the harvest, storage, or feeding processes, we would expect that concentrations of *Map* found in naturally infected colostrum on commercial dairy farms should be significantly lower than those used in this study, lending confidence to the conclusion that heat-treatment at 60°C for 60 min should be sufficient to control the transmission of this organism in most situations. Also, it is still undetermined whether low concentrations of the organism fed in a single feeding of colostrum could represent an infective dose to a newborn calf. Thus, if heat-treatment of colostrum at 60°C for 60 min can eliminate, or at least significantly reduce, the concentration of viable *Map* in colostrum, then adoption of the practice of feeding heat-treated colostrum could prove to be an effective means of preventing, or at least reducing, the transmission of *Map* on commercial dairy farms. However, these questions require further investigation.

This work was done on colostrum that was collected, frozen, thawed, inoculated with potential pathogens, and then pasteurized, although we would expect similar results with fresh colostrum. The results of this research are very promising; however, these studies have thus far been confined to a controlled laboratory setting, and many questions remain to be investigated. For example, even though this longer, lower-temperature approach may not harm the colostral antibody concentration or activity, it has yet to be determined whether the heat-treatment process can harm other important immune factors found in colostrum (e.g., vitamins, white blood cells). Furthermore, large-scale prospective controlled field studies will be needed to 1) describe whether the practice of feeding heat-treated colostrum can be successfully adopted on commercial dairy farms without interfering with passive transfer in calves, 2) describe and quantify any short-term or long-term health or performance benefits in calves fed heat-treated colostrum (e.g., reduced preweaning morbidity and mortality, improved growth rates, reduced transmission of *Map*), and finally, 3) describe and quantify whether there is any economic benefit from feeding heat-treated colostrum on commercial dairy farms (e.g., cost–benefit and break-even analyses).

CONCLUSIONS

Large (30-L) batches of moderate- to high-quality bovine colostrum can be heat-treated in a commercial on-farm batch pasteurizer at 60°C for at least 120 min without affecting the IgG concentration or activity. *Mycoplasma bovis, L. monocytogenes, E. coli* O157:H7, and *S. enteritidis* added to colostrum could not be recovered after colostrum was heat-treated at 60°C for 30 min. Heat-treatment at 60°C for 60 min should be sufficient to eliminate *Map* from colostrum in most situations. However, the latter findings should be further investigated to determine whether they can be replicated by additional studies. Further research is also needed to determine whether these results can be replicated on commercial dairy farms, and to describe any health performance or economic benefits that may come from feeding heat-treated colostrum on commercial dairy farms.

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Heat-treated colostrum and reduced morbidity in preweaned dairy calves: Results of a randomized trial and examination of mechanisms of effectiveness

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ABSTRACT

A randomized controlled clinical trial was conducted using 1,071 newborn calves from 6 commercial dairy farms in Minnesota and Wisconsin, with the primary objective being to describe the effects of feeding heat-treated colostrum on serum immunoglobulin G concentration and health in the preweaning period. A secondary objective was to complete a path analysis to identify intermediate factors that may explain how feeding heat-treated colostrum reduced the risk for illness. On each farm, colostrum was collected each day, pooled, and divided into 2 aliquots; then, one aliquot was heat-treated in a commercial batch pasteurizer at 60°C for 60 min. Samples of fresh and heat-treated colostrum were collected for standard microbial culture (total plate count and total coliform count, cfu/mL) and for measurement of immunoglobulin G concentrations (mg/mL). Newborn calves were removed from the dam, generally within 30 to 60 min of birth, and systematically assigned to be fed 3.8 L of either fresh (FR, n = 518) or heat-treated colostrum (HT, n = 553) within 2 h of birth. Venous blood samples were collected from calves between 1 and 7 d of age for measurement of serum IgG concentrations (mg/mL). All treatment and mortality events were recorded by farm staff between birth and weaning. Regression models found that serum IgG concentrations were significantly higher in calves fed HT colostrum (18.0 ± 1.5 mg/mL) compared with calves fed FR colostrum (15.4 ± 1.5 mg/ml). Survival analysis using Cox proportional hazards regression indicated a significant increase in risk for a treatment event (any cause) in calves fed FR colostrum (36.5%, hazard ratio = 1.25) compared with calves fed HT colostrum (30.9%). In addition, we observed a significant increase in risk for treatment for scours in calves fed FR colostrum (20.7%, hazard ratio = 1.32) compared with calves fed HT colostrum (16.5%). Path analysis suggested that calves fed HT colostrum were at lower risk for illness because the heat-treatment process caused a significant reduction in colostrum total coliform count, which was associated with a reduced risk for illness as a function of improved serum IgG concentrations.

Key words: bacteria, colostrum, morbidity, heat-treatment

INTRODUCTION

The neonatal calf relies on the passive absorption of colostral immunoglobulins within the first few hours after birth to provide protection against infectious disease challenge early in life (Robison et al., 1988; Banks, 1993). Serum IgG concentrations ≥10 mg/mL at 24 to 48 h are associated with reduced risk for morbidity and mortality in the preweaning period, improved daily weight gain and feed efficiency, reduced age at first calving, and improved milk production later in life (Robison et al., 1988; DeNise et al., 1989; Wells et al., 1996; Donovan et al., 1998; Weaver et al., 2000; Faber et al., 2005). Despite the health and nutritional benefits for the calf, colostrum is a potential early source of exposure to microbial pathogens. Microorganisms may be present in colostrum from multiple sources, including secretion from the mammary gland; contamination during milking, storage, or feeding; or bacterial proliferation in stored colostrum (Fecteau et al., 2002; McGuirk and Collins, 2004; Stewart et al., 2005). Contamination of colostrum with microbial pathogens may result in acute or chronic disease, depending on the nature of the pathogen: *Salmonella* spp., *Escherichia coli*, *Mycobacterium avium* ssp. *paratuberculosis* (**MAP**), *Mycoplasma* spp., and bovine leukemia virus are just a few of the pathogens that may be isolated from colostrum (Ferrer and Piper, 1981; Streeter et al.,

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1995; González and Wilson, 2003; CDC, 2008; Houser et al., 2008; Nielsen et al., 2008; Pithua et al., 2009). As an additional concern, some studies have reported that high concentrations of bacteria in colostrum may be associated with decreased immunoglobulin absorption, thereby contributing to failure of passive transfer (James et al., 1981; Poulson et al., 2002).

Heat treatment (**HT**) may be one approach to reduce microbial contamination in colostrum. The history of developing a technique to heat-treat colostrum has been reviewed elsewhere (Donahue et al., 2012). Briefly, HT at 60°C for 60 min significantly reduces or eliminates inoculated pathogens, including *Mycoplasma bovis*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enteritidis*, and MAP, and significantly reduces total bacteria counts and total coliform counts in colostrum, while maintaining colostrum IgG concentrations and nutrient composition. This HT protocol has been validated in laboratory-based inoculation studies, in 2 separate single-herd university trials and, more recently, in a multiherd study conducted on 6 large commercial Upper Midwest dairy farms (McMartin et al., 2006; Godden et al., 2006; Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009a; Donahue et al., 2012).

Two single-herd university trials reported that calves fed HT colostrum experienced improved efficiency of IgG absorption and higher serum IgG concentrations as compared with calves fed the untreated, fresh (**FR**) colostrum (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009a). The mechanism to explain the relationship between feeding HT colostrum and improved passive transfer of IgG has not yet been determined. Although it has been suggested that bacteria present in the small intestine may interfere with the process of passive absorption of colostral immunoglobulins (James et al., 1976; James and Polan, 1978; James et al., 1981) to date, equivocal and limited evidence support this hypothesis (Poulson et al., 2002; Elizondo-Salazar and Heinrichs, 2009b).

In theory, reducing microbial exposure and enhancing the serum IgG status of calves fed HT colostrum should result in improved calf health. However, studies to date have lacked sufficient sample sizes to investigate this hypothesis. The first objective of the current study was to conduct a multiherd controlled clinical trial to describe the direct effects of feeding HT colostrum on passive transfer of IgG and on calf health, the hypothesis being that calves fed HT (vs. FR) colostrum would have improved serum IgG concentrations and would be at reduced risk for morbidity and mortality in the preweaning period. Assuming that a treatment effect was discovered, a secondary objective was to complete a path analysis to identify intermediate factors that may explain how feeding HT colostrum reduced risk for illness. The authors hypothesized that feeding HT colostrum would result in reduced illness either by reducing bacterial pathogen exposure or by enhancing serum IgG concentrations or both.

MATERIALS AND METHODS

Farm Enrollment

This study was approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC). Six large commercial dairy farms in Minnesota and Wisconsin were enrolled in the study during the summer of 2007. This was a convenience sample of herds, based on their proximity to the University of Minnesota (~250 km radius), willingness to adhere to study protocols, and with at least one animal in the herd that tested positive for infection with MAP by fecal culture or serum ELISA within the previous 3 yr. Calf enrollment was from June to August 2007. The target sample size of $\geq$500 calves per treatment group was calculated to allow for a sufficient number of adult animals to still be present in the herd 3 to 5 yr after enrollment, to address a longer term objective: evaluate the effect of feeding HT colostrum on risk for transmission of MAP.

Colostrum Preparation

First-milking colostrum was collected by farm personnel within 2 h postcalving and refrigerated at 4°C for 24 to 48 h before pooling. Refrigerated colostrum was pooled into one batch, thoroughly mixed, and divided into equal aliquots. One aliquot was left untreated whereas the other was heat-treated in a commercial batch pasteurizer at 60°C for 60 min (DairyTech Inc., Windsor, CO). Pasteurizers were programmed to heat colostrum to a target temperature of 60°C for 60 min, with a maximum allowable fluctuation of 0.56°C during the 60-min holding phase. Colostrum was then automatically cooled to 15.6°C. Times and temperatures were reported by digital display on the equipment during the heat-treatment process, but were not recorded due to lack of availability of electronic recording equipment for the batch pasteurizers used at the time the study was conducted (Note: electronic data loggers and paper chart recorders are currently available as an option for use with this equipment). Colostrum was agitated during the entire heat-treatment process. Duplicate 50-mL aliquots of FR and HT colostrum were aseptically collected from each batch and frozen at −20°C before analysis. The effects of HT on colostrum characteristics

are reported in a companion manuscript (Donahue et al., 2012). Fresh and HT colostrum were transferred to sanitized 3.8-L bottles and refrigerated for later feeding to calves enrolled in the study. Colostrum was generally fed within 24 to 48 h of preparation.

Calf Enrollment, Management, and Data Collection

Newborn calves were enrolled between May 30 and August 23, 2007. All farms had 24-h supervision of the maternity area. Newborn calves were separated from the dam shortly after birth and before suckling. All liveborn heifer calves were eligible for enrollment on 5 farms. Both heifer and bull calves were enrolled on the sixth farm, which raised all calves to weaning age. Calves were systematically assigned by birth order to receive 3.8 L of HT or FR colostrum as soon as possible after birth (goal <2 h). Five farms fed the entire 3.8-L volume of colostrum by esophageal tube feeder, whereas 1 farm fed colostrum first by nipple bottle, with any remaining unconsumed colostrum fed via an esophageal tube feeder. One farm offered a second feeding of 1.9 L of colostrum by nipple bottle approximately 12 h after the first feeding, whereas the remaining 5 farms fed only a single colostrum feeding. Farm personnel recorded calf enrollment information, including dam identification number, calf identification number, birth date and time, colostrum treatment group assigned, colostrum batch fed, time of colostrum feeding, sex and number of calves born (single/twin), and calving ease score (1 = unassisted, 2 = easy pull, 3 = moderate pull, 4 = hard pull, 5 = caesarian section).

After the colostrum was fed, calves were moved to individual calf housing, either hutches (3 farms) or individual pens within a barn (3 farms), where they were managed according to each farm's routine feeding and management protocols. Farm personnel responsible for calf enrollment and colostrum feeding were not blinded to treatment assignment; however, farm personnel responsible for calf care to weaning were at a different physical location from the calf enrollment area, and were therefore effectively blinded to treatment. Farm staff recorded all treatment events, types of treatments administered, and mortality events between birth and weaning.

Sample Collection and Analysis

University technicians visited the study farms once per week to collect enrollment information, treatment and mortality records, and frozen colostrum samples. In addition, they collected a 1-mL blood sample from the jugular vein from all calves between 1 and 7 d of age. Blood and colostrum samples were transported on ice to the University of Minnesota (St. Paul) for analysis. Blood samples were centrifuged and serum collected and frozen at −20°C until it could be analyzed for serum IgG concentration (mg/mL) by turbidometric immunoassay (Etzel et al., 1997). Frozen colostrum was thawed and colostrum IgG concentration was determined by turbidometric immunoassay. The second (duplicate) colostrum sample was submitted to the Laboratory for Udder Health (University of Minnesota) for bacterial culture. Colostrum samples were thawed to 4°C, mixed by vortex, and serially diluted 1:10 for 5 dilutions. Each dilution was plated on plate count agar for total plate count (**TPC**) and on MacConkey agar for total coliform count (**TCC**). Plates were incubated for 48 h at 37°C and the number of colonies recorded (cfu/mL).

Statistical Analysis for Objective 1: Effect of Feeding HT Colostrum on Serum IgG Concentration and Calf Health

All analyses for this objective were performed using SAS software (version 9.2; SAS Institute, Cary, NC), with final significance declared at $P < 0.05$. A calf was excluded from the analysis if it was reported as stillborn (i.e., died within 24 h of birth) or if it could not be matched to a specific batch and sample of colostrum fed (FR or HT) for which colostrum bacterial culture and IgG results were available. Descriptive statistics were generated to describe characteristics of study calves and the colostrum fed for both treatment groups.

General mixed linear regression (Proc MIXED) was used to describe the effect of treatment (FR vs. HT) on calf serum IgG (mg/mL). Initially, univariate models were created to test the effect of treatment (FR vs. HT), age at first colostrum feeding (min), calving ease score (1 through 5), IgG concentration in the colostrum fed (mg/mL), and age at blood sample collection (d). Sex of the calf was not considered in the models because bull calves were only enrolled on one farm. Even if differences existed in study outcomes between bulls and heifers, this still would not introduce a bias to study findings, as bull calves were randomly assigned to both FR and HT groups. Covariates significant at $P < 0.20$ in a univariate model were carried forward into a multivariate model. A backward stepwise approach was then used to create the final model to predict the outcome serum IgG. Farm was included in all models as a random effect to control for the clustering of calves within farm. All possible 2-way interaction terms were explored between significant covariates remaining in the model.

Cox proportional hazards regression (Proc PHreg) was used to test the effect of treatment on the risk for (1) treatment for any disease event; (2) treatment for a scours event; (3) treatment for a respiratory disease event; or (4) death, in the preweaning period. Calves were censored at 56 d of age (weaning) if they did not experience the event of interest by that time. The covsandwich estimator was used to estimate robust standard errors that accounted for clustering at the farm level.

Statistical Analysis for Objective 2: Path Analysis to Investigate How Feeding HT Colostrum Results in Reduced Risk for Illness

We hypothesized that feeding HT colostrum would result in reduced risk for illness either by reducing pathogen exposure (i.e., lower TPC or TCC) or by enhancing serum IgG concentrations, or both. Preliminary steps for this analysis used SAS software (version 9.1) and involved bivariate analyses to investigate all possible unconditional relationships between the variables describing colostrum treatment (FR vs. HT), colostral TPC ($\log_{10}$ cfu/mL), colostral TCC ($\log_{10}$ cfu/mL), serum IgG (mg/mL), and risk for a treatment event in the preweaning period (treated/not treated). Linear regression (Proc MIXED) was used to develop those univariate models in which the outcome was continuous in nature (i.e., TPC, TCC, or IgG). Cox proportional hazards regression (Proc PHreg) was used to develop the univariate models in which the outcome was time to an illness event. For these models, the proportional hazards assumption was evaluated using standard graphical methods and a statistical assessment of the residuals (Kleinbaum and Klein, 2005). Herd was controlled for as a random effect in all models.

A series of path models was used to evaluate the hypotheses pursuant to the temporal effects of the experiment. In the first set of models, we considered whether TPC, TCC, or both functioned as intermediate variables of the effect of randomization to FR or HT colostrum and subsequent IgG measures. Mediation was assessed by examining the attenuation of the initial estimate between randomization and IgG, the statistical significance of the component paths, and the estimated indirect effect (product of the component pathways) from the *model indirect* subcommand in M*plus* version 6.1 (Muthén and Muthén, 2009). These initial mediation models were extended with 2 time-to-event outcomes: time to any illness and time to scours. Because these models specified all possible relationships between the variables included in the model, absolute model fit was, by definition, perfect, so standard tests of model fit for path analyses are not reported.

RESULTS

Farm, Calf, and Colostrum Characteristics

Mean (SD, range) herd size, rolling herd average, bulk tank SCC, and dry period length for the 6 participating herds were 1,617 (454; 1,200 to 2,500) milking cows, 12,891 (1,167; 11,280 to 14,512) kg of milk, 280,983 (99,986; 149,900 to 430,000) cells/mL, and 44 (3.7; 37 to 48) days dry, respectively. All 6 herds used freestall housing and housed predominantly Holstein cows, although a few crossbreds were represented. Five farms used group maternity pens and 1 used individual maternity pens. Four farms housed preweaned calves on the main farm, whereas 2 farms housed calves off-site. Five farms and 1 farm fed pasteurized whole milk or commercial powdered milk replacer (20:20 fat:protein, whey protein base, nonmedicated), respectively, until weaning. Free-choice starter pellet and water were offered on all farms by 3 to 5 d of age, and calves were routinely weaned between 7 and 8 wk of age.

Of 1,093 calves originally enrolled in the study, 6 were excluded from analysis because they were later defined as being stillborn (i.e., died within 24 h of birth). An additional 16 calves were excluded from analysis because they could not be matched to a specific batch and sample of colostrum fed (FR or HT) for which colostrum bacterial culture and IgG results were available. Final analysis was completed for 1,071 calves (FR = 518; HT = 553). Calving ease scores, age at first feeding, colostrum IgG concentrations, and age at blood sample collection were not different between treatment groups (Table 1; $P > 0.05$). As expected, the $\log_{10}$ cfu/mL TPC and TCC concentrations in the colostrum fed were significantly lower for calves fed HT (TPC = 3.5, TCC = 2.1) compared with calves fed FR (TPC = 5.6, TCC = 4.7; Table 1; $P < 0.05$). Because of 24-h supervision of the calving area on all farms, removal from the dam and feeding of colostrum was generally very consistent and prompt: the mean age at first feeding for both treatment groups was 47.8 min, with 95% of calves fed colostrum within 2 h of birth.

Objective 1: Effect of Feeding HT Colostrum on Serum IgG Concentration and Calf Health

The analysis of effect of treatment on serum IgG concentration considered only 1,064 calves because serum samples were not available for 7 of the 1,071 calves, 5 of these because the calf died before a blood sample could be collected at the technician's weekly herd visit, and 2 because the sample went missing (lost or broken) in the laboratory. Calves with missing serum IgG

Table 1. General characteristics of calves and the fresh and heat-treated colostrums[1]

Variable	Fresh	Heat-treated
Calves (no.)	518	533
Calving ease score (1–5)	1.4 (0.74, 1 to 5)	1.4 (0.74, 1 to 4)
Age at first feeding (min)	47.5 (42.8, 0 to 520)	50.0 (36.9, 0 to 195)
Age at blood sample collection (d)	4.0 (2.2, 1 to 9)	4.0 (2.2, 1 to 8)
In colostrum fed:		
IgG (mg/mL)	63.9 (20.4, 16.3 to 139.9)	61.1 (19.2, 12.1 to 139.5)
Total plate count (log_{10}, cfu/mL)	5.6 (0.9, 2.6 to 9.1)	3.5 (1.1, 1 to 8.4)
Total coliform count (log_{10}, cfu/mL)	4.7 (1.3, 0 to 6.8)	2.1 (1.6, 0 to 7.0)
Total plate count[2] (cfu/mL)	515,000 (390 to 1.2×10^9)	2,100 (10 to 2.4×10^8)
Total coliform count[2] (cfu/mL)	51,500 (0 to 6.7×10^6)	90 (0 to 9.5×10^6)

[1]Mean (SD, range).
[2]Median (range).

measures were equally distributed between treatment groups (FR = 3; HT = 4). Covariates associated with the dependent variable serum IgG (mg/mL) in univariate models included colostrum treatment [estimate(SE)$_{\text{Fresh}}$ = −2.39 (0.37); $P < 0.0001$], IgG concentration in the colostrum fed [estimate(SE) = 0.11 (0.0098); $P < 0.0001$], age at first feeding [estimate(SE) = −0.012 (0.0062); $P = 0.056$], and age at blood sample collection [estimate(SE) = −0.27 (0.10); $P = 0.0064$]. However, only colostrum treatment group, IgG concentration in the colostrum fed, and age at blood collection were associated with serum IgG in the final multivariate model (Table 2). We observed no interactions among these 3 variables. The least squares means (SE) serum IgG concentration for calves fed FR and HT colostrum was 15.4 (1.5) and 18.0 (1.5) mg/mL, respectively ($P < 0.0001$; Table 2). Failure of passive transfer, defined as serum IgG <10 mg/mL, occurred in 30.1% (156 of 518) and 18.6% (103 of 553) of calves fed FR and HT colostrum, respectively.

The analysis of effect of treatment on calf health outcomes considered all 1,071 calves. Colostrum treatment group (FR vs. HT: forced) was the only covariate remaining in any of the final survival models predicting risk for an illness or death event. Treatment had no effect on risk for treatment for a respiratory disease event or risk for a death event in the first 8 wk of life (Table 3). However, we observed a significant increase in risk for treatment for any illness in calves fed FR colostrum [36.5%, hazard ratio $(\mathbf{HR})_{\text{Fresh}} = 1.25$] compared with calves fed HT colostrum (30.9%; $P = 0.002$; Table 3). In addition, we found a significant increase in risk for treatment for scours in calves fed FR colostrum (20.7%, $\text{HR}_{\text{Fresh}} = 1.32$) compared with calves fed HT colostrum (16.5%; $P = 0.0003$; Table 3).

Objective 2: Path Analysis to Investigate How Feeding HT Colostrum Results in Reduced Risk for Illness

Preliminary bivariate analyses identified significant associations for each of the possible unconditional relationships examined among the variables describing colostrum treatment group, TPC, TCC, serum IgG, and risk for treatment for any illness or for scours in the preweaning period (Table 4). Specifically, in unconditional models, raw colostrum (FR) had a positive relationship with TPC, TCC, and risk for treatment for any illness or for scours, but a negative relationship with serum IgG (Table 4). Colostral TPC had a positive association with TCC and risk for treatment for any illness or for scours, but a negative association with serum IgG (Table 4). Colostral TCC had a positive association with risk for treatment for any illness or for scours, but a negative association with serum IgG (Figure 1, Table 4). Finally, serum IgG had a negative relationship with risk for treatment for any illness or for scours.

Table 2. Final multivariate model describing the effect of colostrum treatment (fresh vs. heat-treated) on calf serum IgG (mg/mL)[1]

Covariate	Level	LSM (SE)	Estimate (SE)	*P*-value
Intercept			11.84 (1.66)	0.0009
Colostrum treatment	Fresh	15.4 (1.5)	−2.61 (0.34)	<0.0001
	Heat-treated	18.0 (1.5)	Referent	
Colostrum IgG (mg/mL)			0.11 (0.0096)	<0.0001
Age at blood sample (d)			−0.22 (0.09)	0.017

[1]Herd controlled for as random effect.

Table 3. Results from final models describing the effect of treatment (fresh, FR, vs. heat-treated, HT) on morbidity and mortality risk in preweaned dairy calves[1]

Outcome parameter	Treatment group	Affected, % (no.)	Estimate (SE)	Hazard ratio$_{Fresh}$	*P*-value
Treatment for any disease	FR (n = 518)	36.5 (189)	0.22 (0.073)	1.25 (1.08, 1.44)	0.0022
	HT (n = 553)	30.9 (171)	Referent		
Treatment for scours	FR (n = 518)	20.7 (107)	0.27 (0.076)	1.32 (1.14, 1.53)	0.0003
	HT (n = 553)	16.5 (91)	Referent		
Treatment for respiratory disease	FR (n = 518)	11.4 (59)	−0.00062 (0.012)	0.99 (0.98, 1.02)	0.96
	HT (n = 553)	9.4 (52)	Referent		
Death	FR (n = 518)	1.7 (9)	0.15 (0.20)	1.16 (0.78, 1.72)	0.46
	HT (n = 553)	2.4 (13)	Referent		

[1]Herd controlled for as random effect in all models.

The negative relationships between TPC or TCC and serum IgG were linear in nature (not curvilinear), with no optimal TPC or TCC cutpoint identified. Moreover, the strength and nature of this relationship (i.e., the slope of the regression lines) was the same for calves in both colostrum treatment groups (FR vs. HT; analysis not shown). The negative relationship between serum IgG concentration and risk for treatment for illness was also linear in nature (not curvilinear), with no optimal IgG cutpoint being evident for predicting illness. Finally, it should also be noted that when multivariate models were created predicting the outcome "risk for treatment for illness," which included variables describing TPC, TCC, or serum IgG as covariates along with colostrum treatment (FR/HT) in the model statement, the variable describing colostrum treatment consistently became nonsignificant and fell out of the model when using backward stepwise elimination (analysis not shown). This suggested that the association between colostrum treatment and risk for treatment for illness was confounded by TPC, TCC, and serum IgG, supporting the hypothesis that the protective effect of feeding heat-treated colostrum on calf health was mediated through the effect of reducing pathogen exposure (TPC, TCC) or improving serum IgG concentrations in the calf, or both. The results of this preliminary modeling led to the secondary objective of completing a formal path analysis to investigate how feeding heat-treated colostrum results in reduced risk for illness.

Data from all 1,071 calves were used in developing the path models, as calves with missing data on outcomes included in the path models (i.e., 7 calves missing serum IgG measures) were retained for estimation through full information maximum likelihood. The initial path models indicated that TCC was superior to TPC in explaining the covariation between treatment assignment and serum IgG (Figure 2). With TPC specified as the intermediate variable, we observed a statistically significant association only between treatment assignment and TPC. The remaining direct effect between treatment and serum IgG was attenuated and not statistically significant. The estimated indirect association between treatment and serum IgG as a function of TPC was −1.52 (95% CI = −4.00, 0.96). In contrast, speci-

Table 4. Unconditional (crude) relationships among variables describing colostrum treatment group (fresh vs. heat-treated), colostral total plate count (TPC), colostral total coliform count (TCC), calf serum IgG, and risk for treatment for any illness event or for scours in the preweaning period (treated/not treated)[1,2]

	Outcome variable				
Explanatory variable	TPC (log_{10} cfu/mL)	TCC (log_{10} cfu/mL)	Serum IgG (mg/mL)	Treated for any illness	Treated for scours
Fresh vs. heat-treated (referent)	2.09 (0.06)	2.58 (0.08)	−2.39 (0.37)	0.22 (0.07)	0.27 (0.08)
	<0.0001	<0.0001	<0.0001	0.0022	0.0003
TPC (log_{10} cfu/mL)		1.11 (0.02)	−0.70 (0.13)	0.09 (0.03)	0.13 (0.05)
		<0.0001	<0.0001	0.0042	0.015
TCC (log_{10} cfu/mL)			−0.69 (0.10)	0.10 (0.03)	0.13 (0.05)
			<0.0001	0.0009	0.0085
Serum IgG (mg/mL)				−0.04 (0.014)	−0.06 (0.024)
				0.0019	0.0011

[1]Values reported are the parameter estimates (SE in parentheses) and *P*-values (below) for the explanatory variable, as derived from bivariate linear regression models where outcomes were TPC, TCC, or serum IgG, or from Cox proportional hazards regression models, where the outcome was risk for treatment for any illness or for scours.

[2]Herd controlled for as random effect in all models.

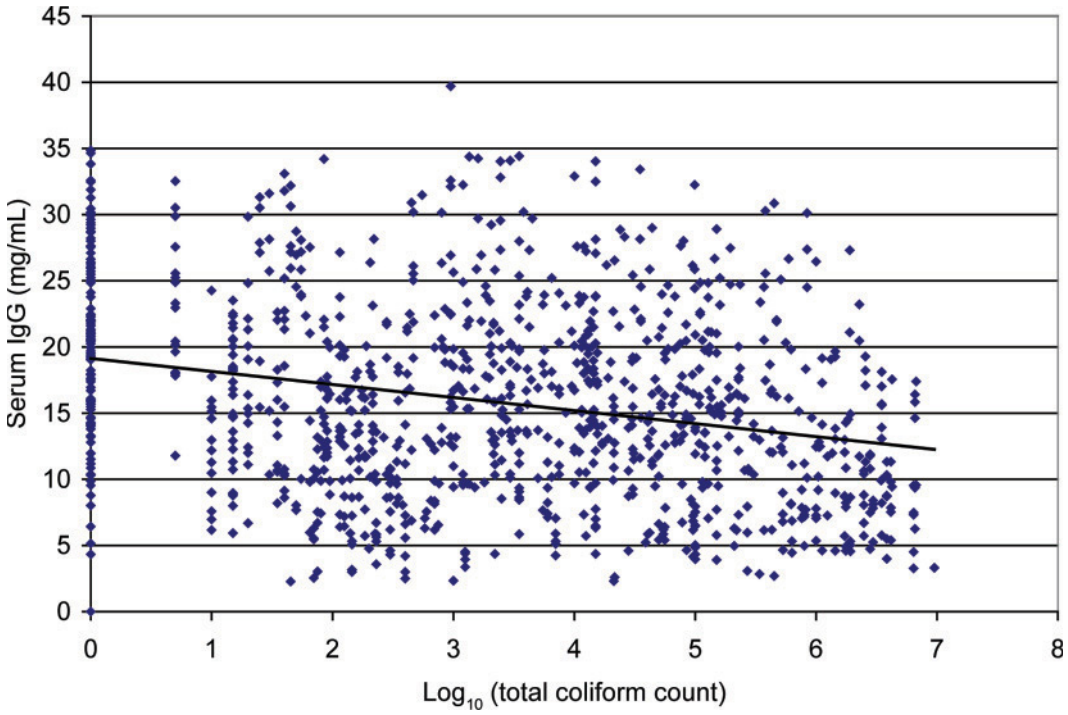

Figure 1. Scatter plot showing negative relationship between colostrum total coliform count ($\log_{10}$ cfu/mL) and calf serum IgG (mg/mL); $P < 0.0001$. Color version available in the online PDF.

fication of TCC as the intermediate variable yielded statistically significant associations between treatment and TCC and between TCC and IgG. The attenuation of the direct association between treatment assignment and serum IgG was stronger than when using TPC as the intermediate variable. The estimated indirect effect was −2.47 (95% CI = −5.45, 0.50). Given the reduced power associated with statistical inference of compound pathways (MacKinnon et al., 2000), the significance of both constituent pathways and the attenuation of the effect of treatment assignment on serum IgG to almost unity led us to conclude that TCC was the superior explanatory variable for the lower IgG levels observed for calves randomized to raw colostrum.

Subsequent path models that included time-to-event outcomes (days to treatment for any illness, days to treatment for scours) demonstrated that the association between treatment assignment, TCC, and the outcome (any illness or scours) was a function of serum IgG (Figure 3). For both outcomes, calves assigned to raw colostrum had higher TCC values, which were associated with lower serum IgG values. Accounting for the association between treatment assignment and TCC, and between TCC and serum IgG attenuated the observed total effect of treatment assignment on rates of any illness or scours to values indistinguishable from unity. This suggests that the higher infectious load and subsequent reduction in immunity in the FR calves is a plausible explanation for the observed effect of the treatment on calf health.

DISCUSSION

This is the first clinical trial designed to investigate the effect of feeding HT colostrum on calf health. Although this was a convenience sample of 6 large Midwest Holstein herds, the study results should be reasonably generalizable to other large commercial North American dairy herds. National US statistics estimate that the proportions of preweaned calves with failure of passive transfer, treated for scours or other digestive problems, and treated for respiratory disease are 19.2, 17.9, and 11.4%, respectively (USDA-APHIS, 2007a; Beam et al., 2009). By comparison, the proportions of control calves (FR group) in the current study with failure of passive transfer, treated for scours, and treated for respiratory disease were 30.1, 20.7, and 11.4%, respectively. Study herds did differ from national reported averages in some respects. For example, although the 6 study herds reported an average of 44 d dry, national US statistics report an average of 57.8 d dry (USDA-APHIS, 2007b). Furthermore, even though preweaning morbidity rates were comparable, the preweaning mortality rate in the control group (1.7%) was lower than that in national

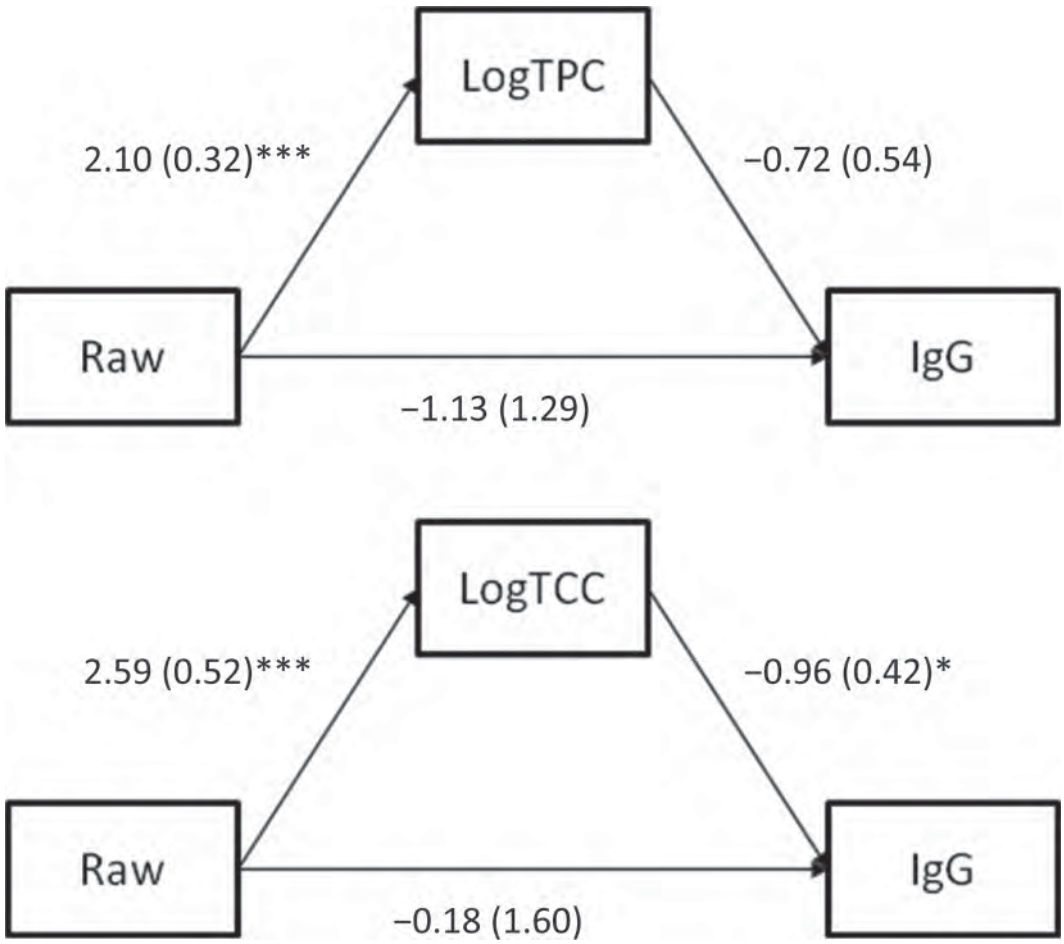

Figure 2. Path models of the direct and indirect effects of feeding fresh (raw) colostrum on serum IgG as a function of total plate count (TPC) and total coliform count (TCC). Feeding heat-treated colostrum was the referent. Values reported are the parameter estimates (SE) for the explanatory variable. $^{*}P < 0.05$; $^{***}P < 0.001$.

US statistics (7.8%; USDA-APHIS, 2007a). The latter could reflect differences in the level of management on study dairies, but probably also reflects the fact that the current study was conducted in the summer months, when mortality rates in upper Midwest dairies are typically lowest. By comparison, the national statistics report an annual mortality rate.

In the current study, serum IgG concentrations were higher for calves fed HT colostrum (18.0 mg/mL) than for calves fed FR colostrum (15.4 mg/mL). These results are consistent with 2 earlier single-herd studies (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009a). This is an important finding as it demonstrates that the results of the aforementioned, tightly controlled, single-herd university studies were repeatable when the protocol for heat-treating colostrum at 60°C for 60 min was conducted by farm staff on multiple commercial dairy herds. Because the aforementioned studies measured birth weights on calves, they were able to calculate and report that the apparent efficiency of absorption of IgG was significantly improved in calves fed HT colostrum. It was a limitation of the current study that electronic birth weights were not available for calves from the 6 study herds, and so the apparent efficiency of absorption of IgG could not be calculated. Another limitation of the study was that colostrum was not resampled for microbiological culture immediately before feeding, as this would have added significantly to project costs and to protocol complexity for calf feeders. Although some bacterial growth is expected to occur between initial preparation and later feeding of a batch, this additional growth should be low and, because it would occur in both HT and FR colostrum, should not bias the study findings.

A novel and important finding of the current study was that calves fed FR colostrum were at significantly higher risk for treatment for illness (any cause) (HR = 1.25; $P = 0.0022$) and for treatment for scours (HR =

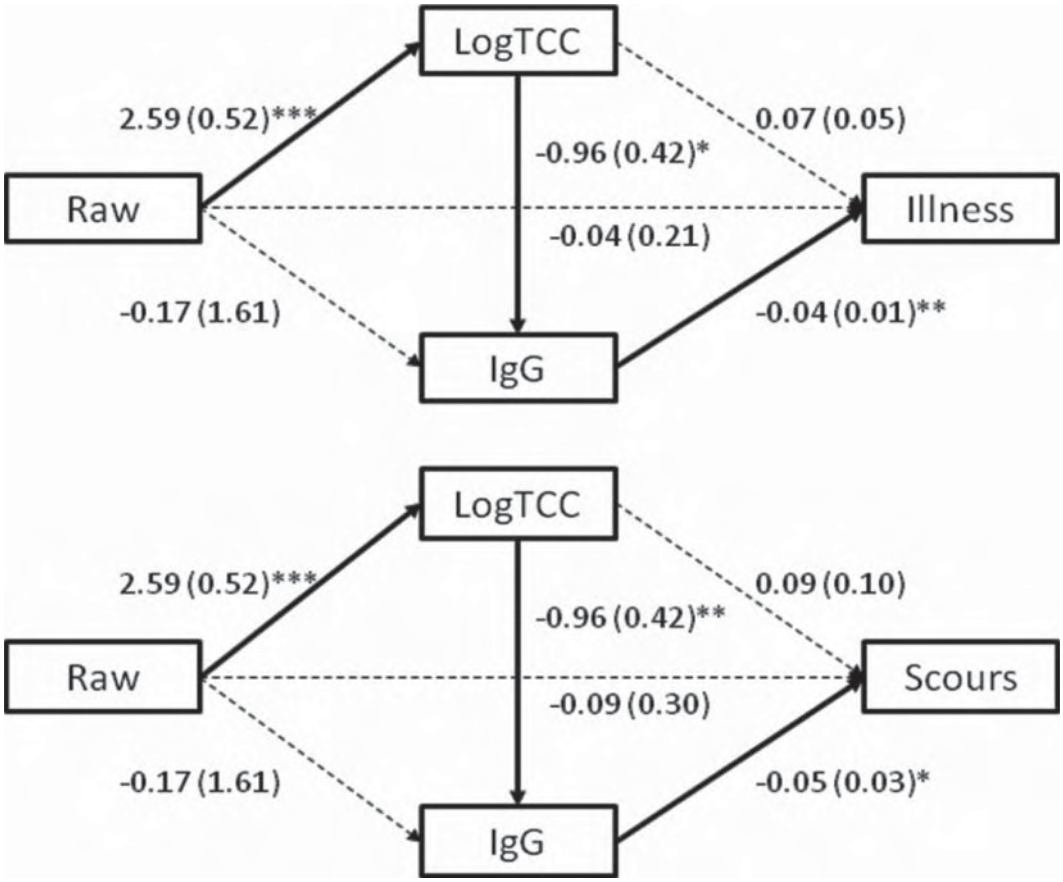

Figure 3. Final path models of feeding fresh (raw) colostrum and time-to-event outcomes as a function of total coliform count (TCC) and serum IgG. Feeding heat-treated colostrum was the referent. Values reported are the parameter estimates (SE) for the explanatory variable. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

1.32; $P = 0.0003$) compared with calves fed HT colostrum. This finding prompted the authors to complete a path analysis to identify intermediate factors that may explain the relationship between feeding HT colostrum and calf health, the hypothesis being that this was attributable to reduced bacterial pathogen exposure, enhanced serum IgG concentrations, or both. In the original work credited with originating this hypothesis, it was reported that when viable bacteria were present in the small intestine at the time of arrival of γ-globulin protein, the number of bacteria per gram of tissue was negatively correlated with globulin uptake, and that γ-globulin uptake was lowest in segments of intestine receiving live bacteria compared with segments receiving sterile inocula (James et al., 1981). This hypothesis was later supported by an observational study of 101 calves, in which calves fed raw colostrum with a high bacteria count experienced lower levels of passive transfer compared with calves fed raw colostrum with a low bacteria count (Poulson et al., 2002). However, a more recent study reported no difference in serum IgG concentrations in 10 calves fed high-bacterial-load raw colostrum (mean SPC = 5.61 $\log_{10}$ cfu/mL) compared with 10 calves fed low-bacterial-load raw colostrum (mean SPC = 3.97 $\log_{10}$ cfu/mL; Elizondo-Salazar and Heinrichs, 2009b). However, it is possible that the latter study failed to detect a relationship between colostral bacteria counts and IgG absorption due to a relatively small sample size or a relatively narrow biological range in colostrum bacterial counts for the 2 treatment groups compared.

The path analysis conducted in the current study supports the hypothesis that IgG absorption is negatively associated with colostrum bacteria counts. It was interesting that the variable TCC (and not TPC) was most strongly associated with serum IgG and, subsequently, with calf health. Although TPC and TCC were highly correlated (Pearson r = 0.85; $P < 0.0001$), TCC may have produced a better model fit to predict IgG and risk for illness because a greater proportion of the total microbial population contributing to TCC would include pathogenic coliforms, compared with TPC,

in which the pathogenic coliforms would be relatively more diluted by noncoliform bacteria. Although this hypothesis cannot be further investigated in the current study, the fact that TCC was most strongly associated with reduced serum IgG and impaired health is biologically consistent with previously proposed mechanism(s) that might explain the negative relationship between colostrum bacteria counts and IgG absorption: (1) pathogenic bacteria may be bound and neutralized by colostral immunoglobulins in the lumen of the small intestine, thereby decreasing the total mass of fed immunoglobulin available for absorption; (2) pathogenic bacteria (e.g., *E. coli*) may attach to and damage intestinal epithelial cells, thereby reducing permeability to immunoglobulin molecules; (3) damage to intestinal epithelial cells by pathogenic bacteria (e.g., *E. coli*) may result in enhanced replacement of permeable cells by cells incapable of macromolecular uptake (i.e., accelerated gut closure); or (4) nonspecific pinocytosis of bacteria could physically block absorption of immunoglobulin molecules (Corley et al., 1977; James et al., 1981). Although the mechanism(s) to explain the negative relationship between colostral bacteria counts and passive transfer of IgG requires further study, the current study shows this relationship to be real. Furthermore, this explains why calves fed HT colostrum had improved health compared with calves fed FR colostrum, because it is widely accepted that improved serum IgG status results in reduced risk for health problems in neonatal calves (Robison et al., 1988; Banks, 1993; Wells et al., 1996).

Of interest is that the path analysis suggested that the entirety of the health benefits observed in calves fed HT colostrum was mediated through the reduction in TCC and its association with improved serum IgG concentrations. Unexpectedly, the analysis did not show any additional direct association between TCC and risk for an illness event. It would be reasonable to expect that the latter relationship might still be true in some herds and under some conditions in which specific pathogenic bacteria might be shed intermittently or in disease outbreaks in fresh cows (e.g., *Salmonella* spp., *Mycoplasma* spp.). It is possible that the current study did not observe such a relationship because cows calving during the 3-mo enrollment window for the current study might not have been shedding important pathogens in their colostrum with enough frequency or concentration to cause significant calfhood diseases. Unfortunately, this hypothesis cannot be further investigated because, due to budget constraints, we were unable to culture colostrum for specific known pathogens such as *Salmonella* spp. or *Mycoplasma* spp.

One consideration requiring further study is the effect of heat treatment on viability of colostral leukocytes and the clinical significance of this to the calf. Colostral leukocytes are known to be absorbed by the neonatal intestine and reach the lymphoid tissue and circulation (Liebler-Tenorio et al., 2002; Reber et al., 2006), where they may play a role in enhancing the development of the neonatal immune system (Donovan et al., 2007; Reber et al., 2008a,b). However, studies are lacking to describe the potential clinical effect of not feeding maternal leukocytes. A recent laboratory-based study that used a prototype colostrum pasteurizer reported that the heat-treatment process significantly reduced the viability of colostral leukocytes (Koewler et al., 2010). Because the HT calves in the current study experienced reduced morbidity compared with FR calves, it must be concluded that the benefit of reducing colostrum bacteria counts outweighed the potential (and as yet unmeasured) cost of reducing the concentration of viable colostral leukocytes. Studies are needed to learn more about the role and clinical significance of colostral leukocytes to neonatal calves.

Given that calves fed HT colostrum had improved serum IgG concentrations and reduced risk for illness in the preweaning period, other longer-term benefits from feeding HT colostrum might exist. Several studies have reported that, besides the reduced risk for preweaning morbidity and mortality, additional long-term benefits associated with successful passive transfer include reduced mortality in the postweaning period, improved rate of gain and feed efficiency, reduced age at first calving, improved first- and second-lactation milk production, and reduced tendency for culling during the first lactation (Robison et al., 1988; DeNise et al., 1989; Wells et al., 1996; Faber et al., 2005). Furthermore, because MAP can be shed in the colostrum of subclinically infected cows, another potential benefit of feeding HT colostrum could be reduced risk for transmission of MAP (Streeter et al., 1995; Godden et al., 2006; Nielsen et al., 2008; Pithua et al., 2009). We are currently in the process of investigating this hypothesis by following study animals through to the end of their third lactation to collect records of milk production and longevity, and to conduct annual testing for infection with MAP.

Implications from the current study are that producers should strive to reduce the level of bacterial contamination in colostrum fed to newborn calves. This may be achieved through a variety of management strategies, including discarding colostrum from known infected cows; not feeding pooled raw colostrum; proper udder preparation before colostrum harvest; proper cleaning and sanitation of all colostrum milking, storage, or feeding equipment; rapid chilling or freezing of stored colostrum; the use of preservatives; feeding commercial colostrum replacement products; and, the

topic of the current study, heat treatment of colostrum (Stewart et al., 2005; Godden et al., 2006; McMartin et al., 2006; Nielsen et al., 2008; Pithua et al., 2009; Donahue et al., 2012). Experts have suggested that a goal should be to harvest and feed colostrum with TPC and TCC of <100,000 and <10,000 cfu/mL, respectively (McGuirk and Collins, 2004). Even though TPC and TCC are known to be highly correlated, the results of this study suggest that if a producer were to select only one parameter to monitor, then periodic culture of colostrum for TCC might be a better predictor of disease. Furthermore, although consistently achieving a TCC <10,000 cfu/mL ($\log_{10} = 4.0$) would be a reasonable goal for many producers (McGuirk and Collins, 2004) (the mean $\log_{10}$ TCC in FR colostrum from the current study was 4.7), the fact that we observed a negative linear relationship between TCC and serum IgG suggests there is no optimal cutpoint for TCC: the lower the better.

CONCLUSIONS

Calves fed HT colostrum had significantly higher serum IgG concentrations and were at significantly lower risk for treatment for scours and treatment for any illness in the preweaning period compared with calves fed FR colostrum. Path analysis illustrated that calves fed HT colostrum were at lower risk for illness because the heat-treatment process caused a significant reduction in colostrum TCC, which was associated with a reduced risk for illness as a function of improved serum IgG concentrations.

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Colostrum Management for Dairy Calves

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KEYWORDS

• Calf • Colostrum management • Passive immunity • Monitoring

KEY POINTS

- Colostrum management is the single most important management factor in determining calf health and survival.
- Although good progress has been made in the past 20 years, there remains a considerable opportunity for many dairy producers to improve their colostrum management practices, resulting in improved short-term and long-term health and performance of the animals.
- Producers should provide calves with a sufficient volume of clean, high-quality colostrum within the first few hours of life.

INTRODUCTION

The syndesmochorial placenta of the cow separates the maternal and fetal blood supplies, preventing in utero transmission of protective immunoglobulins (Ig) (**Fig. 1**).[1] Consequently, the calf is born agammaglobulinemic and so is almost entirely dependent on the absorption of maternal Ig from colostrum after birth. Achieving early and adequate intake of high-quality colostrum is widely recognized as the single most important management factor in determining the health and survival of neonatal calves.[2–4] The absorption of maternal Ig across the small intestine during the first 24 hours after birth, termed passive transfer, helps to protect the calf against common disease organisms until its own immature immune system becomes functional. In addition to reduced risk for preweaning morbidity and mortality, additional long-term benefits associated with successful passive transfer include reduced mortality in the postweaning period, improved rate of gain, reduced age at first calving,

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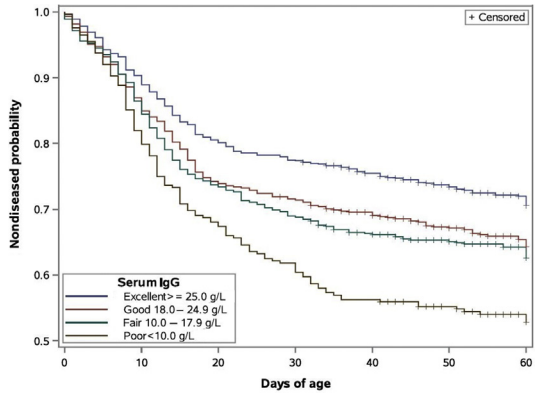

Fig. 1. Nondiseased probability for preweaned heifer calves by days of age and serum IgG concentration categories. Corresponding with serum IgG levels of greater than or equal to 25.0 g/L, 18.0 to 24.9 g/L, 10 to 17.9 g/L, and less than 10.0 g/L were serum total protein categories of greater than or equal to 6.2 g/dL, 5.8 to 6.1 g/dL, 5.1 to 5.7 g/dL, and less than 5.1 g/dL, and Brix score categories of greater than or equal to 9.4%, 8.9% to 9.3%, 8.1% to 8.8%, and less than 8.1%, respectively.[131]

improved first and second lactation milk production, and reduced tendency for culling during the first lactation.[5–8] Benefits from colostrum may be attributed to protective Ig as well as high levels of nutrients and bioactive compounds that stimulate postnatal growth and development.[9]

Calves have historically been defined as having failure of passive transfer (FPT) if the serum IgG concentration is less than 10 g/L when sampled between 24 and 48 hours of age, based on increased mortality risk below this threshold.[10–12] However, this definition of FPT needs to be reevaluated, given that recent studies have described reduced morbidity in calves to be associated with incrementally higher serum IgG levels (**Fig. 1**).[4,12,13] Although the US dairy industry has shown steady improvement in colostrum and calf management over the past few decades, a recent national dairy study reported FPT to affect 15.6% of calves tested,[14] indicating a need for continued efforts to improve colostrum management. This article reviews the process of colostrogenesis and colostrum composition, and discusses the key components of developing a successful colostrum management program. In addition, it discusses methods for monitoring and presents new goals for passive immunity in dairy herds.

COLOSTROGENESIS AND COLOSTRUM COMPOSITION

Bovine colostrum consists of a mixture of lacteal secretions and constituents of blood serum, most notably Ig and other serum proteins, which accumulate in the mammary gland during the prepartum dry period.[15] This process begins several weeks before calving, under the influence of lactogenic hormones including prolactin, and ceases abruptly at parturition. Important constituents of colostrum include Ig, leukocytes, growth factors, hormones, nonspecific antimicrobial factors, and nutrients. Concentrations of many of these components are greatest in the first secretions harvested after calving (first milking colostrum), then decline steadily over the next 6 milkings (transition milk) to reach the lower concentrations routinely measured in saleable whole milk (**Table 1**).[15]

Table 1
Composition of colostrum, transition milk, and whole milk of Holstein cows

	Colostrum	Transition Milk (Milking Postpartum)		
Parameter	**1**	**2**	**3**	**Milk**
Specific gravity	1.056	1.040	1.035	1.032
Total solids (%)	23.9	17.9	14.1	12.9
Fat (%)	6.7	5.4	3.9	4.0
Total protein (%)	14.0	8.4	5.1	3.1
Casein (%)	4.8	4.3	3.8	2.5
Albumin (%)	6.0	4.2	2.4	0.5
Immunoglobulins (%)	6.0	4.2	2.4	0.09
IgG (g/100 mL)	3.2	2.5	1.5	0.06
Lactose (%)	2.7	3.9	4.4	5.0
IgGF-I (μg/L)[9]	341	242	144	15
Insulin (μg/L)[9]	65.9	34.8	15.8	1.1
Ash (%)	1.11	0.95	0.87	0.74
Calcium (%)	0.26	0.15	0.15	0.13
Magnesium (%)	0.04	0.01	0.01	0.01
Potassium (%)	0.14	0.13	0.14	0.15
Sodium (%)	0.07	0.05	0.05	0.04
Chloride (%)	0.12	0.1	0.1	0.07
Zinc (mg/100 mL)	1.22	—	0.62	0.3
Manganese (mg/100 mL)	0.02	—	0.01	0.004
Iron (mg/100 g)	0.20	—	—	0.05
Copper (mg/100 g)	0.06	—	—	0.01
Cobalt (μg/100 g)	0.5	—	—	0.10
Vitamin A (μg/100 mL)	295	190	113	34
Vitamin D (IU/g fat)	0.89–1.81	—	—	0.41
Vitamin E (μg/g fat)	84	76	56	15
Thiamine (μg/mL)	0.58	—	0.59	0.38
Riboflavin (μg/mL)	4.83	2.71	1.85	1.47
Biotin (μg/100 mL)	1.0–2.7	—	—	2.0
Vitamin B_{12} (μg/100 mL)	4.9	—	2.5	0.6
Folic acid (μg/100 mL)	0.8	—	0.2	0.2
Choline (mg/mL)	0.7	0.34	0.23	0.13
Ascorbic acid (mg/100 mL)	2.5	—	2.3	2.2

Adapted from Foley, J.A. and D.E. Otterby. Availability, storage, treatment, composition, and feeding value of surplus colostrum: A review. J. Dairy Sci. 1978; 61:1033-1060; with permission and *data from* Hammon, H.M., I.A. Zanker, and J.W. Blum. Delayed colostrum feeding affects IGF-1 and insulin plasma concentrations in neonatal calves. J. Dairy Sci. 2000; 83:85-92.

Immunoglobulins

IgG, IgA, and IgM account for approximately 85% to 90%, 5%, and 7%, respectively, of the total Ig in colostrum, with IgG_1 accounting for 80% to 90% of the total IgG.[16]

Although levels are highly variable among cows, one study reported that mean colostral concentrations of IgG, IgA, and IgM were 75 g/L, 4.4 g/L, and 4.9 g/L, respectively.[17] IgG, and IgG_1 in particular, is transferred from the blood stream across the mammary barrier into colostrum by a specific transport mechanism; receptors on the mammary alveolar epithelial cells capture IgG_1 from the extracellular fluid, and the molecule undergoes endocytosis, transport, and eventually release into the luminal secretions.[16] The alveolar epithelial cells cease expressing this receptor, most likely in response to increasing prolactin concentrations, at the onset of lactation.[18] Smaller amounts of IgA and IgM are largely derived from local synthesis by plasmacytes in the mammary gland.[16] Although not well understood, colostral transfer of IgE also occurs and may be important in providing early protection against intestinal parasites.[19] After absorption into the calf's circulation, the duration of passive immunity from maternal Ig is highly variable and depends to a great extent on the total mass of Ig consumed and absorbed within the first 24 hours of life. The rate of decay of colostral antibodies can be influenced by multiple factors, including active viral infections or vaccination.[20–22]

Maternal Leukocytes

Fresh colostrum contains leukocytes of maternal origin; in cattle, macrophages and lymphocytes (mononuclear cells) make up the largest proportion of maternal colostral leukocytes.[23] Maternal colostral leukocytes enter the tissues of neonates following ingestion or enteral delivery in a variety of species, including rats, sheep, swine, and cattle,[24–26] and feeding colostrum containing maternal leukocytes has been associated with modified neonatal immune responses.[27–31] Blood mononuclear cells from calves fed colostrum containing maternal leukocytes developed the ability to activate cell-mediated immune responses by the time calves were 1 week of age, compared with 3 weeks of age for calves fed leukocyte-free colostrum.[27] Significant differences in percentage and degree of blood mononuclear cell activation were measured in calves receiving colostrum containing maternal leukocytes, compared with calves fed leukocyte-free maternal colostrum or frozen colostrum.[29–31] Both freezing[28] and heat treatment (Godden, unpublished, 2010) of colostrum kill most if not all colostral leukocytes. Blood mononuclear cells from 1-day-old calves fed colostrum containing maternal leukocytes were significantly more responsive to bovine viral diarrhea virus, compared with day-old calves that received frozen colostrum or leukocyte-free colostrum.[28] In contrast, there was no difference between treatment groups in the response to a mycobacterial antigen that the calves' dams had not encountered, suggesting that antigen-specific responses measured in a calf following ingestion of maternal colostral leukocytes are related to specific immune memory in the dam. In support of this, cell-mediated immune responses in piglets that nursed maternal colostrum containing leukocytes were significantly higher if their dams had been vaccinated against the tested antigen than if their dams had not been vaccinated.[32] Although research has not evaluated the degree of difference in responses induced by colostral leukocytes from a calf's own dam versus colostral leukocytes from another cow, cross-fostering experiments in piglets suggest that effects of colostral leukocytes on neonatal cell-mediated immunity are greatest when the colostrum contains leukocytes from the neonate's dam.[33]

Although multiple studies have confirmed that colostral leukocytes modify immune responses in calves in ways that seem relevant to protective immunity, to date research has not clearly shown an unequivocally beneficial effect of colostral leukocytes on practical outcomes such as calf respiratory or enteric morbidity, or induction of specific and measurable protective immunity following vaccination. Colostral

leukocytes fed alone are not sufficient to protect calves from fatal disease in the neonatal period,[34] and recent studies comparing proportions of calves affected by naturally occurring diarrhea or respiratory disease after calves consume fresh maternal colostrum containing leukocytes, or frozen colostrum from their own dam[31] or other cows,[35] have shown small or variable differences in disease between the groups. Regarding the effect of colostral leukocytes on vaccine responses, Meganck and colleagues[36] evaluated humoral and cell-mediated responses to tetanus toxoid vaccination at 2, 5, or 10 days of age in calves fed pooled colostral whey with maternal leukocytes added, or calves fed only pooled colostral whey; this work suggested that colostral leukocytes influenced both tetanus toxoid–specific cell-mediated and humoral responses in calves, but the number of calves tested was small, and the effects measured varied substantially for calves vaccinated at 2, 5, or 10 days of age. Langel and colleagues[37] evaluated total (ie, not antigen-specific) monocyte and lymphocyte responses by measuring relative numbers and activation state of calf blood mononuclear cell subsets after routine calfhood vaccination; these investigators found significant differences between groups at certain time points over the months following vaccination. However, the clinical relevance of these differences for immunity against specific pathogens, or resistance to disease, was not defined. In summary, colostral leukocytes modify calf immune responses, and these effects may affect cow health and immunity months or years later. However, to date, effects of colostral leukocytes on practically important health outcomes have not been unequivocally identified, which may in part because it is logistically challenging and expensive to conduct research to measure effects of colostral leukocytes on calf immunity and health, so trials to date may not have included enough calves to provide adequate statistical power to identify small but important health differences.

Nutrients and Nonnutritive Factors

In addition to Ig for passive immunity, colostrum also contains high amounts of nutrients and nonnutritive biologically active factors that stimulate maturation and function of the neonatal gastrointestinal tract (GIT).[9] The total solids content (percentage) in first milking colostrum and whole milk in Holstein cows was reported to average 23.9% and 12.9%, respectively (see **Table 1**). Much of the increase in colostrum solids content is attributed to a more than 4-fold increase in protein content of colostrum versus milk, this being caused by significant increases in both Ig and casein content.[2] The crude fat content of first milking Holstein colostrum (6.7%) is also significantly higher than for milk (3.6%).[15] Energy from fat and lactose in colostrum is critical for thermogenesis and body temperature regulation. Certain vitamins and minerals, including calcium, magnesium, zinc, vitamin A, vitamin E, carotene, riboflavin, vitamin B12, folic acid, choline, and selenium, are also found in increased concentrations in bovine colostrum.[15,38]

Nonnutritive factors found in increased levels in colostrum include, but are not limited to, growth factors, hormones, cytokines, and nonspecific antimicrobial factors. Trypsin inhibitor, a compound found in colostrum in concentrations nearly 100 times greater than in milk, serves to protect Ig and other proteins from proteolytic degradation in the intestine of the neonatal calf. Bioactive components with antimicrobial activity include lactoferrin, lysozyme, and lactoperoxidase.[39–41] Oligosaccharides may provide protection against pathogens by acting as competitive inhibitors for the binding sites on the epithelial surfaces of the intestine.[38] It has also been suggested that certain oligosaccharides in colostrum may contribute to gut microbiome development by serving as a substrate to beneficial microorganisms such as *Bifidobacterium*, although this hypothesis requires further study.[42]

Growth factors in bovine colostrum include transforming growth factor beta-2, growth hormone, and insulin. Colostral insulinlike growth factor I and II may be key to regulating development of the GIT of bovine neonates, including stimulation of mucosal growth, brush-border enzymes, intestinal DNA synthesis, and increased villus size, resulting in enhanced absorptive capacity and glucose uptake.[9,43]

Another intriguing and potentially beneficial factor found in high levels in colostrum may be microRNAs (miRNAs). MiRNAs are short, noncoding RNA molecules that can regulate gene expression at the posttranscriptional level, and could represent one possible method of postnatal signaling from the mother to the neonate. Although studies are needed to describe their functional significance in calves, early research in other species suggests that, once absorbed by the neonate, MiRNAs from colostrum may be important in the differentiation and functional development of the intestinal epithelium,[44] and could also play an important role in the maturation of the neonate's immune system.[45]

These nutrients and nonnutritive factors, combined with benefits of disease protection from Ig, may contribute to the short-term and long-term benefits from improved colostrum intake, including improved rate of gain, reduced age at first calving, improved first and second lactation milk production, and reduced tendency for culling during the first lactation.[5–8] Further research is needed to investigate the concept of epigenetic programming or imprinting effects of colostrum on both short-term and long-term health and performance.[9]

COMPONENTS OF A SUCCESSFUL COLOSTRUM MANAGEMENT PROGRAM

To achieve successful passive transfer, calves must consume a sufficient mass of IgG in colostrum, and then successfully absorb a sufficient portion of IgG into their circulation. In order to achieve acceptable passive transfer (APT) in greater than or equal to 90% of calves fed, using the traditional definition of APT (serum IgG >10 g/L), it has been estimated that a minimum of 150 to 200 g of IgG needs to be delivered to the calf shortly after birth. In order to achieve the more ambitious goals for excellent passive transfer, presented later in this article, the authors estimate that producers need to deliver greater than or equal to 300 g of IgG shortly after birth. The 2 major factors affecting the mass of Ig consumed are the quality and volume of colostrum fed. Factors affecting the absorption of Ig molecules into circulation include the quickness with which the first colostrum feeding is provided after birth, bacterial contamination of colostrum, and metabolic status of the calf. This article next discusses these key factors, strategies for minimizing bacterial contamination of colostrum, the use of colostrum supplements (CSs) and replacers, benefits of multiple feedings, and benefits of extended colostrum or transition milk feeding after intestinal closure.

FACTORS ASSOCIATED WITH COLOSTRUM QUALITY AND YIELD

Although it is recognized that colostrum contains a wide spectrum of important immune and nutritional components, the concentration of IgG in colostrum has traditionally been considered the hallmark for evaluating colostrum quality, with high quality defined as IgG levels greater than 50 g/L. Colostrum IgG levels can vary dramatically among cows; in an observational study that tested 2253 colostrum samples from 104 farms in 13 states, mean colostral IgG level was 74.2 g/L, with the 5th and 95th percentiles ranging from 24.9 to 130.2 g/L. A total of 77.4% of samples had colostrum IgG level greater than 50 g/L.[46] Factors affecting colostrum quality and yield are reviewed next and methods for testing colostrum quality are discussed.

Breed

Comparative studies have reported that there can be a breed effect on colostrum quality.[47,48] In one study, Holstein cows produced colostrum with total Ig content (5.6%) that was lower than for Guernsey (6.3%), Brown Swiss (6.6%), Ayrshire (8.1%), or Jersey (9.0%) breed cows.[48] Breed differences could be caused by genetics and/or dilutional effects.

Age of Dam

Most, but not all, studies report a tendency for older cows to produce higher-quality colostrum, presumably /because of older animals having had a longer period of exposure to farm-specific pathogens.[46,49,50] In a study by Shivley and colleagues,[46] colostrum from first and second lactation cows had similar colostrum quality (73.2 and 71.7 g/L of IgG), whereas colostrum from third lactation and older cows was of higher quality (83.3 g/L IgG). Producers should test and record the quality of all colostrum fed. Producers should not automatically discard colostrum from first calf heifers without first testing, because it may be of very good quality.

Nutrition in the Preparturient Period

Studies have generally reported that Ig content of colostrum is not greatly affected by restricting prepartum maternal nutrition.[51–53] Mann and colleagues[54] reported that feeding a controlled energy diet that met, but did not exceed, energy requirements during the dry period increased colostral IgG but did not affect colostrum yield, compared with diets that offered increased energy. Lacetera and colleagues[55] reported that cows supplemented with injections of selenium and vitamin E in late pregnancy produced a greater volume of colostrum than unsupplemented cows, when all cows were fed a prepartum diet that was deficient in vitamin E and selenium. Aragona and colleagues[56] reported that supplementation with nicotinic acid for 4 weeks prepartum increased IgG concentration in colostrum from 73.8 to 86.8 g/L. More research is needed to investigate whether and how nutrition of the dam during the preparturient period may affect colostrum yield and quality. Producers should feed rations balanced according to National Research Council 2001 guidelines.[57]

Season of Calving

The relationship between season and colostrum quality or volume remains unclear. Although some studies have reported that exposure to high ambient temperatures during late pregnancy is associated with poorer colostrum composition, including lower mean concentrations of colostral IgG and IgA,[49,58] others have reported the opposite.[46] It has been suggested that any negative effects of heat stress on colostrum quality might be associated with reduced dry matter intake or reduced mammary blood flow resulting in impaired transfer of IgG and nutrients to the udder.[58] Season may also have an impact on colostrum yield, although this is less well described. In a year-long study of a 2500-cow Jersey dairy in Texas, colostrum yield was highest in June but declined during the fall and winter months.[59] A low-temperature humidity index and a shortened photoperiod 1 month before and at calving were both highly correlated with reduced colostrum yield. The investigators hypothesized that shortened photoperiod may reduce colostrum production because of its impact on melatonin and prolactin, hormones known to be involved with colostrogenesis. However, a study that experimentally manipulated photoperiod reported no effect of photoperiod during the dry period on colostrum

quality or yield.[60] Producers should adopt heat-abatement strategies for prepartum cows and heifers and are advised to bank frozen colostrum to meet needs during low colostrum production months.[59]

Preparturient Vaccination of the Dam

Although vaccination is not likely to increase total IgG in colostrum, a large body of research has established that vaccinating pregnant cows and heifers during the final 3 to 6 weeks preceding calving results in increased concentrations of antigen-specific protective colostral antibodies, and increased passive antibody titers in calves of vaccinated dams, specific for some common pathogens including *Pasteurella haemolytica*, *Salmonella typhimurium*, *Escherichia coli*, rotavirus, and coronavirus.[61–64]

Dry Period Length

Cows with excessively short dry periods (<21 days) produce colostrum with lower IgG concentration.[65] Furthermore, cows with shorter dry periods produce lower yields of colostrum.[59,66] One controlled field study reported cows with a short (40-day) dry period produced 2.2 kg less colostrum than did cows with a conventional (60-day) dry period.[67]

Volume of Colostrum Produced at First Milking

Pritchett and colleagues[68] observed that cows producing less than 8.5 kg of colostrum at first milking were more likely to produce high-quality (>50 g/L) colostrum than higher-producing cows, presumably because of dilutional effects. However, more recent studies report that there is no strong predictable relationship between colostrum IgG concentration and weight of colostrum produced at first milking.[67,69,70]

Delayed Colostrum Collection

Most studies report that the concentration of Ig in colostrum is highest immediately after calving but begins to gradually decrease over time if harvest is delayed.[60,71] In an experimental study, Morin and colleagues[60] reported that colostral IgG concentration decreased by 3.7% during each subsequent hour that milking was delayed after calving, because of postparturient secretion (dilution) by the mammary glands. In another study, delaying harvest of colostrum for 6, 10, or 14 hours after calving resulted in a 17%, 27%, and 33% decrease in colostral IgG concentration, respectively.[72]

Cow-Side Testing of Colostrum Quality

It is difficult to predict, based on such factors such as visual consistency, which colostrum collected will be of high (>50 g/L IgG) versus low quality.[69] The colostrometer, a hydrometer instrument that estimates IgG concentration by measuring specific gravity, can be useful to differentiate high-quality from low-quality colostrum (specific gravity >1.050 approximates IgG >50 g/L). However, factors such as content of fat and colostrum temperature affect the hydrometer reading.[73] More recently, several studies have validated use of the Brix refractometer, an instrument that measures percentage solids in a solution, to indirectly estimate IgG level in colostrum. The Brix refractometer is less affected by temperature and more durable than the glass colostrometer. Studies have reported that a value between 18% and 23% Brix is an appropriate cut point for determining good-quality colostrum (IgG >50 g/L).[74–77] An achievable herd-level goal is to harvest high-quality colostrum (IgG $\geq$50 g/L or Brix $\geq$22%) in greater than or equal to 90% of samples tested.

VOLUME OF COLOSTRUM CONSUMED AT FIRST FEEDING

It is recommended that calves be fed 10% to 12% of their body weight (BW) of colostrum at first feeding (3–4 L for a Holstein calf). In one study, mean serum IgG level at 24 hours was significantly higher for calves fed 4 L of colostrum at 0 hours and a further 2 L at 12 hours (serum IgG = 31.1 g/L) compared with calves fed only 2 L of high-quality colostrum at 0 hours and a further 2 L at 12 hours (serum IgG = 23.5 g/L).[78] Another study reported that Brown Swiss calves fed 3.8 L (vs 1.9 L) of colostrum at first feeding experienced significantly higher rates of average daily gain and greater levels of milk production in both the first and second lactations.[8] The method of delivering colostrum deserves consideration. Suckling the dam is the least preferred approach, because delays in suckling and failure to control quality and volume ingested can result in higher rates of FPT.[79] When colostrum is delivered with an esophageal tube feeder, the esophageal groove reflex is not triggered, resulting in fluid being deposited into the forestomachs. However, this is not a significant limitation because outflow of colostrum from the forestomachs to the abomasum and small intestine occurs for the most part within 3 hours.[80] As such, equal and acceptable levels of passive transfer are achieved when colostrum is delivered by nipple bottle or esophageal tube feeder, provided that a sufficient volume of colostrum is delivered.[81,82] One study reported that calves drinking from a nipple bottle consumed an average of only 2.2 L (range, 1–4 L).[83] As such, producers feeding colostrum by nipple bottle should be prepared to deliver any remaining colostrum using a tube feeder, or provide a second bottle feeding within 6 hours, for those calves that do not voluntarily consume their whole allotment. Veterinarians should train staff on how to safely administer colostrum using tube feeders. Equipment sanitation and maintenance are important for both bottles and tube feeders.

EFFICIENCY OF ABSORPTION OF IMMUNOGLOBULINS

The term open gut refers to the unique ability of the neonatal enterocyte to nonselectively absorb intact large molecules, such as Ig, by pinocytosis.[84] From there, Ig molecules are transported across the cell and released into the lymphatics by exocytosis, after which they enter the circulatory system through the thoracic duct.[85] In a process referred to as closure, the absorption of Ig across the intestinal epithelium decreases linearly with time from birth to completely close at approximately 24 hours.[11] Factors affecting the apparent efficiency of absorption (AEA) of Ig for the first colostrum feeding are discussed here, as well as the value of extended colostrum feeding and feeding colostrum or transition milk after gut closure.

Time to First Colostrum Feeding

The efficiency of Ig transfer across the gut epithelium is optimal soon after birth, with a progressive decline in Ig absorption over time until gut closure.[86,87] Delaying the first colostrum feeding can only slightly postpone gut closure (36 hours).[88] In a study that randomized newborn calves to provide the first feeding of colostrum (7.5% BW; approximately 200 g of IgG) at different times, higher efficiency of absorption and maximum serum IgG levels were achieved for calves fed at 45 minutes of age (AEA = 51.8%; IgG = 25.5 g/L), compared with calves fed at 6 hours (AEA = 35.6%; IgG = 18.2 g/L) or 12 hours (AEA = 35.1%; IgG = 18.5 g/L).[87] Earlier feeding also resulted in more rapid bacterial colonization of the intestine with organisms such as *Bifidobacterium* spp. Producers should aim to feed all calves within 1 to 2 hours after birth.

Bacterial Contamination of Colostrum

High levels of bacteria in colostrum, and particularly coliform bacteria, may bind free Ig in the gut lumen and/or directly block uptake and transport of Ig molecules across intestinal epithelial cells, thus interfering with passive transfer.[89] Strategies to minimize bacterial contamination of colostrum are discussed next.

Metabolic Disturbances

Decreased colostral Ig absorption in the first 12 hours has been reported in calves with postnatal respiratory acidosis, associated with prolonged parturition.[90,91] Hypothermia may also be responsible for a delay in Ig absorption.[92] Although hypoxic calves may have delayed IgG absorption initially, studies have reported that there is no difference in overall absorptive capacity between hypoxic and normoxic calves, and that there is no difference in serum IgG concentrations by the time of gut closure.[93,94] Producers should provide adequate supportive care to newborns, including warming and drying calves born during cold weather, and providing supplemental heat, blankets, and deep straw bedding. Pain management, through the provision of a nonsteroidal antiinflammatory, has been shown to improve calf vigor and enhance IgG absorption for low-vigor calves following difficult calvings.[95–97]

Presence of the Dam

Ig absorption was improved when calves were housed with the dam.[98] However, considering that acceptable levels of serum IgG can be achieved without housing the calf with the dam, and given that the latter practice may increase the calf's risk of exposure to pathogens in the dam's environment, it is currently recommended that the calf be removed from the dam within 1 to 2 hours of birth and hand-fed colostrum.[3]

Value of Extended Colostrum Feeding

Although it is well recognized that maximal efficiency of absorption of IgG is achieved when the first colostrum feeding is provided within 2 hours after birth, the neonatal intestine is still permeable to IgG past 12 hours. Providing a second feeding sometime after the first postnatal meal can further increase passive transfer of IgG. In a recent study in which calves were randomly assigned to be fed a second feeding (5% BW) of either colostrum, a 1:1 colostrum/milk mixture, or milk at 12 hours of age, calves achieved a higher maximum serum IgG concentration if they were fed either colostrum (30 g/L) or mixture (25.0 g/L) at the second feeding, compared with milk (22.4 g/L).[99]

Value of Feeding Colostrum or Transition Milk After Gut Closure

Feeding colostrum after the gut has closed still offers benefits, even though Ig absorption no longer occurs. One benefit may be that bioactive compounds, such as hormones or oligosaccharides, may stimulate development of the GIT.[42,100] In one recent study, calves that were transitioned directly onto milk after the first colostrum meal had less overall gastrointestinal mass and less development of villi in the small intestine compared with calves fed either colostrum or transition milk for the first 3 days of life.[100] This improved GIT development could be beneficial for nutrient absorption and gut health. Another benefit may be local protection of the GIT by colostral antibodies. Challenge studies and field trials have reported health and growth benefits from supplementing the milk diet with colostrum for the first 14 days of life. One controlled field trial that added 70 g of colostrum powder containing 10 g of IgG into milk replacer twice daily for 14 days reported improved growth, reduced diarrhea

days, and reduced antimicrobial use in treated calves.[101] In another field trial, supplementation of milk replacer with 150 g of bovine colostrum powder containing 32 g of IgG, for the first 14 days, resulted in reductions in diarrhea, respiratory disease, umbilical enlargement, and antibiotic therapy in treated dairy calves.[102] Producers feeding pasteurized whole milk are encouraged to include transition milk in the pool.

STRATEGIES FOR REDUCING BACTERIAL CONTAMINATION OF COLOSTRUM

Although it is an important source of nutrients and immune factors, colostrum can also represent one of the earliest potential exposures of dairy calves to infectious agents, including *Mycoplasma* spp, *Mycobacterium avium* subsp *paratuberculosis*, and *Salmonella* spp.[103,104] Furthermore, high levels of bacteria in colostrum may interfere with Ig absorption.[89] A negative association between colostrum bacteria levels and Ig absorption has been described in several studies.[105–107] Fresh/raw colostrum fed to calves should contain less than,100,000 colony-forming units (cfu)/mL total plate count (TPC) and less than 10,000 cfu/mL total coliform count.[3] However, bacteria levels in colostrum frequently exceed these goals in dairies. In an observational study that tested 827 colostrum samples from 67 farms in 12 states, almost 43% of samples had TPC greater than 100,000 cfu/mL and 17% of samples had greater than 1 million cfu/mL.[104] Strategies for minimizing bacterial contamination of colostrum are discussed next.

Preventing Contamination During Colostrum Harvest, Storage, and Feeding

Producers should avoid feeding colostrum from known infected cows (eg, Johne disease) and should avoid pooling raw colostrum. Contamination during colostrum harvest, storage, or feeding processes can be reduced by properly cleaning and sanitizing udders before harvesting colostrum; milking into a clean, sanitized bucket; and transferring colostrum into clean, sanitized storage or feeding equipment.

Minimizing Bacterial Growth in Stored Colostrum

Bacteria can multiply rapidly if colostrum or milk is stored at warm ambient temperatures. Unless colostrum is to be fed right away, it should be frozen or refrigerated within 1 hour after collection. Colostrum may be frozen for up to 1 year, provided repeated multiple freeze-thaw cycles do not occur. When thawing frozen colostrum, producers should avoid overheating colostrum (avoid temperatures >60°C) or some denaturation of Ig can occur.[108] Options for storing fresh colostrum include refrigeration with or without the use of US Food and Drug Administration–approved preservatives such as potassium sorbate (0.5% final solution in colostrum). In one study, average bacterial counts in raw refrigerated colostrum reached unacceptably high levels (TPC >100,000 cfu/mL) after 2 days of refrigeration. By comparison, average colostrum TPC remained less than,100,000 cfu/mL for 6 days of refrigeration when colostrum was preserved with potassium sorbate.[109]

Heat-Treated Colostrum

Although pasteurization at higher temperatures can damage Ig, colostrum may be safely heat treated (HT) using a lower-temperature, longer-time approach (60°C [140 F] for 60 minutes), maintaining IgG levels and fluid characteristics while eliminating important pathogens, including *E coli*, *Salmonella enteritidis*, and *Mycoplasma bovis*, and significantly reducing risk of exposure to *M avium* subsp. *paratuberculosis*.[108,110,111] Calves fed HT colostrum have improved efficiency of IgG absorption, presumably caused by reduced bacterial interference with IgG

absorption.[105,112] In a field study of 1071 newborn calves in 6 Midwest dairy herds, calves fed HT colostrum had higher serum IgG level (18.0 g/L) and reduced risk for diarrhea (30.9%) compared with calves fed fresh colostrum (15.4 g/L; 36.5%).[106] Possibly contributing to these health benefits, Malmuthuge and colleagues[113] reported that feeding HT colostrum enhanced GIT colonization with *Bifidobacterium* but reduced colonization with *E coli* within the first 12 hours. If refrigerated in a clean covered container, the shelf life of HT colostrum is at least 8 days.[114] Goals for bacteria levels in HT colostrum are TPC less than 20,000 cfu/mL and coliform count less than 100 cfu/mL, respectively.

USE OF COLOSTRUM SUPPLEMENTS OR REPLACEMENT PRODUCTS

Although feeding high-quality, clean maternal colostrum is considered the gold standard, the use of high-quality CSs or colostrum replacements (CRs) may be attractive to producers for a variety of reasons, including availability, consistency, convenience, and as a means of breaking the transmission cycle of pathogens such as *M avium* ssp. *paratuberculosis*.[115] Supplements typically contain less than or equal to 60 g of IgG per dose and are intended to supplement (not replace) existing colostrum. There is no added benefit of feeding CS if already feeding 3 to 4 L of high-quality maternal colostrum.[116] By comparison, CR products are designed to completely replace maternal colostrum. They should provide a minimum of 100 g of IgG per pack and should also provide sufficient levels of nutrients to the calf to support metabolic needs in the first day of life. In Canada and the United States, CS and CR products may be licensed through the Canadian Food Inspection Agency, Canadian Center for Veterinary Biologics (Ottawa, ON), or through the US Department of Agriculture (USDA) Center for Veterinary Biologics (CVB; Ames, IA), respectively. In addition to other requirements, licensed products must originate from bovine colostrum; must be processed using accepted protocols to guarantee efficacy, safety, purity, and potency (minimum IgG content); and every serial made for sale and distribution must be tested for purity and potency.[117,118] Many products that are not CVB-licensed are produced in the United States, using a variety of manufacturing techniques, and with Ig sources including spray-dried bovine colostrum, milk, whey, bovine serum, or plasma. Nonlicensed products are not legally able to claim to supply IgG or to purport to be used for the prevention of FPT, although their use for this purpose is widespread in the United States.

A major consideration when feeding CR products is delivering an adequate dose of IgG to the calf. Many products provide only 100 to 150 g of IgG per pack, although some products provide label directions that suggest feeding increased masses of IgG, at the discretion of the producer. Although not true of all products, studies have shown that several commercially available CR products, when administered at a high enough IgG mass (150–200 g of IgG) within a few hours after birth, can provide acceptable serum IgG concentrations when using a conventional goal for APT (eg, $\geq$90% of calves with serum IgG $\geq$10 g/L).[119–122] However, if producers hope to achieve the more ambitious goals for passive transfer that are proposed in relation to monitoring, the authors suggest that they may need to deliver at least 300 g of IgG in a CR product. Research is required to investigate this hypothesis. Apart from dose, there can also be differences among CR products in Ig absorption, with studies generally reporting greater AEA percentage for lacteal-derived CR compared with serum-derived or plasma-derived CR.[123,124] Because of variable performance among products, veterinarians should review results of peer-reviewed controlled trials when recommending CR products to producers.

ON-FARM MONITORING AND GOALS FOR PASSIVE TRANSFER

A dairy's colostrum management program is one of very few processes in the animal health world that can be easily evaluated and should be routinely reviewed by veterinarians. Although serum IgG measured via radial immunodiffusion (RID) assay is considered the gold standard for evaluating passive transfer in calves,[11] it is expensive and generally requires that samples be tested at a laboratory. Other analytes, such as serum total protein (STP), have been extensively validated, are easily measured at the farm level, and are more economical than measuring IgG directly.[125,126] STP levels in healthy calves should be evaluated from blood samples collected from 24 hours after the first colostrum feeding to 10 days of age.[127] The earlier in this sampling window that samples are collected, the more accurately the results reflect true IgG absorption and the less likely it is for results to be influenced by IgG distribution/decay or dehydration. The use of a standard optical refractometer to measure STP or an optical or digital Brix refractometer, both of which are field friendly, is becoming more common. Optical refractometer values of 5.0 to 5.5 g/dL and Brix readings of 8.1% to 8.5% have been used as the cutoff for FPT.[3,128–131]

The individual calf standard for FPT (serum IgG <10 g/L) has been used for more than 35 years and is mainly based on a decreased risk of mortality when values are greater than or equal to 10 g/L.[7,10,12] Although strategies to evaluate colostrum management programs have traditionally been based on the individual calf standard, McGuirk and Collins[3] proposed sampling a minimum of 12 healthy calves and defined a successful program as one in which 80% of calves had an STP value of 5.5 g/dL or higher. From a study by Calloway and colleagues,[128] Tyler proposed (Personal Communication, 2002) that a successful passive transfer program was one in which 90% of sampled calves test 5.0 to 5.2 g/dL or higher.[132] However, one concern with this approach to setting goals includes the notion that "failure" should be used to describe calves with no measurable IgG, whereas "adequate" does not convey whether an optimal amount of IgG has been absorbed by the calf. In addition, a single cutoff that expresses failure versus adequate passive transfer is too simplistic, because it fails to recognize that increasing concentrations of IgG or STP are associated with reducing morbidity risk and improved calf performance. Studies by Furman-Fratczak and colleagues[13] and Windeyer and colleagues[12] showed that dairy calves with serum IgG levels greater than or equal to 15 g/L and STP greater than or equal to 5.7 g/dL, respectively, experienced lower rates of respiratory disease. In beef calves, Dewell and colleagues[133] reported lower morbidity rates when serum IgG level was greater than or equal to 24 g/L. Based on these and other studies, including the USDA National Animal Health Monitoring System's Dairy 2014 study,[4,46] a reevaluation of the FPT individual and herd-based cut points was conducted. A group of calf experts from the United States and Canada convened in 2018 to review and propose revised individual and herd-based evaluation standards. The proposed consensus standard is based on the association of lower morbidity and higher values of serum IgG, because mortality risk is associated with serum IgG values less than 10 g/L. The proposed standard includes 4 categories: excellent, good, fair, and poor. These categories can be applied to individual calves and to the operation for herd-based evaluation based on the percentage of calves that should be represented in each category (**Table 2**). Because serum IgG level is not commonly measured, equivalent STP and Brix levels are provided for the 4 categories. The proposed consensus standard is meant to set higher goals for calf health in the US dairy industry.

Producers feeding CR products should be aware that the relationship between STP and serum IgG can vary dramatically for calves fed different CR products, depending

Table 2
Proposed categories for immunoglobulin G levels and equivalent total protein and Brix measurements, and percentage of calves recommended in each category

Proposed Categories	Proposed IgG Levels (g/L)	Equivalent STP Levels (g/dL)	Equivalent Serum Brix Levels (%)	Proposed Calves in Each Category (%)
Excellent	≥25.0	≥6.2	≥9.4	>40
Good	18.0–24.9	5.8–6.1	8.9–9.3	~30
Fair	10.0–17.9	5.1–5.7	8.1–8.8	~20
Poor	<10.0	<5.1	<8.1	<10

on manufacturing techniques, the Ig source, level of inclusion, and level of absorption of Ig and non-Ig proteins. As such, the STP and Brix cut points suggested for monitoring passive transfer in calves fed maternal colostrum are frequently inaccurate for calves fed CR. Veterinarians are encouraged to use STP or serum Brix measures to monitor the effectiveness of a CR feeding program only if independently conducted studies are available describing the relationship between STP or serum Brix measures and serum IgG for the specific commercial CR product in use on the farm. If this information is not available for specific CR products, veterinarians are advised to periodically submit frozen serum samples for laboratory analysis of IgG using direct methods such as RID.

SUMMARY

Colostrum management is the single most important management factor in determining calf health and survival. Although good progress has been made in the past 20 years, there remains a considerable opportunity for many dairy producers to improve their colostrum management practices, resulting in improved short-term and long-term health and performance of the animals. Producers should provide calves with a sufficient volume of clean, high-quality colostrum within the first few hours of life. Additional benefits may be captured by providing multiple feedings and by extended feeding of colostrum or transition milk after gut closure. Colostrum replacers are useful tools if clean, high-quality maternal colostrum is not available. Ongoing monitoring helps producers to more quickly identify and correct problems within the colostrum management program.

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Symposium review: Colostrum management and calf nutrition for profitable and sustainable dairy farms*

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ABSTRACT

Multistate dairy management research project NC-2042, which is part of the National Institute of Food and Agriculture, has devoted an ongoing objective to calf and heifer nutrition and management. Within this objective, colostrum research has been a priority due to continued opportunities to improve this area on US dairy farms. Research has focused on heating colostrum to reduce bacterial populations and pathogens while increasing IgG absorption. Research also identified other proteins that are reduced when heating colostrum. Studies indicated an apparent upper limit to IgG absorption from colostrum fed to calves. Additional studies have shed light on absorption of IgG from colostrum replacers and evaluated the use of lactoferrin and sodium bicarbonate in both maternal colostrum and colostrum replacers. Milk replacer formulation, feeding strategies, and the effect of calfhood nutrition on future performance have also been researched. Finally, water quality and its effect on calves and heifers have been studied. This review focuses on research done by multistate research project NC-2042 member states in the area of colostrum and calf nutrition and management.
Key words: dairy calf, colostrum, milk replacer

HEAT TREATMENT OF COLOSTRUM

Colostrum is a most important aspect of dairy calf health and management and is a critical item for all dairy farms to use. A survey of colostrum management practices (Kehoe et al., 2007b) showed that although colostrum composition on dairy farms in 2006 was similar to that in previous years (Foley and Otterby, 1978), the variation found on Pennsylvania farms was extremely large. Several samples clearly were transition milk and not colostrum, with ranges of 2.0 to 26.5% fat, 7.1 to 22.6% protein, and 11.8 to 74.2% IgG observed (Kehoe et al., 2007b). The most troubling aspect of the survey was the bacterial analysis of colostrum samples (Houser et al., 2008), which showed very high coliform and standard plate counts in 51% of samples. This and the increased awareness of colostrum as a transmission pathway for Johnne's disease led to many studies related to heating colostrum. Elizondo-Salazar et al. (2010) and Godden et al. (2006) studied the time and temperature requirements for reducing bacteria numbers while preserving viscosity and IgG levels in colostrum. They concluded that 60°C and 30 min (Elizondo-Salazar et al., 2010) to 60 min (Godden et al., 2006) of batch-process heating were optimal for colostrum. Heat treatment generally reduced colostrum IgG levels by approximately 5%, but it was noted that blood IgG levels were markedly improved when feeding this product. It was determined that the apparent efficiency of absorption (**AEA**) of Ig was the key issue related to improved concentrations of IgG in calves (Elizondo-Salazar and Heinrichs, 2009b). Several studies that followed were consistent in implicating lower bacterial populations in heat-treated colostrum as the underlying factor improving IgG absorption (Elizondo-Salazar and Heinrichs, 2009a; Gelsinger et al., 2014). A critical paper then proved that it was a reduction in bacteria and not some other element of the heat-treating process that was improving IgG absorption (Gelsinger et al., 2015). This study (Gelsinger et al., 2015) used a large number of calves fed from a single batch of pooled colostrum that had either high or low bacteria levels and was either unheated or heat-treated. Results showed that bacteria were the reason for reduced IgG absorption in colostrum with high bacterial counts and that the improved IgG absorption from heat-treated colostrum was due to lower bacteria counts rather than

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an intrinsic effect of heat treatment. Additional studies with heat-treated colostrum demonstrated that heat treatment may not increase IgG levels or AEA when initial colostrum quality is very high and bacteria levels are low (Gelsinger and Heinrichs, 2017; Saldana et al., 2019a). A further study also looked at heat treatment and its effects on reducing immunity-related proteins in the young calf (Gelsinger and Heinrichs, 2017). A total of 62 proteins found in colostrum samples differed in abundance by more than 2-fold in heat-treated (for 30, 60, or 90 min) samples compared with the unheated control. The majority of proteins affected by heat treatment were involved in immunity, enzyme function, and transport-related processes (Tacoma et al., 2017). Although heat treatment of colostrum is an excellent means to reduce bacteria and increase IgG absorption, it is not without issues that should be understood.

Additives and Colostrum Replacer

Several studies have evaluated colostrum replacer (**CR**) feeding and the use of additives to enhance IgG uptake in calves. Shea et al. (2009) evaluated feeding 1 or 2 doses (105 g of IgG/dose) of a lacteal-based CR with or without supplemental lactoferrin (**LF**) to 80 Holstein bull calves. Lactoferrin has been shown to improve intestinal development and immunity in mice and humans (Shah, 2000; Zhang et al., 2001). Calves fed 2 doses of CR had greater serum IgG ($P = 0.005$) and AEA at 24 h ($P < 0.0001$). These results were likely due to more IgG being provided in the 2-dose treatment. Lactoferrin addition resulted in a negative quadratic response for AEA and xylose area under the curve. Xylose absorption tests are a means of determining intestinal development. These data suggest that LF affected intestinal absorption of IgG. A similar study was conducted using maternal colostrum; no benefits of added LF on IgG uptake, AEA, or xylose absorption were observed in that experiment (Connelly and Erickson, 2016). Heating colostrum in the manufacture of CR could have reduced the LF in CR. Paulsson et al. (1993) showed that heating LF from 60 to 100°C resulted in 10 to 100% denaturation of LF.

The diet fed to the prepartum dam can positively or negatively affect colostral IgG and other components in colostrum, along with the ability for the calf to absorb Ig and nutrients from colostrum. Feeding prepartum cows a negative-DCAD diet may reduce the ability of the calf to absorb IgG (Joyce and Sanchez, 1994). This may be due to the neonate suffering from metabolic acidosis. To test this hypothesis, adding $NaHCO_3$ to CR was evaluated to determine whether the absorption of IgG was enhanced. Prepartum Holstein cows were placed on diets with a DCAD of −100 mEq/kg or +77 mEq/kg, and calves received lacteal-based CR (198 g of IgG) with or without $NaHCO_3$ to increase the pH of the CR to 7.0. Results indicated that a negative-DCAD diet did not affect IgG absorption, but adding $NaHCO_3$ increased IgG uptake by 23% (serum IgG of 16 g/L vs. 13 g/L for treatment and control, respectively; Morrill et al., 2010). To determine the optimum dose of $NaHCO_3$ to add to CR, in a follow-up study 52 calves were fed CR containing 0, 15, 30, or 45 g/d $NaHCO_3$ (Cabral et al., 2011). Results indicated a tendency for IgG to decrease in plasma IgG as $NaHCO_3$ increased ($P = 0.08$). A linear reduction ($P < 0.05$) in AEA and area under the curve for IgG was observed. The results appeared to be primarily driven by the greatest amount of $NaHCO_3$, possibly indicating metabolic alkalosis in those calves. These results were contrary to the earlier results of Morrill et al. (2010). A major difference between these studies was that Morrill et al. (2010) fed CR in 2 feedings, whereas Cabral et al. (2011) fed CR in a single feeding. Therefore, Cabral et al. (2012) fed CR in 1 or 2 feedings to 40 calves, with calves receiving either 0 or 30 g of $NaHCO_3$ in a randomized block design. An interaction for number of feedings and $NaHCO_3$ was observed ($P = 0.02$) for 24-h IgG concentration. Calves fed CR once with $NaHCO_3$ had 7% greater IgG compared with calves fed once without $NaHCO_3$. These data indicated that in calves fed a single feeding of CR, supplementing 30 g of $NaHCO_3$ improved IgG absorption. In another study to investigate IgG uptake in calves fed CR with or without $NaHCO_3$, 80 newborn calves were fed CR once or twice with or without $NaHCO_3$ and milk replacer (**MR**) in the same day with or without $NaHCO_3$. Results indicated that every calf fed $NaHCO_3$ had reduced blood IgG, possibly due to the dams being fed an alkalotic diet resulting in the calves fed $NaHCO_3$ having metabolic alkalosis (Cabral et al., 2014). Results were similar to an earlier study in which 45 g of $NaHCO_3$ added to CR caused a negative linear effect on blood IgG concentration at 24 h (Cabral et al., 2011).

One study was conducted to evaluate the use of $NaHCO_3$ in maternal colostrum. Results indicated no benefit of adding 30 g of sodium bicarbonate ($NaHCO_3$) to high-quality maternal colostrum (82.05 ± 8.45 g/L IgG) with regard to IgG uptake or AEA by calves (Chapman et al., 2012).

Influence of the Dam on Colostrum IgG Content

Other studies attempted to predict colostrum IgG content from information about previous lactation performance and weather (Cabral et al., 2016). Data from

111 cows from 9 Holstein dairy farms in New Hampshire were used to predict colostrum quality from cows with at least 1 previous lactation. Previous production of milk and components along with component content, SCS, parity, and predicted transmitting abilities from DHI data were used. Weeks on pasture during the dry period, previous lactation fat content, previous lactation total protein yield, and predicted transmitting ability for milk had negative effects on the prediction of colostrum quality, whereas previous lactation fat yield, previous lactation protein content, parity, and days during the 3 wk immediately prepartum with the environmental temperature >23°C had positive effects on the prediction of colostrum quality (Cabral et al., 2016). The model was validated using 27 colostrum samples from 9 dairy farms. Data from DHI, local weather stations, and time from calving to colostrum harvest were collected. All data were subjected to the variance inflation factor assessment (SAS version 9.4; SAS Institute Inc., Cary, NC). For each iteration, the highest value was removed until all variables entering the model were ≤10. It was determined that the best fit was when data were converted to natural logarithms, after which all variables are evaluated using the backward elimination procedure until all remaining variables had $P \leq 0.10$. Results indicated that the model modestly predicted ($R^2 = 0.56$) colostrum IgG concentration.

Additional studies investigated how nutrition of the dam prepartum may affect colostrum quality or IgG absorption by the calf. Nicotinic acid may enhance microbial protein synthesis, which may have positive effects on colostrum quality. Aragona et al. (2016) fed either 0 or 48 g of nicotinic acid/d to 26 multiparous Holstein cows for 4 wk prepartum and observed similar colostrum yield but about 18% greater IgG content in colostrum from cows supplemented with nicotinic acid. Another potential strategy to improve colostrum is utilizing probiotics and enzymes during the dry period to increase intake and result in more nutrients available for colostrogenesis. Ort et al. (2018) fed 36 multiparous Holstein cows no additives, a direct-fed microbial (**DFM**) containing *Enterococcus faecium* (1.323 billion cfu/g) and *Saccharomyces cerevisiae* (45.4 g/d), or a combination of DFM, cellulase, and amylase for 3 wk prepartum. Results indicated no benefit on colostrum IgG concentration or yield or any effect on calves fed the colostrum. Colostral fat yield was reduced ($P = 0.05$) for supplemented cows, whereas IgA was reduced for cows fed the DFM ($P = 0.04$). Nicotinic acid supplementation to prepartum cows improved colostrum quality, whereas DFM and enzymes did not affect the colostrum. These studies demonstrate that many additives may not affect colostrum IgG concentration.

Milk Feeding Potentially Affecting Future Production

Cooperators in the dairy management research project also have researched feeding frequency and the amount fed to calves. In the area of feeding frequency, feeding 2 or 4 times per day was compared using traditional 20% CP, 20% fat replacers or 26% CP, 18% fat products (Kmicikewycz et al., 2013). Offering the low-protein diet at 1.5% of BW or the high-protein diet at 2.0% of BW had no effect on growth or feed efficiency to 42 or 56 d. Using whole-milk diets, Kehoe et al. (2007a) found that starter intake was increased when feeding once versus twice daily for calves weaned at 4, 5, or 6 wk. Body weight and withers height were the same for all groups regardless of weaning age or number of times fed per day with starter and water offered free choice at all times. The same effect of no difference in weight gain or structural growth was observed when Saldana et al. (2019b) fed calves whole milk either once or twice daily at the same rate per day for all calves. These studies show that management changes can be made to minimize labor with no detrimental consequences on calf growth. Frequency of feeding milk or MR was not related to calf grow in any of these studies.

The management question of the amount of liquid feed offered to calves and its effect on future milk production has also been addressed by NC-2042 stations. An early report based primarily on abstracts, meeting proceedings, unpublished data sets, and personal communication (Soberon and Van Amburgh, 2013) led to interesting conclusions and prompted much research in this area. Using 11 peer-reviewed studies, Gelsinger et al. (2016) published a meta-analysis of these experiments. The meta-analysis showed that every 100 g of gain/d during the preweaning period yields 150 kg of milk during the first lactation. By accounting for energy and protein intakes from milk or MR and grain, the authors showed that 19.6% of this first-lactation milk production variation was related to milk feeding amounts and 80.4% was related to increased grain intakes. However, an important highlight of the study was that ADG during the milk feeding portion of the animal's life accounted for only 2.3% of the variation in first-lactation milk production. A further large study by Chester-Jones et al. (2017) using 2,880 individual animal observations and milk and grain intakes up to 8 wk also showed small but significant improvements in first-lactation milk, fat, and protein production when calves consumed more energy and protein from grain intake. In another large study, Rauba et al. (2019) collected MR and starter protein intake, ME intake, and growth data from birth to 195 d for 4,534 Holstein heifer calves born from 2004 to 2014 on 3 commercial

dairies. First-lactation production data were available for 3,627 of these animals. Protein and ME intake from starter affected growth more than the same nutrients from MR, which was fed at a conventional level for a high percentage of calves. Starter protein and ME intake during the first 6 and 8 wk had a positive relationship with first-lactation 305-d milk, fat, and protein production.

The effect of grain intake on future milk has also been shown in a large management study that looked at calf factors related to 305-d first-lactation production (Heinrichs and Heinrichs, 2011) and showed that health events and grain intake at weaning were the 2 largest calfhood factors related to first-lactation milk production.

Finally, Van De Stroet et al. (2016) summarized a large group of calf studies to look at the effects of young calf growth on production parameters in the first lactation. Results showed that calf growth during the preweaning period was related to future potential. The shortest calves (based on hip height) had lower milk production potential and were most likely to be removed from the herd before first lactation. In addition, weaned calves with medium or tall hip height had a greater potential for milk production than short calves across all lactations.

MR Feeding

Guindon et al. (2015) studied reducing weaning stress in calves fed a high-protein MR at high levels. Calves were fed either a 20% CP, 20% fat MR (454 g of powder/d; 4 L/d; **Conv**) or a 28% CP, 20% fat MR (916 g/d from d 2 to 8 and 1,134 g/d from d 9 to 41; **HP**). Calves fed HP consumed more MR as expected, more water ($P = 0.007$), and less starter ($P < 0.006$). The HP-fed calves gained more ($P = 0.01$) weight and were more efficient ($P = 0.007$) compared with the Conv calves during the preweaning period. During weaning week, Conv calves consumed more starter ($P = 0.003$) and less MR than HP calves ($P < 0.001$). No other differences were detected among the treatments. The increase in starter intake in the Conv-fed calves continued into the postweaned week ($P = 0.08$). Results of this study indicated that calves fed HP gained at a greater rate, consumed more water, and were more efficient preweaning, but these differences went away during the weaning week and postweaning.

Chapman et al. (2017a) fed calves 446 g of a 20% CP, 20% fat MR (**Con**), 680 g/d of a 26% CP, 18% fat MR (**Mod**), or 908 g/d of the Mod treatment (**AAG**). During wk 5, all calves were fitted with a urinary catheter and urine was collected for 3 d. Simultaneously, nutrient digestibility was determined using chromium oxide as an external marker. As in the study of Guindon et al. (2015), calves responded by consuming less starter as MR increased ($P = 0.01$), and ADG ($P = 0.002$) and feed efficiency ($P = 0.02$) increased as MR feeding rate increased. Nitrogen efficiency was 15.3% greater in the Con treatment compared with the other treatments ($P = 0.01$). Calves fed the Mod and AAG treatments produced more urine (1.13 and 2.23 kg/d more, respectively, than Con calves; $P < 0.001$) and excreted more N in their urine than Con calves (12.1, 17.0, and 18.2 g/d for Con, Mod, and AAG, respectively; $P = 0.02$). Calves fed the Con program had less percentage N in their feces than calves fed the AAG treatment (3.93% vs. 5.21%; $P = 0.02$). Using calves from the same study (Chapman et al., 2017b), on d 50 calves received an intravenous dose of deuterium oxide (300 mg/kg) to develop a new means of determining body composition evaluating the decay of deuterium over the subsequent week and analyzing blood using nuclear magnetic resonance spectroscopy. Results indicated that using nuclear magnetic resonance is a valid method for determining body composition, and there were no differences among treatments with regard to body composition during the last week of the experiment.

Alternative Proteins for MR

In the past 25 yr, as human demand for dairy proteins has created competition for high-quality proteins (Thornsberry et al., 2016), all-milk protein MR formulas have replaced casein or skim milk with whey protein, whey protein concentrate, delactosed whey, or whey protein isolates. The fluctuating costs of MR have increased interest in cheaper alternatives. Other plant and animal protein options include modified wheat such as hydrolyzed wheat gluten protein, blood plasma proteins (**PL**), egg, and peptide protein powder (**PP**), although antinutritional factors and lower AA profiles plus decreased digestibility are a concern (Raeth et al., 2016). The NC-2042 group has evaluated many alternative proteins in MR. Thornsberry et al. (2016) summarized alternative protein studies at the University of Minnesota. Many of the studies used a 20% CP, 20% fat MR fed at 0.57 kg/d for 35 d and half this amount from d 36 to weaning at 42 d. Studies offered water and 18% CP (as-fed) calf starter free choice. Typically, up to 50% of milk proteins were replaced by plant sources. Three University of Minnesota study examples were implemented between 2004 and 2014 (Raeth et al., 2016). The 100% all-milk protein (20% CP, 20% fat) MR was compared with 30% wheat gluten (**WG**), 50% WG, 50% soy protein concentrate (**SPC**), and a 1:1 ratio of 50% WG and SPC. In one study, the alternative protein MR reduced preweaning ADG compared with

the control. The intake of calf starter was not compromised by MR protein sources. A second study replaced all-milk whey protein concentrate with 50% WG 1:1 with PL, 50% SPC and PL, or 50% from PL, WG, and SPC and fed as in previous studies. Calf performance across diets was not different. In the third study, whey protein concentrate in MR was partially replaced by 25% PL, 25% PP, or 25% PL and PP. Milk replacer was fed at 0.68 kg in 4.76 L of water twice daily for 35 d and once daily with half the amount from d 36 to 42. No differences occurred across MR treatments with comparable growth and health. Frame growth and mortality standards were met for all MR treatments.

Water Preferences of Young Calves

A final area of calf management studied has been drinking water preferences by calves. Senevirathne et al. (2016) evaluated the drinking preference of young calves for reverse-osmosis water (**RW**), municipal city water (**MW**), or untreated well water. Preference was greatest for RW, with MW a close second. However, a follow-up study comparing RW and MW showed similar health yet less water intake of RW with similar animal growth (Senevirathne et al., 2018).

The work done in colostrum management, heat treatment, and use of supplements and additives has been important and helpful to dairy farmers. Showing that milk feeding amounts and frequency of feeding have minimal effects on first-lactation performance and the importance of grain feeding and MR ingredients is economically valuable for dairy farmers. As new developments in nutrition and management arise, researchers will continue to study best nutrition and management for dairy calves for profitable and environmentally efficient dairy farms.

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The effect of various heat-treatment methods on colostrum quality, health and performance of dairy calves

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ABSTRACT. To investigate the effect of feeding heat-treated colostrum at different duration on the health and performance, 48 Holstein calves were enrolled randomly into four treatment groups before first feeding and consumed untreated colostrum (H0, n = 12), heat-treated colostrum at 60ºC for 30 min. (H30, n = 12), heat-treated colostrum at 60ºC for 60 min. (H60, n = 12) and heat-treated colostrum at 60ºC for 90 min. (H90, n = 12). Blood samples were collected for analyses of IgG and protein profile at 0, 6, and 24h of age. The colostrum sample from treated and untreated batches and feces sample from each calf also were taken. The results showed heat-treatment of colostrum at 60ºC for 60 (p = 0.03) and 90 min. (p = 0.01) reduced total bacterial count, while colostral IgG concentration maintained up to 60 min. Serum total protein (p = 0.02), IgG concentrations (p = 0.03), and apparent efficiency of IgG absorption (p = 0.02) were significantly greater at 6 and 24h in calves that were fed heat-treated colostrum (H90) compared to calves fed unheated colostrum (H0). General health status of calves that were received heat-treated colostrum was better and the prevalence of diarrhea-induced pathogens was lower than calves were fed unheated colostrum. In conclusion, the consumption of heat-treated colostrum had a positive effect on health, growth characteristics, and performance of calves during the suckling period.

Keywords: IgG; growth; health; Holstein calf; heat-treating; suckling period.

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Introduction

The newborn calf is born into a world laden with challenges it must overcome in order to grow and develop into a healthy, productive adult. 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 transmission of Ig in utero (Elizondo-Salazar & Heinriches, 2009; Constable, Hincheliff, Done, & Gruenberg, 2016; Godden, Lombard, & Woolums, 2019). Thereupon calf borne agammaglobulinemic and in the early days of life are highly dependent on colostrum consumption till growth, health, and future economic performance be guaranteed (Arguello, Castro, & Capote, 2005; Wheeler, Hodgkinson, Prosser, & Davis, 2007; Zakian et al., 2018). Immunoglobulins, which are highest in concentration at first milking, are critical to protect the newborn from the transmission of pathogenic infections in early life (Godden, 2008). Colostrum also contains a high concentration of nutrients including protein (much of which consists of the immunoglobulins), lipids, carbohydrates, minerals, and vitamins. Nearly all components of colostrum are present at higher concentrations compared to those measured in mature milk, with the main exception being lactose (Blum & Hammond, 2000). Inadequate or improper colostrum feeding and management cause a significant portion of the calf morbidity and mortality on dairy farms. The importance of consumption of high-quality colostrum with low microbial pollution in the critical first 24h of life is well documented (Staley & Bush, 1980; Godden 2008; Zakian et al., 2018). In addition to reduced risk for preweaning morbidity and mortality, additional long-term benefits associated with successful passive transfer include reduced mortality in the postweaning period, improved rate of weight gain, reduced age at first calving, improved first and second lactation milk production, and reduced tendency for culling during the first lactation (Wells, Dargatz, & Ott, 1996; Faber, Faber, McCauley, & Ax, 2005).

Although it is an important source of nutrients and immune factors, colostrum can also represent one of the earliest potential exposures of dairy calves to infectious agents, such as *Mycobacterium avium subsp paratuberculosis* (Streeter, Hoffsis, Bech-Nielson, Shulaw, & Rings, 1995; Stabel & Goff, 2004), *Salmonella* spp. (Houser, Donaldson, Kehoe, Heinrichs, & Jayarao, 2008), *Escherichia coli* (Farber, Sanders, & Malcolm, 1988; Steele et al., 1997) *Listeria monocytogenes* (Farber et al., 1988; Doyle et al., 1987) and *Campylobacter* spp. (Lovett, Francis, & Hunt, 1983) that can cause infections that may directly via colostrum or during the time of collection and storage colostrum to be disposed and transferred to neonates. Microbial contamination and lower colostrum immunoglobulin G [IgG ⩽ 50 g L^{-1}] are two important factors which affecting the quality of bovine colostrum; and if contaminated colostrum consumed for calf feeding, colostrum bacteria bonded with free Ig in the small intestine and IgG cannot absorb as well as reducing the apparent efficiency of absorption (AEA) (James, Polan, & Cummins, 1981; Morrill et al., 2012).

A dairy's colostrum management program is one of the very few processes in the animal health world that can be easily evaluated and should be routinely reviewed by veterinarians. One of the best methods for reduction or elimination of infectious pathogens is heat-treatment (McMartin et al., 2006; Godden 2008). A disadvantage of this method is the potential heat denaturation of colostral immunoglobulins and other essential constituents which could increase calf morbidity and mortality rates. Previous studies showed colostrum heating at 60°C as safe and without affecting at immunoglobulin G concentration of colostrum and colostrum viscosity (Godden et al., 2006; McMartin et al., 2006), but still, about the most effective duration of this treatment process (30, 60, 75, 90 or 120 min.) there are many discussion and disagreement (Godden et al., 2006; McMartin et al., 2006; Elizondo-Salazar, Jones, & Heinrichs, 2010).

Recently, there is a growing body of literature that recognises the importance of failure of passive transfer of immunity (FPTI) in the management of the dairy cow industry. The current hypothesis suggests that the presence of pathogen in gastrointestinal tract at the time of colostrum consumption could interfere with the absorption of Ig biomolecule (Staley & Bush, 1985). To our knowledge, there is a lack of published data on the difference between feeding heat-treated colostrum and unheated to calves and the effect of heat-treated colostrum consumption on growth, health and performance of dairy calves in pre-weaned period (Elizondo-Salazar & Heinriches, 2009; Rafiei, Ghoorchi, Toghdory, Moazeni, & Khalili, 2019). Hence, the specific objective of this study was to determine the effects of feeding heat-treated colostrum at different time duration (30, 60, and 90 min.) on apparent efficiency of Ig absorption, growth, health, and skeletal development of Holstein calves. In addition, the second purpose of this investigation is to explore the effects of heat-treatment at different time duration on colostrum composition, bacterial contamination, and pathogen viability at the on-farm situation.

Material and methods

Farm selection and herd management

The present research was conducted in summer season at Fazeel, a large dairy farm in the Isfahan province, Iran where 3,321 Holstein cows were milked two times a day with a herd average of 12107 Liters milk (305-day mature equivalent (305ME) fat, and protein yields: 3.3 ± 0.33% and 3.2 ± 0.14%, respectively). Geographical conditions of the study period were included ambient air temperature 29.23 ± 5.68°C, and relative humidity 26.33 ± 3.72. The study was confirmed according to the guidelines of the Iranian Council on Animal Care [ICAC] (1995). Cows were kept in free-stall barns and were fed a total mixed ration with free access to fresh water. When parturition was imminent, cows were moved from free stalls into shared maternity pens, which were bedded with dry straw and supervised 24hours per day.

Colostrum harvesting, batch preparation, and heat-treatment

First milking colostrum of 50 multiparous Holstein (Dry period: 80 ± 13.46 day) during the month before starting study were collected and 240 liters colostrum were stored in 60 sterile bags with 4 liters volume frozen at -20°C. Colostrum was thawed at 15-20°C and thoroughly hemogenized together into a plastic tub with 300-liter capacity and gently shaken for 2 min via the sterile plastic applicator. Then, hemogenized colostrum was divided into 4 batches of 60 L; unheated batch (3 × 20 L; H0), and three batches of colostrum

heat-treated at 60°C for 30 min. (3 × 20 L; H30), heat-treated at 60°C for 60 min. (H60; 3 × 20 L) and heat-treated at 60°C for 90 min. (H90; 3 × 20 L) by an on-farm colostrum batch pasturizator (20 liters capacity; Shir-mak Company, Isfahan, Iran). It should be noted pasteurizers were programmed to heat colostrum to a target temperature of 60°C for 30, 60 and 90 min., with a maximum allowable fluctuation of 0.71 ± 0.02ºC during the 30 min., 0.84 ± 0.02ºC during 60 min. and 0.96 ± 0.03ºC during 90 min. holding phase. The colostrum was then automatically cooled to 37ºC (approximately 20 minutes for each heating cycle) ready for feeding to calves. Times and temperatures were reported by the digital display on the equipment during the heat-treatment process and were recorded each pasteurization cycle and time-dependent temperature change of pasturizator at 30, 60, and 90 min. observed completely. Colostrum was agitated during the entire heat-treatment process.

Calf enrollment, experimental design, feeding and weaning protocol

Forty-eight female calves were immediately removed from the maternity pen after birth and transferred into individual calf units from birth to weaning (bedded with fresh wood-shavings), 2 m apart from each other, and then dipped navels with 7% (vol:vol) iodine. Calves eligible for inclusion in the study were born from multiparous cows with similar body condition score (3.5 on the scale of 1-5; according to the method of Edmonson, Lean, Weaver, Farver and Webster, 1989) at calving time, without dystocia and obvious congenital abnormalities based on clinical examination by farm veterinarian. Male calves and twins were excluded from enrollment. The animals were assigned randomly into 4 experimental groups with 12 animals each. For the first and second feeding, 3 and 2 L of colostrum prepared as described above (H0, H30, H60, and H90) was fed by 2 and 12h of age, respectively. From the second day of experiment to weaning, the milk was supplied as follows: calves were fed 3.0 L of milk twice daily from day 2 to 10±2, then received 4.0 L of milk twice daily from day 10 ± 2 to 40 ± 2, then received 5.0 L of milk twice daily from day 40 ± 2 to 65 ± 2, then received 2.0 L of milk in the morning from day 65 ± 2 to 70 ± 2 and were weaned from milk at day 70 ± 2. All milk was from the farm's hospital herd and pasteurized before being fed to the calves at 63°C for 30 min. Calves were offered starter grain (Table 1) and water (20-25°C) *ad libitum* from day 2 to 70 ± 2.

Table 1. Ingredients and chemical composition of the starter ration of all calves consumed in this study during suckling period.

Ingredients	(% DM)
Corn Grain, ground, dry	39
Barley Grain, ground	15
Wheat grain, ground	5
Soybean, Meal, solv.44%CP	26
Soybean, Seed, whole roasted	5
Canola Meal, mech. Extract	3
Molasses Beet	3
Calcium Carbonate	0.8
Sodium Bicarbonate	0.7
Salt	0.5
Mineral supplement[1]	1
Vitamin premix[2]	1
Chemical Analysis	
DM (% as fed)	90
Energy (Mcal Kg^{-1})	2.95
NEg (Mcal Kg^{-1})	1.35
Crude Protein (%)	20.7
EE (%)	4
Ash (%)	6.7
NDF (% DM)	13.2
ADF (% DM)	6.93
NFC* (%)	55.4

[1]Contained 4 g Mn, 65 g Ca, 4.5 g Zn, 30 g P, 44 g Mg, 60 g Na, 75 g Cl, 10.5 F, 42 g S, 10 mg Co, 1 g Cu, 24.6 mg I, and 48 mg Se per kg supplement. [2]Contained 1 300 000 IU vit A, 80000 IU vit D_3, 6600 IU vit E, 880 mg vit B_1, 850 mg vit B_2, 1740 mg vit B_5, 1345 mg vit B_5, 870 mg vit B_6, 76 mg vit B_9, 9.4 mg vit B_{12}, 13.4 mg vit H_2, and 16500 mg vit C per kg premix. *NFC = 100 – (%NDF + %CP + %ether extract + %ash).

Colostrum composition, IgG concentration, and bacterial contamination

13-mL colostrum aseptically collected from each batch in sterile 15-mL centrifuge tubes (Jet Biofil, Guangdong Co., China) for colostrum composition, IgG measurement, and microbial contamination.

The concentration of IgG (MonoScreen quantitative C-ELISA; Bio-X diagnostic Co., Belgium), and pH (Portable pH-meter, Cyber Scan, Eutech Co., Germany) were measured in duplicate and mean of each sample was recorded. To measure IgG concentration, calf serum samples were diluted 1:100, and colostrum samples were diluted 1:1000 using by dilution buffer. Briefly, in the dilution microplate wells, 100 µL of the calibration curve dilutions and diluted samples were transferred, and diluted conjugate was added to each well, mixed, and 100 µL of the content were transferred to the original microplate wells. The microplate was incubated at 21-24°C for 1h. samples between all dilution steps vortexed well. Subsequently, the microplate was rinsed 3 times with a washing solution, then 100 µL of chromatogen solution were added to each followed by incubation at 21-24°C and away from light for 10 min. The reaction was stopped by adding 50 µL of stop solution to each well. The optical density of the evaluated samples was determined using an ELISA reader and the Ig concentrations were calculated using Log/Logit computer software.

Colostrum samples were also diluted (vol:vol) using phosphate buffer solution (PBS; pH 7.4) to reduce sample viscosity and prevent technical difficulties usually encountered with undiluted and highly viscous samples (Maunsell, Morin, & Constable, 1998), then samples were analyzed for concentrations of fat and protein (milk-O-scan 133B infra-analyzer, FOSS Electric Co., Denmark).

For microbial investigation, 50 µL of 10-fold serial diluted colostrum samples were cultured into McConkey, blood agar, and Preston enrichment broth medium using an inoculating loop. Then by using biochemical tests listed in the FDA Bacteriological Analytical Manual (BAM) methods the presence or absence of viable *E. coli*, *Salmonella Enteritidis,* and *Campylobacter* spp. in suspected colonies was proved. Plates for identification *E. coli, Salmonella Enteritidis* and *Campylobacter* spp. were incubated at 37°C for 24 to 48h and 41°C, respectively for 24h. To enumerate total plate count (TPC) and total coliform count (TCC) of colostrum batches, samples were also incubated at 32 and 37°C for 48h, respectively. The overall number of bacteria was calculated for each sample and expressed in a TPC and TCC as colony-forming units per milliliter (CFU ml^{-1}).

Health, biochemical parameters, performance, and growth characteristics

All calves were evaluated daily for the respiratory score (from score 1 to 6) and the feces score (from score 1 to 4) based on Larson, Owen, Albright, Appleman and Muller (1977). Respiratory scoring of each calf evaluated based on the type of symptom, as follows: 1: normal; 2: runny nose; 3: heavy breathing; 4: cough-moist; 5: cough-dry; 6: fever accompanied with one of the above-mentioned symptoms. Fecal scoring was also performed daily based on physical appearance, as follows: 1: normal (firm but not hard); 2: soft (piles but spreads slightly); 3: runny (spreads readily to about 6 mm); and 4: watery (liquid consistency, splatters). Fresh feces sample (5 gr) of each calf was collected on sterile sample container at days 3, 7, and 14 post-birth for the total fecal aerobic bacterial count and diarrhea-induced pathogens. All fecal samples were examined for the presence of *E. coli* K99, Rotavirus, Coronavirus and *Cryptosporidium parvum* by a commercial immunochromatography rapid on-farm kit (Rainbow Calf Scour 4, Bio-X Diagnostics Co., Belgium) following manufacturer's instructions and after 10 minutes, the results were read (Klein et al., 2009). In the test red-lined strip, yellow-lined strip, blue-lined strip, green-lined strip were corresponds to Rotavirus, Coronavirus, *E. coli* attachment factor K99, and *C. parvum* respectively. To investigate fecal total aerobic bacterial, 50 µL of homogenized and diluted stool samples by PBS (pH = 7.4) cultured into nutrient agar media and incubated for 24 to 48h at 37°C; then by colony counter number of growth colony in each media counted and recorded.

For measurement of biochemical parameters, blood samples were taken by venipuncture and partially evacuated 6 mL sterile tubes without anticoagulant (BD Vacutainer Precision Glide, Becton Dickinson Co., Franklin Lakes, NJ) at 0, 6 and 24h of life. The samples were allowed to clot (15 min.) and sera was separated within one hour of collection by centrifugation at 1,800 $\times$ *g* for 15 min. The serum was harvested in a microtube (Easy-lock, 1.5 mL, FL Medical Technology, Italy) at -20ºC until evaluation. The concentrations of serum total protein (STP) and albumin using commercially available kits (Pars Azmoon Company, Iran) were measured. The concentration of globulin was calculated by subtracting total protein value from albumin, then A:G ratio was assessed. Serum IgG concentration by a commercial C-ELISA kit (MonoScreen quantitative; Bio-X diagnostic Co., Belgium) also, was measured according to the manufacturer's guidelines in duplicates and mean of each sample was recorded.

The efficiency of IgG absorption was determined by multiplying the estimated plasma volume of the calf by its serum IgG concentration and dividing this product by the mass of colostral IgG that was fed based on liter. Plasma volume at 6 and 24h was estimated to be 0.089 × BW (Quigley & Drewry, 1998), and birth BW was used to estimate BW at 6 and 24h.

To evaluate the performance of each calf in the current study, dry matter intake (DMI), feed efficiency rate (FER) and feed conversion rate (FCR) of all calves calculated from the second day of age to weaning time. Calves had their body weight (BW) recorded at birth and every ten days till weaning using by electronic on-farm scale (Sepehr-Alborz Co., Iran) and then, average daily weight gain (ADWG) and total weight gain (TWG) of each calf calculated. Growth characteristics of all calves such as body length (BL), the height of the withers (HW), height of the hip (HH), width of the hip (WH), breast circumference (BC) and abdominal circumference (AC) assessed at day 3, 15, 30, 45 and 60 of suckling period based on the method of Kolkman et al. (2010).

Statistical analyses

Of the 48 enrolled calves, 47 calves (one calf death at 10 days of age from H30 group) were survived at the end of study, and respective data were used for statistical analyses. Data were expressed as mean±standard error of the mean (SEM) and $p < 0.05$ was considered significant. Repeated measures ANOVA was used to detect the main effects of treatment, time, and the interaction between treatment and time for evaluation of skeletal growth characteristics and performance parameters of calves during the suckling period. The relative and absolute numbers of calves between different groups was compared by the Chi-square test and Fisher's exact coefficient. All analyses were carried out using statistical software programs (Statistical Analysis System [SAS], 2015). The statistical model also used was

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

where, Y_{ij} is the outcome variable (SPC, TCC, IgG, STP, ...), μ is overall mean, α_i is the effect of treatment, and e_{ij} is residual.

Results

Colostrum composition, IgG concentration, and bacterial contamination

Composition and bacterial count of colostrum batches after the various time (H30, H60, H90) at 60°C in heat-treated and unheated (H0) treatments are presented in Table 2. The concentrations of fat, protein, and F:P ratio of heat-treated colostrum batches were not shown any significant change after heat-treatment at different duration in comparison to raw colostrum ($p > 0.05$). The pH of colostrum batches after heat-treatment increased slightly (from 6.77 ± 0.003 to 6.79 ± 0.003), although this increment was not statistically significant ($p > 0.05$).

IgG concentration of H0, H30, H60, and H90 colostrum batches was equal to 67.63 ± 0.08, 66.17 ± 0.08, 63.07 ± 0.08, and 59.53 ± 0.09 g L^{-1} which indicated a significant difference between H90 compared to other batches ($p = 0.02$). Colostral IgG concentration decreased significantly ($p = 0.01$) by 2.16 ± 0.62, 6.74 ± 0.78, and 11.98 ± 1.03% when batches heat-treated for 30, 60, and 90 min., respectively.

Counts for TPC and TCC declined as time and temperature increased (Table 2) compared with bacterial counts in unheated colostrum, but after 30 min. treatment of batches at 60°C *E.coli* was destructed and not detected. Colostral TPC decreased significantly ($p = 0.02$) up to 18.59 ± 0.66, 49.36 ± 0.9, and 62.18 ± 0.11% when batches heat-treated for 30, 60, and 90 min, respectively. Besides, the TCC reduced significantly ($p = 0.04$) after heating at 60°C for 30, 60, and 90 min. to 26.15 ± 0.54, 45.67 ± 1.2, and 62.65 ± 1.03%, respectively.

Results of pathogen viability determined all batches were infected with *Salmonella enteritidis*, *E. coli,* and *Campylobacter* Spp. naturally. For all batches, *Salmonella enteritidis*, *Campylobacter* Spp. were consistently not found in any of the examined colostrum batch samples collected upon reaching the target temperature of 60°C, which indicating this organisms had not survived after the 60-min heat-up phase (Table 2). *E. coli* was consistently not detected from any colostrum batches after heating at 60°C for 30 min., which indicating the organism had not survived in colostrum samples treated for 30 min.

Table 2. The effect of different heat-treatment methods on colostrum composition, bacterial count and pathogens viability (Mean ± SEM). Totally 240 L of pooled colostrum divided to one of four treatment batches; unheated colostrum (H0; 60 L); heat-treated colostrum at 60°C for 30 min (H30; 60 L); heat-treated at 60°C for 60 min (H60; 60 L); heat-treated at 60°C for 90 min (H90; 60 L).

	Treatment			
Colostrum composition	H0	H30	H60	H90
Volume (L)	60 (3 × 20L)	60 (3 × 20L)	60 (3 × 20L)	60 (3 × 20L)
IgG (g L^{-1})	67.63 ± 0.08^{a}	66.17 ± 0.08^{a}	63.07 ± 0.2^{a}	59.53 ± 0.09^{b}
Loss of IgG (%)	-	2.16 ± 0.62^{a}	6.74 ± 0.78^{a}	11.98 ± 1.03^{b}
Fat (%)	4.15 ± 0.003	4.14 ± 0.005	4.16 ± 0.006	4.18 ± 0.003
Protein (%)	17 ± 0.06	17 ± 0.1	17.03 ± 0.07	17 ± 0.1
Fat:Pro	0.24 ± 0.001	0.24 ± 0.003	0.24 ± 0.003	0.24 ± 0.003
pH	6.77 ± 0.003	6.78 ± 0.002	6.78 ± 0.006	6.79 ± 0.003
Total palate count (log_{10} Cfu ml^{-1})	6.24 ± 0.05^{a}	5.08 ± 0.1^{a}	3.16 ± 0.07^{b}	2.36 ± 0.03^{b}
Total palate count decrement (%)	-	18.59 ± 0.66^{a}	49.36 ± 0.9^{b}	62.18 ± 0.11^{b}
Total coliform count (log_{10} Cfu ml^{-1})	5.89 ± 0.03^{a}	4.35 ± 0.23^{a}	3.2 ± 0.2^{b}	2.2 ± 0.1^{b}
Total coliform count decrement (%)	-	26.15 ± 0.54^{a}	45.67 ± 1.2^{b}	62.65 ± 1.03^{b}
Salmonella enteritidis	G	G	ND	ND
E. Coli	G	ND	ND	ND
Campylobacter Spp	G	G	ND	ND

$^{a\text{-}b}$Means within each row with a dissimilar letter(s) are significantly different from each other at 5% level ($p < 0.05$). G: growth; ND: not detected.

Health status, biochemical parameters, performance and growth characteristics

Observation of fecal and respiratory score based on every ten days and an overall score of each treatment showed calves that were fed heat-treated colostrum for 90 min. (H90) had significantly lower ($p = 0.02$) fecal score (1.37 ±0.02; firm feces) than calves fed unheated colostrum (1.56 ±0.02; soft feces) and heat-treated for 30 min. (1.59 ±0.02; soft feces) during the suckling period. Although from point of respiratory score was not found any significant difference ($p > 0.05$) between treatments. The analyses of the fecal samples with the immunochromatographic rapid test to the detection of diarrhea-induced pathogens were positive in 42.55% of the fecal samples in studied calves. In the H0 group all enteropathogens were detected as days 3, 7, and 14, but in groups of H60 and H90 prevalence of diarrhea-induced pathogens was lower (Table 3) when compared to other treatments.

Table 3. The effect of consumption of unheated (H0) and heat-treated (H30, H60, and H90) colostrum on definitive and relative frequencies of infection by various diarrhea-induced pathogens by using immunochromatography method and total aerobic bacterial count of feces during first 2 weeks of neonatal period. Group H0 received unheated colostrum; Group H30 received heat-treated colostrum at 60°C for 30 minutes; Group H60 received heat-treated colostrum at 60°C for 60 minutes; Group H90 received heat-treated colostrum at 60°C for 90 minutes.

	Day 3 (N = 48)				Day 7 (N = 48)				Day 14 (N = 47)			
	Treatment											
Infective agents	H0	H30	H60	H90	H0	H30	H60	H90	H0	H30	H60	H90
C. Parvum (%)	2 (16.66%)	1 (8.33%)	-	-	5 (41.66%)	1 (8.33%)	1 (8.33%)	-	6 (50%)	3 (25%)	1 (8.33%)	1 (8.33%)
Coronavirus	2 (16.66%)	2 (16.66)	2 (16.66%)	-	3 (25%)	3 (25%)	2 (16.66%)	1 (8.33%)	4 (33.33%)	3 (25%)	1 (8.33%)	-
Rotavirus	3 (25%)	3 (25%)	1 (8.33%)	-	4 (33.33%)	2 (16.66%)	1 (8.33%)	-	6 (50%)	2 (16.66%)	1 (8.33%)	-
E. coli K99	3 (25%)	3 (25%)	2 (16.66%)	-	1 (8.33%)	2 (16.66%)	-	-	1 (8.33%)	1 (8.33%)	-	-
Total infected	10 (20.83%)	9 (19.15%)	5 (10.42%)	-	13 (27.08%)	8 (17.02%)	4 (8.33%)	1 (4.8%)	17 (35.42%)	9 (19.15%)	3 (6.25%)	1 (4.8%)
TAB count (log_{10} cfu mL^{-1})	-	-	-	-	8.50^{a} ± 0.36	7.21^{b} ± 0.39	6.35^{bc} ± 0.39	5.97^{c} ± 0.36	9.05^{a} ± 0.41	7.13^{b} ± 0.38	7.27^{b} ± 0.38	7.10^{b} ± 0.41

$^{a\text{-}c}$:Means within each row with a dissimilar letter(s) are significantly different from each other at 5% level ($p < 0.05$). Fecal sample was not collected for bacterial count at day 3. TAB: total aerobic bacterial

Serum total protein and albumin concentrations of all calves varied from 3.6 to 5.9 and 0.09 to 2.9 mg dL^{-1}, respectively. Values above 5.00 mg dL^{-1} of STP were reached by 18 (38.3%) of these calves at hours 6 and by 45 (95.7%) at hours 12 post-birth. Nine animals (19.1%) at hours 6 and one animal (4.7%) at hours 24 had total protein values of ⩾ 5.00 mg dL^{-1}, that implying a moderately successful passive transfer. The remaining were 20 animals (42.5%) at hours 6 and one animal at hours 24 (4.7%) showed insufficient STP

concentrations (≤ 4.9 mg dl^{-1}). STP concentration of calves before colostrum consumption were relatively low and equal to 4.3, 4.21, 4.27, and 4.23 mg dL^{-1} in groups H0, H30, H60, and H90, respectively which were not statistically different ($p > 0.05$; Table 4). Calves in groups H60 ($p = 0.03$) and H90 ($p = 0.02$) had higher concentrations of STP than calves were received untreated (H0) and heat-treated colostrum (H30) at hour 6 of the experiment. The results showed from point of STP concentration were not found the difference between calves fed H0 and calves fed H30 at 6 ($p = 0.07$) and 24h ($p = 0.08$).

A negligible amount of serum IgG was found at birth (0 hour) in calves before colostrum feeding but was not found a significant difference between groups ($p > 0.05$). Feeding calves by heat-treated colostrum (H90) resulted in marked increment in serum IgG concentrations when compared to calves that were fed H30 and H60 ($p = 0.03$). Calves that were consumed heat-treated colostrum (H60) had higher levels of IgG at 6 ($p = 0.02$) and 24h ($p = 0.01$) than calves that were fed heat-treated (H30) and raw colostrum (H0).

As displayed in Table 4, the apparent efficiency of absorption (AEA) of serum IgG was calculated at 6 and 24h to assess the success of the passive transfer of immunity. Calves in group H60 had a higher ($p = 0.03$) means of AEA rates than group H0 (19.99 ± 1.06 vs 16.19 ± 0.73). Similar AEA rates were noted for group H0 at hours 6 and 24 ($p > 0.05$). However, group H30 (18.61 ± 0.84) had a lower AEA rate compared with calves in groups H60 (19.85 ± 1.06) and H90 (22.06 ± 0.9) 6 hours post colostrum consumption. Calves in group H90 had the highest AEA rate on hours 6 ($p = 0.02$) and 24 ($p = 0.02$) in the current study which was significantly different compared to other treatments.

Table 4. The effect of consumption of unheated (H0) and heat-treated (H30, H60, and H90) colostrum on serum concentrations of albumin, globulin, A:G ratio, serum total protein, IgG and AEA rate of calves at 0, 6, and 24h after colostrum feeding (Mean ± SEM). Group H0 received unheated colostrum; Group H30 received heat-treated colostrum at 60°C for 30 minutes; Group H60 received heat-treated colostrum at 60°C for 60 minutes; Group H90 received heat-treated colostrum at 60°C for 90 minutes.

	Treatment			
Parameters/Time	H0	H30	H60	H90
Albumin (mg dL^{-1})				
0	1.49^{b} ± 0.056	1.46^{b} ± 0.054	1.43^{b} ± 0.047	1.3^{a} ± 0.073
6	1.65^{c} ± 0.035	1.75^{b} ± 0.037	1.81^{b} ± 0.035	1.96^{a} ± 0.035
24	2.12^{b} ± 0.065	2.16^{b} ± 0.068	2.20^{b} ± 0.065	2.55^{a} ± 0.065
Globulin (mg dL^{-1})				
0	2.9^{bc} ± 0.1	3.03^{ab} ± 0.096	2.83^{c} ± 0.11	2.92^{bc} ± 0.11
6	3.17 ± 0.051	3.19 ± 0.053	3.22 ± 0.051	3.10 ± 0.051
24	3.09 ± 0.068	3.14 ± 0.072	3.13 ± 0.069	3.08 ± 0.068
A:G (%)				
0	0.52 ± 0.024	0.5 ± 0.027	0.52 ± 0.033	0.46 ± 0.043
6	0.53^{b} ± 0.016	0.54^{b} ± 0.018	0.57^{b} ± 0.016	0.64^{a} ± 0.016
24	0.71^{b} ± 0.037	0.69^{b} ± 0.039	0.71^{b} ± 0.037	0.83^{a} ± 0.037
STP (mg dL^{-1})				
0	4.3 ± 0.077	4.51 ± 0.084	4.27 ± 0.093	4.23 ± 0.075
6	4.83^{b} ± 0.038	4.84^{b} ± 0.042	5.07^{a} ± 0.038	5.15^{a} ± 0.038
24	5.21^{c} ± 0.043	5.28^{bc} ± 0.047	5.33^{b} ± 0.043	5.65^{a} ± 0.043
IgG (g L^{-1})				
0	0.008 ± 0.008	0.088 ± 0.07	0	0.12 ± 0.05
6	8.67^{b} ± 0.49	9.63^{ab} ± 0.52	9.68^{ab} ± 0.49	10.1^{a} ± 0.49
24	13.7^{c} ± 0.59	15.2^{bc} ± 0.62	16.2^{b} ± 0.59	18.1^{a} ± 0.59
AEA (%)				
6	16.36^{c}±1.13	18.61^{b}±0.84	19.85^{b}±1.06	22.06^{a}±0.9
24	16.19^{b}±0.73	17.9^{b}±0.96	19.99^{c}±0.84	23.32^{a}±0.57
FPTI (*n*)				
6	8 (66.6%)	6 (54.5%)	7 (58.3%)	6 (50%)
24	1 (12%)	-	-	-

[a-c]Means within each row with a dissimilar letter(s) are significantly different from each other at 5% level ($p < 0.05$).

The BW and ADWG changs of animals during the suckling period shown in Figures 1a and b. Results indicated that the consumption of heat-treated colostrum did not have any negative effect on daily and total weight gain during the suckling period, but was not found a significant difference between heat-treated and unheated groups ($p > 0.05$) at weaning time. The mean and SD of FCR at weaning time showed that in H0, H30, H60, and H90 groups were equal to 1.55 ± 0.05, 1.62 ± 0.05, 1.74 ± 0.05, and 1.64 ± 0.05%, respectively, which indicated a significant difference between heat-treated and unheated groups ($p = 0.04$), but was not found a significant difference from point of FER in various treatments

($p > 0.05$; Figures 1c and d). Also, between treatments during the suckling period from point of DMI was not seen as a statistically significant difference ($p > 0.05$). Furthermore, groups H60 and H90 have significantly higher performance in comparison to calves that were fed unheated colostrum. Skeletal growth characteristics demonstrated WH of calves that were received heat-treated colostrum at 60°C for 90 min. (23.0 ± 0.24 cm) was significantly higher ($p = 0.03$) than calves were fed raw colostrum (22.6 ± 0.24 cm) or heat-treated colostrum at 60°C for 30 min. (22.0 ± 0.24 cm), although from point of HW ($p > 0.05$), AC ($p > 0.05$), and BC ($p > 0.05$) calves in group H90 have higher growth rate at weaning time, this difference was not significant (Figures 2a, b, c, and d). The performed linear regression tests demonstrated that no significant relationship existed between serum total protein concentration and birth weight ($R^2 = 0.07$), BW ($R^2 = 0.04$), BL ($R^2 = 0.05$), BC ($R^2 = 0.06$), HW ($R^2 = 0.06$), FCR ($R^2 = 0.1$), FER ($R^2 = 0.3$) and ADWG ($R^2 = 0.06$). While, was found a significant relationship between serum IgG concentration and overall BL ($R^2 = 0.09$), BC ($R^2 = 0.08$), and HW ($R^2 = 0.08$) of studied calves during the suckling period.

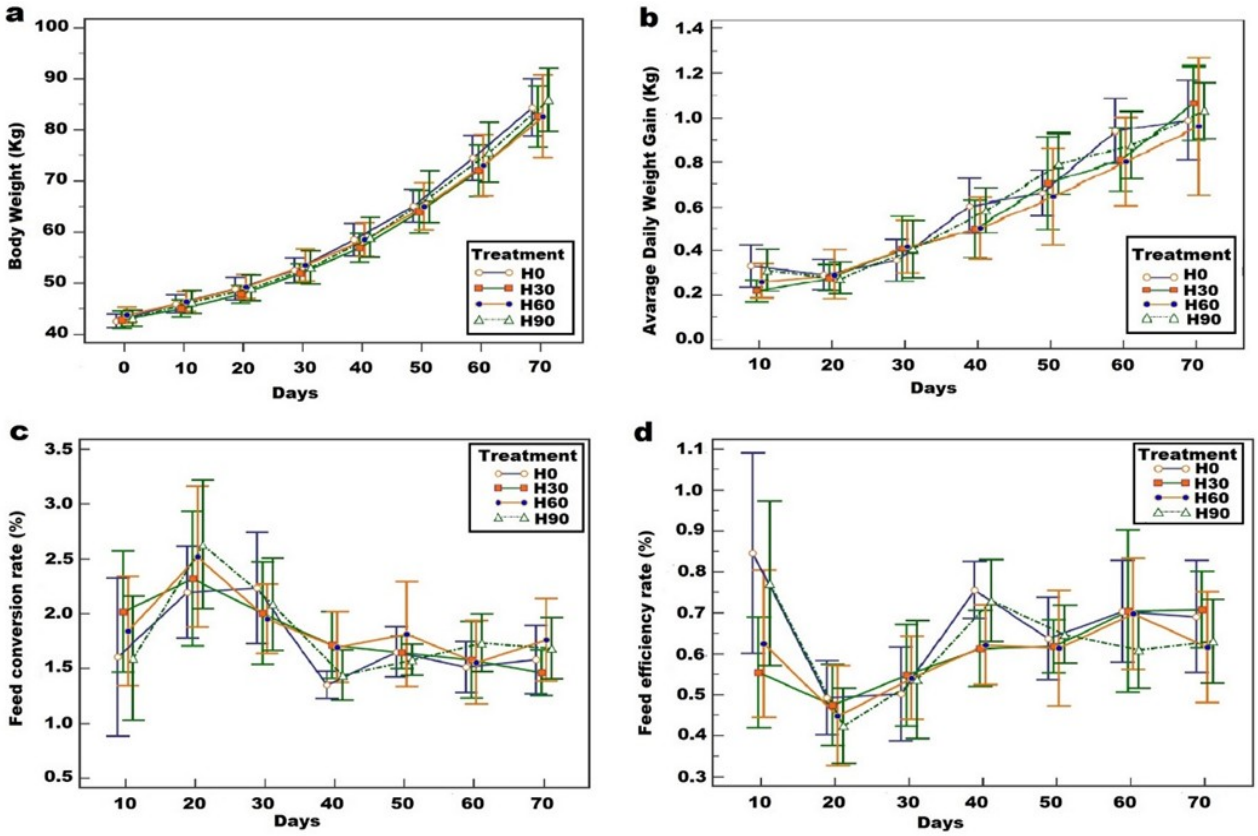

Figure 1. Calculated body weight (BW, a), average daily weight gain (ADWG, b), feed conversion rate (FCR, c) and feed efficiency rate (FER, d) of 48 Holstein calves every 10 days from birth to weaning time. Calves were randomly assigned to one of four treatment groups of 12 animals; group H0 fed unheated colostrum; group H30 fed heat-treated colostrum at 60°C for 30 min.; group H60 fed heat-treated colostrum at 60°C for 60 min.; group H90 fed heat-treated colostrum at 60°C for 90 min. Data is presented as mean ± SD.

Discussion

Colostrum composition, IgG concentration, and bacterial contamination

Results showed that colostrum heat-treatment at 60°C till 90 min. have not a significant effect on fat and protein concentrations, although IgG of the unheated batches with 67.63 g L^{-1} after 30, 60 and 90 min. heat-treatment was reduced in 2.16, 6.74 and 11.98%, respectively. The proteins of milk are probably the constituents most affected by heating (Fox & McSweeney, 1998). Typically, decreased whey protein solubility is founded when milk is heated for 40 to 60°C. Over this range, whey proteins denature due to disruption of noncovalent bonds stabilizing the secondary and tertiary structure (Pelegrine & Gasparetto, 2005). In a study, Tacoma et al. (2017) evaluated the effects of heat-treatment (at 60°C) for 0, 30, 60, and 90 min. on colostrum protein profile.

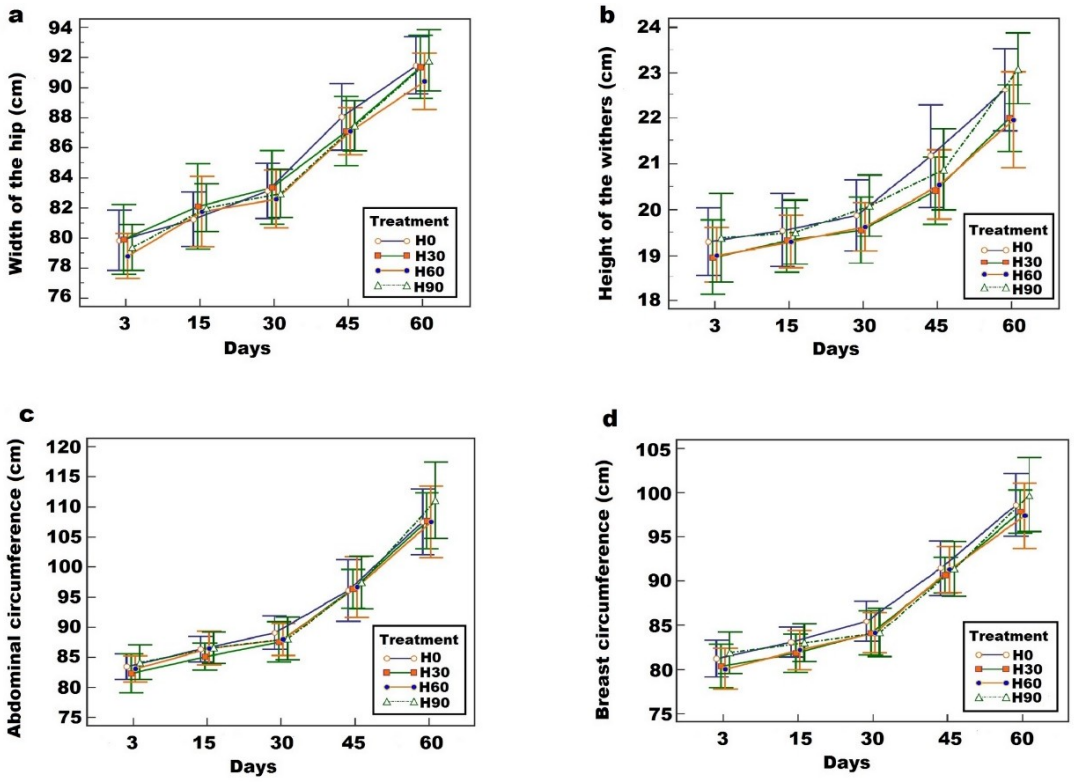

Figure 2. Calculated width of the hip (WH, a), height of the withers (HW, b), breast circumference (BC, c), and the abdominal circumference (AC, d) assessed at day 3, 15, 30, 45 and 60 of suckling period based on the method of Kolkman et al. (2010). Fourteen-eight Holstein calves were randomly assigned to one of four treatment groups of 12 animals; group H0 fed unheated colostrum; group H30 fed heat-treated colostrum at 60°C for 30 min.; group H60 fed heat-treated colostrum at 60°C for 60 min; group H90 fed heat-treated colostrum at 60°C for 90 min. Data is presented as mean ± SD.

They indicated that the length of heat-treatment changed the composition of high and low abundance proteins within bovine colostrum, and the majority of low abundance proteins affected by heat were involved in cellular and immune processes. deWit and Klarenbeek (1984) studied the effects of heat-treatment on the structure and solubility of the Ig fraction of whey. They demonstrated that Ig are among the most heat-stable whey proteins, which was attributed to the high content of disulfide bonds and whey components such as fats, lactose, carbohydrates, salts, and other proteins that help stabilize antibodies during thermal treatment (Indyk, Williams, & Patel, 2008). Fat is the main energy providing component of milk. The mean percentage of fat in colostrum is 6.7%, compared to 4.0% in mature milk (Foley & Otterby, 1978). Proteins of the milk fat globule membrane start to denature at temperatures above 70°C. This damage to the milk fat globule membrane leads to the formation of free (non-globular) fat. However, of milk's principal constituents, lipids are the least affected by heat (Fox & McSweeney, 1998).

With denaturation temperatures between 65 and 70°C (Topel, 2004), immunoglobulins (IgG, IgG, IgM, and IgA) are very thermolabile whey proteins (dewit and Klarenbeek, 1984). IgG, which accounts for 85 to 90% of the total immunoglobulin mass in bovine colostrum (Larson, Heary, & Devery, 1980), exhibits two transitions when exposed to heat. The isolated Fab fragment shows a transition at 61°C, while the Fc fragment displays a transition at 71°C, which means that these individual transitions represent the denaturation of the IgG's Fab and Fc domains, respectively (Vermeer & Norde, 2000). Donahue et al. (2012) findings indicated that when IgG change was expressed as an absolute change (mg mL^{-1}), batches of colostrum with ⩾ 70 to 79.9 mg mL^{-1} initial IgG concentration experienced a 6.7 mg mL^{-1} or 8.8% loss of IgG after heat-treatment for 60 min., and batches of colostrum with ⩾ 80 mg mL^{-1} initial IgG concentration experienced a 9.8 mg mL^{-1} or 9.8% loss of IgG after heat-treatment. Therefore, results of the present study were in agreement with Donahue et al. (2012) and Gelsinser, Jones and Heinrichs (2015) where IgG concentration of unheated batches showed a high decrement rate at 60°C for 60 min. but were in contrast to the results of Godden et al. (2006), McMartin et al. (2006), Johnson, Godden, Molitor, Ames and Hagman, (2007); Elizondo-Salazar, and Heinrichs (2009), where colostrum heat-treated at 60°C up to 120 min. was not expressed any significant change in IgG concentration.

Minimizing bacteria counts in colostrum requires the knowledge of conditions and practices that cause these elevations of counts. TPC and TCC are standard methods to survey the quality of dairy products. Fresh/raw colostrum fed to calves should contain less than 100,000 cfu mL^{-1} TPC and less than 10,000 cfu mL^{-1} TCC (McGuirk & Collins, 2004). However, goals for bacteria levels in heat-treated colostrum are TPC less than 20,000 cfu mL^{-1} and coliform count less than 100 cfu mL^{-1}, respectively (Godden et al., 2019). In an observational study that tested 827 colostrum samples from 67 farms, almost 43% of samples had TPC greater than 100,000 cfu mL^{-1}, and 17% of samples had greater than 1 million cfu mL^{-1} (Walz et al., 1997). The results of the study Donahue et al. (2012) shown heat-treatment of colostrum at 60°C for 60 min. decreased TPC by 2.25 log_{10} and TCC by 2.49 log_{10} cfu mL^{-1}. Accordingly, the results of our study indicated unheated colostrum with 6.24 log_{10} TPC and 5.89 log_{10} cfu mL^{-1} TCC after 30, 60, and 90 min. heat-treatment, was observed 18.59, 49.36 and 62.18% loss of TPC and 25.16, 45.67 and 62.65% of TCC, respectively. The findings of the present study were also verified in 4 clinical trials, each conducted on individual dairies by university personnel (Johnson et al., 2007; Elizondo-Salazar & Heinrichs, 2009; Kent-Dennis, 2014; Rafiei et al., 2019). Godden et al. (2006) reported that after 15 and 30 min. of heat treatment at 60°C, *E.coli* and *Salmonella enteritidis* were not detected at colostrum batches, respectively. Our study was in agreement with Godden et al. (2006) findings and not detected aformentioned colostrum-borne pathogens at 60°C for 60 min.

Health, biochemical parameters, performance and growth characteristics

Absorption of IgG from colostrum is higher when the gastrointestinal tract have a proper health condition and this led to reduction of diarrhea incidence (Corley, Staley, Bush, & Jones, 1977; English et al., 2007; Yang, Zou, Wu, Li, & Cao, 2015) and respiratory diseases. Elizondo-Salazar et al. (2010) indicated calves that were received heat-treated colostrum at 60°C for 60 min. have lower feces score and higher health status than calves consumed unheated colostrum at birth. The results of the study Rafiei et al. (2019) indicated the rate of incidence to pneumonia during suckling period between treatments that were fed unheated colostrum had greater rate than calves fed unheated colostrum. Our findings showed that consumption of heat-treated colostrum was not significant effect on respiratory score, while fecal score of calves that were received heat-treated colostrum (especially H60 and H90) was lower when compared to those that received unheated colostrum during suckling period. Higher IgG concentration of serum during first days of calf life resulted in general and local immunity against *E.coli*, *Rotavirus*, *Coronavirus* and *Cryptosporidium parvum* because these are important agents that led to diarrhea in neonatal ruminants (Acres, 1985; Arthington, Cattell, & Quigley, 2000; Crouch et al., 2000; Constable et al., 2016). Decreasing bacterial exposure to calves through colostrum while maintaining colostrum quality (IgG concentrations) should result is a healthier calf due to the higher AEA rate of IgG. The result of current study showed when bovine colostrum heated for up to 90 min. approximately most of the infective agents omitted from colostrum, thereupon the risk of transmitting of diarrhea-induced pathogens reduced within the first 2 weeks of age or so after and prevalence of neonatal diseases in calves of heat-treated groups was lower than those were received unheated colostrum. Returning to the question posed at the beginning of this study, it is now possible to state that colostrum heat-treatment with reduction of microbial count leads to the increment of AEA rate of Ig and health of newborn calves.

Different studies showed calves that were fed heat-treated colostrum at 60°C for 30 or 60 min. have higher STP and IgG concentrations during first 24h of age than calves fed unheated colostrum (Johnson et al., 2007, Elizondo-Salazar & Henrichs, 2009; Gelsinger et al., 2015; Rafiei et al., 2019). It is generally accepted that FTPI is indicated when a blood Ig and STP concentration is less than 10 g L^{-1} or 5.2 g dL^{-1} at 48h of age, respectively (Elsohaby & Keefe, 2015; Elsohaby et al., 2019). Tyler (personal communication, 2002) proposed that a successful passive transfer program was one in which 90% of sampled calves test 5.0 to 5.2 g dL^{-1} or higher of STP. In the current study 92, 87, 76, and 71% of calves in H90, H60, H30, and H0 had STP concentration of 5.0 g dL^{-1} or higher at 24h of age, respectively. Calves with FTPI are more susceptible to infectious diseases and have higher morbidity and mortality (Windeyer et al., 2014). Pathogenic bacteria may be bound and neutralized by colostral Ig in the lumen of the small intestine, thereby decreasing the total mass of fed IgG available for absorption. Pathogenic bacteria (e.g., *E.coli*) may attach to and damage intestinal epithelial cells, thereby reducing permeability to IgG molecules. Mean AEA rate from maternal colostrum typically averages 20 to 35% and the concentration of IgG in the colostrum may influence AEA

(Stott & Fellah, 1983; Quigley & Drewry, 1998), but in our study AEA rate was between 10 to 27.32%. Also, the AEA rate of calves in group H0 was significantly lower than calves in groups H30, H60, and H90 at hours 6, that was indicated the effects of consumption of heat-treated colostrum on IgG absorption. This finding was in agreement with other studies (Elizondo-Salazar & Henrichs, 2009; Moazeni, Rasooli, Nouri, Ghorbanpoor, & Mosavari, 2017; Rafiei et al., 2019).

Rebelein (2010) showed that calves fed raw colostrum at first feeding had an average weight gain of 0.79 ± 0.12 kg per day, while calves fed heat-treated colostrum gained 0.87 ± 0.12 kg per day. Results of Kent-Denis (2014) indicated that consumption of heat-treated colostrum at 60°C for 60 min. led to higher ADWG and total gaining at suckling period than calves fed unheated colostrum. Different studies (Elizondo-Salazar & Henrichs, 2009; Rafiei et al., 2019) confirmed that consumption of heat-treated colostrum have not a negative effect on body weight and skeletal growth of calves in the suckling period. Results of our study were in agreement and confirmed the finding of previous studies (Elizondo-Salazar & Heinrichs, 2009; Rafiei et al., 2019) and showed that consumption of heat-treated colostrum improves growth characteristics and average daily weight gain during suckling period which indicated that heat-treatment of colostrum at 60°C up to 90 min. have not any damage on essential compositions of colostrum such as hormones, growth factors, and minerals.

Conclusion

To put it in a nutshell, the current study proved that the best duration for colostrum heat-treatment was 90 min. Since it not only did not significantly reduced the pH, fat and protein contents of colostrum, but also significantly increased Alb, STP, and IgG concentrations of blood and AEA rate of calves that were fed H90. This research has thrown up many questions in need of further investigation. It would be interesting to assess the effects of heat-treatment at 60°C for 60 and 90 min. on macro and micro mineral compositions and also active immunologic substances of colostrum and serum of calves that have consumed this colostrum.

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Heat Treatment of Bovine Colostrum. I: Effects of Temperature on Viscosity and Immunoglobulin G Level

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ABSTRACT

The objective of this study was 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. 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, suggested that colostrum could be heated to 60°C for up to 120 min without changing viscosity or IgG concentration. This finding was confirmed by heating 50-mL aliquots from 30 unique batches of colostrum in an RVA for 120 min at 60 and 63°C. 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 pre-heat-treated (73.4 ± 26.5 mg/mL) and post-heat-treated (74.5 ± 24.3 mg/mL) 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 mg/mL) suffered greater losses of IgG and greater viscosity changes when heated to 63°C than did moderate quality colostrum (<73.0 mg/mL). 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 this study indicate that 50-mL volumes of bovine colostrum can be heat treated at 60°C for up to 120 min in an RVA without affecting IgG concentration or viscosity.

Key words: colostrum, pasteurization, viscosity, immunoglobulin

INTRODUCTION

Recent National Animal Health Monitoring System dairy studies have reported that an unacceptably high mortality rate (8.4 to 10.7%) exists among preweaned dairy heifers on US dairy farms (NAHMS, 1993, 1996, 2002). Wells et al. (1996) estimated that 31% of the mortality events occurring within the first 3 wk of life could be attributed to failure of passive transfer of immunity. Although the cornerstones of a successful colostrum management program have traditionally considered colostrum quality, the volume fed, and age of calf at first feeding (Davis and Drackley, 1998), experts have recently suggested that bacterial contamination of colostrum may also be important (McGuirk and Collins, 2004). Some bacterial pathogens that may be transmitted in colostrum and milk (from direct shedding from the mammary gland, postharvest contamination, or bacterial proliferation in improperly stored colostrum) include *Mycoplasma* spp. (Walz et al., 1997), *Mycobacterium avium* ssp. *paratuberculosis* (Streeter et al., 1995), *Escherichia coli* (Clark et al., 1989; Steele et al., 1997), *Salmonella* spp. (McEwen et al., 1988; Giles et al., 1989; Steele et al., 1997), *Listeria monocytogenes* (Farber et al., 1988; Steele et al., 1997), and *Campylobacter* spp. (Lovett et al., 1983; Steele et al., 1997). In one observational study of commercial dairies, Poulsen et al. (2002) reported that 82% of colostrum samples collected exceeded the industry goal of 100,000 cfu/mL total plate count, suggesting that feeding contaminated colostrum is a common occurrence on commercial dairy farms (McGuirk and Collins, 2004).

The first control point in feeding clean colostrum must be to prevent contamination during the harvest, storage, and feeding processes (Stewart et al., 2005).

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Management strategies to prevent bacterial proliferation in stored colostrum may include freezing, refrigeration, and the use of preservative agents such as potassium sorbate in refrigerated fresh colostrum (Stewart et al., 2005). One additional method of reducing or eliminating bacterial pathogens is to heat-treat fresh bovine colostrum. 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; Godden et al., 2005). These on-farm pasteurization systems typically utilize the same pasteurization times and temperatures recognized by the Grade A Pasteurized Milk Ordinance (US Department of Health and Human Services, 1999) to eliminate important pathogens. However, early studies that pasteurized colostrum, using the same times and temperatures recommended for milk, demonstrated that heating resulted in denaturation of from 12 to 30% of colostral IgG and sometimes caused significant increases in viscosity (Meylan et al., 1995; Godden et al., 2003; Green et al., 2003).

The objective of this study was to identify the critical temperature at or below which heat treatment of bovine colostrum would produce no significant change in colostrum viscosity, IgG concentration, or Ig activity.

MATERIALS AND METHODS

Laboratory Methods

First milking colostrum for this study was previously collected from Holstein cows on one commercial dairy farm, and frozen at –20°C for between 2 and 16 wk. Preliminary work involved using a Rapid Visco Analyzer (**RVA**) to heat 50-mL aliquots from 6 unique batches of bovine colostrum to 59, 60, 61, 62, and 63°C. The RVA is a computer-integrated instrument developed by Newport Scientific (Warriewood, Australia) that is capable of rapidly measuring the apparent viscosity (in cP) of products over a range of temperatures and mixing conditions. For each run, the RVA held the sample at 38°C for 10 min, heated it to the target pasteurization temperature over a 30-min period, held it at the target temperature for 120 min, and then cooled it to 38.5°C (approximate feeding temperature) over a 30-min period. Temperature and viscosity were recorded at 8-s intervals during this procedure. Pre- and post-heat-treated colostrum samples were frozen at –20°C.

Frozen colostrum samples were submitted to the Veterinary Diagnostic Laboratory (University of Minnesota) for analysis of IgG concentration and activity. Analysis of colostrum samples for total IgG concentration (mg/mL) was completed using a turbidometric immunoassay using an Olympus AU400e immunoanalyzer (Olympus America Inc., Melville, NY) and reagents from Midland Bioproducts Corp. (Boone, IA). The turbidometric immunoassay is a highly sensitive automated lateral-flow immunoassay that directly measures turbidity of the antigen-antibody complex, producing accurate IgG measures compared with the more time-consuming and labor intensive radial immunodiffusion (RID) method (Etzel et al., 1997; McVicker et al., 2002).

After reviewing the results of this preliminary work, it was determined that the approximate lower critical temperature, at or below which antibodies and viscosity would not be affected, was 60°C. To verify that this was true over a larger number of samples, the above-described RVA heating and testing process was repeated for 50-mL aliquots from 30 unique batches of bovine colostrum, in which the samples were only heated to 60 and 63°C. In addition to measuring viscosity and IgG concentration for these 30 batches, a serum neutralization (**SN**) assay was used to evaluate antibody activity in colostrum samples. Frozen samples of colostrum were thawed at room temperature and then centrifuged at 28,800 $\times g$ for 2 h. Clear whey was used as the starting material for the SN assay. Antibodies against bovine viral diarrhea virus type 1 (**BVDV-1**) were determined using a microtitration SN test. Serial 2-fold dilutions of whey were made in Eagle's mimimal essential medium with Earle's salts, 150 IU/mL of penicillin, 150 μg/mL of streptomycin, 50 μg/mL of neomycin, and 1 μg/mL of fungizone. Each dilution (25 μL) was placed in wells of a 96-well, flat-bottomed microtiter plate followed by the addition of an equal volume of virus suspension containing approximately 300 $TCID_{50}$ (tissue culture infective dose 50%) of BVD-1 (Singer strain). The whey-virus mixture was incubated at 37°C for 60 min. A suspension of bovine turbinate cells (5×10^5 cells/mL) was then added to all wells at 50 μL per well. A drop of mineral oil was placed in all wells to minimize liquid evaporation. The plates were incubated at 37°C in a 5% CO_2 atmosphere for 7 d. Each set of plates had cell controls and a virus back-titration in addition to individual whey controls. Antibody titers were expressed as the reciprocal of the highest dilution that prevented the development of viral cytopathic effects.

Statistical Methods

For the preliminary work using 6 unique batches of colostrum heated to 5 different temperatures (59, 60, 61, 62, and 63°C), descriptive statistics were used to describe measures for viscosity [$\log_{10}$(cP)] and IgG con-

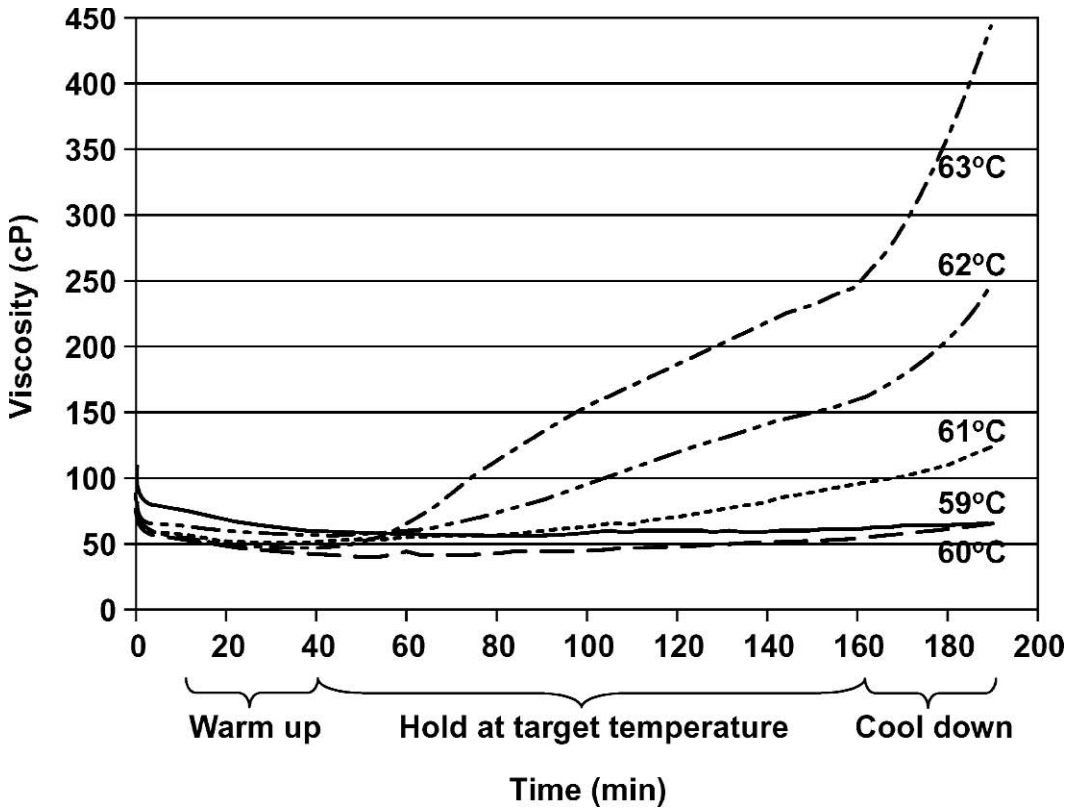

Figure 1. Viscosity changes during heat treatment of bovine colostrum at 5 temperatures for 120 min in a Rapid Visco Analyzer.

centration (mg/mL) in all pre- and post-heat-treated samples at each temperature, as well assess these variables for normality (skew, kurtosis). A linear regression model (PROC MIXED in SAS version 8.2, SAS Institute, 2000) was developed for each of the 5 temperatures studied, to describe the relationship between sample time (pre-heat-treatment vs. post-heat-treatment; explanatory variable) and the following 2 dependent variables of interest: 1) IgG concentration (mg/mL), and 2) viscosity [$\log_{10}$(cP)]. Log-transformed data were used to describe viscosity measures because they are not normally distributed. Batch was controlled for as a random effect in the models.

For the additional work using 30 unique batches of colostrum heated to 2 different temperatures (60 and 63°C), descriptive statistics were produced to describe measures for viscosity [$\log_{10}$(cP)], IgG concentrations (mg/mL), and BVD SN titers in all pre-heat-treated and post-heat-treated samples, as well as the absolute changes and percentage changes in IgG and viscosity between pre- and post-heat-treated samples. A linear regression model (PROC MIXED in SAS version 8.2, SAS Institute, 2000) was developed for each of the 2 temperatures studied, to describe the relationship between sample time (pre- and post-heat-treated; explanatory variable) and the following 3 dependent variables of interest: 1) IgG concentration (mg/mL), 2) viscosity [$\log_{10}$(cP)], and 3) antibody activity [$\log_2$(BVD SN titer)]. Log transformed data were used to describe viscosity measures and BVD SN titer because these measures are not normally distributed. Batch was controlled for as a random effect in the models.

Four more linear regression models were then developed to describe the relationship between temperature (60 or 63°C; explanatory variable) and the following 4 dependent variables: 1) absolute change in IgG concentration (mg/mL) between pre- and post-heat-treated samples; 2) percentage change in IgG concentration (%) between pre- and post-heat-treated samples; 3) absolute change in viscosity [$\log_{10}$(cP)] between pre- and post-heat-treated samples; and 4) percentage change in viscosity (%) between pre- and post-heat-treated samples. A categorical term describing colostrum quality (high = pre-heat-treated IgG ≥73.0 mg/mL; moderate = pre-heat-treated IgG <73.0 mg/mL), as well as an interaction term between the variables describing temperature (60 vs. 63°C) and colostrum quality (high vs. moderate) were offered as covariates in these models. Because only 1 of the 30 batches of colostrum was considered to be of low quality (<50 mg/

Table 1. Mean (SD; range) IgG concentration and viscosity for 6 batches of bovine colostrum after heat treatment at 5 different temperatures for 120 min in a Rapid Visco Analyzer

	Preheating[1]	Postheating[2]
Immunoglobulin G (mg/mL)		
59°C	73.6 (10.9; 57.7 to 85.5)[a]	77.1 (12.7; 58.5 to 92.4)[a]
60°C	71.6 (13.9; 54.6 to 93.0)[a]	70.4 (11.2; 52.5 to 79.6)[a]
61°C	73.0 (18.1; 54.5 to 102.7)[a]	66.1 (9.5; 54.7 to 78.0)[a]
62°C	73.3 (14.6; 58.9 to 98.3)[a]	58.5 (4.9; 53.2 to 67.2)[b]
63°C	76.1 (14.7; 59.7 to 98.7)[a]	43.7 (10.9; 31.1 to 57.6)[b]
Viscosity [$\log_{10}$(cP)]		
59°C	1.93 (0.07; 1.85 to 2.03)[a]	1.80 (0.12; 1.62 to 1.96)[a]
60°C	1.88 (0.10; 1.71 to 1.98)[a]	1.85 (0.19; 1.61 to 2.18)[a]
61°C	1.91 (0.11; 1.79 to 2.09)[a]	2.14 (0.18; 1.99 to 2.47)[b]
62°C	1.91 (0.10; 1.72 to 2.00)[a]	2.38 (0.22; 2.12 to 2.76)[b]
63°C	1.86 (0.07; 1.79 to 1.95)[a]	2.65 (0.20; 2.45 to 3.02)[b]

[a,b]Different superscripts within row indicate a significant difference ($P < 0.05$).

[1]Preheating mean IgG and viscosity measures were taken at approximately 38.5°C, before initiating the heat-treatment process.

[2]Postheating mean IgG and viscosity measures were taken after the heat-treatment process was completed and the colostrum was cooled back down to 38.5°C (approximate feeding temperature).

mL), results from this batch were lumped into the "moderate" quality group. Batch was controlled for as a random effect in all models. Statistical significance was declared at $P < 0.05$.

RESULTS

Effect of Heat-Treatment Temperature on Viscosity and IgG Concentration

For the preliminary work using 6 batches of colostrum heated at 5 different temperatures, regression analysis indicated that IgG concentration was reduced in post-heat-treated samples after colostrum was heated at 62 or 63°C, and that viscosity was increased in post-heat-treated samples after colostrum was heated at 61, 62, or 63°C (Table 1). Viscosity measures, as recorded by the RVA every 8 s during the heating process at 59, 60, 61, 62, and 63°C, are shown in Figure 1. The results of this preliminary work strongly suggested that colostrum could be safely heated to 60°C without affecting either IgG concentration or viscosity. However, to confirm this, 30 unique batches underwent heating in the RVA at both 60 and 63°C.

For the 30 unique batches of colostrum heated in the RVA at 60°C, there were no differences in IgG concentration between pre-heat-treated samples (76.4 ± 26.5 mg/mL) and post-heat-treated samples (74.5 ± 24.3 mg/mL; $P > 0.05$; Table 2 and Figure 2). Similarly, there was no difference in viscosity [$\log_{10}$(cP)] between pre-heat-treated samples (1.90 ± 0.26) and post-heat-treated samples (1.90 ± 0.30; $P > 0.05$; Table 2). However, for the 30 batches heated at 63°C, there was a reduction in mean IgG concentration (mg/mL) in post-heat-treated samples (46.0 ± 13.2) compared with pre-heat-treated samples (77.0 ± 26.1; $P < 0.05$; Table 2 and Figure 3). There was also an increase in mean viscosity [$\log_{10}$(cP)] in post-heat-treated samples (2.62 ± 0.35) compared with pre-heat-treated samples (1.97 ± 0.21; $P > 0.05$; Table 2).

Further regression analysis indicated that heating colostrum to 63°C (vs. 60°C) had an effect on the absolute loss (mg/mL) and the percentage loss of IgG, and on the absolute change [$\log_{10}$(cP)] and the percentage change in viscosity between pre- and post-heat-treated samples (Table 3, Figures 2 and 3). In the same 4 models, it was shown that high quality colostrum suffered from more pronounced losses in IgG and more pronounced increases in viscosity than did moderate quality colostrum (Figures 2 and 3). However, a significant interaction existed between temperature (60 vs. 63°C) and colostrum quality (high vs. moderate) for models predicting absolute changes in IgG or absolute change in viscosity (but not for models predicting the percentage change in IgG or the percentage change in viscosity). As such, the data for the former 2 models were stratified by colostrum quality and reanalyzed (Table 3). For high quality colostrum (≥73.0 mg/mL), the least squares means estimate of the magnitude of IgG change was −31.95 (SE = 2.61) and −4.62 (SE = 0.06) mg/mL for colostrum heated to 63 and 60°C, respectively. Similarly, the least squares means estimate of the change in viscosity was 0.85 (SE = 0.04) and 0.11 (SE = 0.04) $\log_{10}$(cP) for high quality colostrum heated to 63 or 60°C, respectively. By contrast, for moderate quality colostrum (<73.0 mg/mL) the magnitude of IgG change was estimated to be −18.11 (SE = 1.73) and 0.79 (SE = 1.73) mg/mL for colostrum heated to 63 or 60°C, respectively. Similarly, the estimated

Table 2. Descriptive statistics (SD; range) for IgG concentration and viscosity for 30 batches of bovine colostrum after heat treatment at 2 temperatures for 120 min in a Rapid Visco Analyzer

	Preheating	Postheating	Absolute change	Percentage change (%)
Immunoglobulin G (mg/mL)				
60°C	76.4 (26.5; 42.6 to 155.5)[a]	74.5 (24.3; 44.3 to 147.3)[a]	–1.9 (7.4; –27.7 to 13.8)	–1.6 (7.1; –21.6 to 10.3)
63°C	77.0 (26.2; 40.9 to 159.8)[a]	46.0 (13.2; 21.0 to 87.8)[b]	–24.3 (11.1; –42.3 to –4.3)	–33.9 (13.3; –59.3 to –8.6)
Viscosity [$\log_{10}$(cP)]				
60°C	1.90 (0.26; 1.54 to 2.84)[a]	1.90 (0.30; 1.41 to 2.82)[a]	–0.0024 (0.20; –0.46 to 0.36)	0.063 (10.0; –20.72 to 16.54)
63°C	1.97 (0.21; 1.66 to 2.81)[a]	2.62 (0.35; 2.03 to 3.40)[b]	0.65 (0.28; 0.07 to 1.11)	33.35 (14.51; 3.6 to 58.2)

[a,b]Different superscripts within row indicate significant difference between pre- and post-heat-treated measures ($P < 0.05$).

change in viscosity was estimated to be 0.45 (SE = 0.05) and –0.11 (SE = 0.05) $\log_{10}$(cP) for moderate quality colostrum heated to 63 and 60°C, respectively (Table 3).

Effect of Heat-Treatment Temperature on Ig Activity

Twenty-two and 21 paired samples of frozen colostrum, previously heated to 63 or 60°C, respectively, were available for testing with a BVD SN assay to evaluate antibody activity. Unfortunately, this assay was able to produce results for only 18 of the post-heat-treated samples heated at 60°C and for only 10 of the post-heat-treated samples heated at 63°C. The explanation for this is that congealing of the colostrum in the assay precluded determination of a titer. As a result, only 18 and 10 paired pre- and post-heat-treated SN test results were available for samples that had been heated at 63 or 60°C, respectively. For the 18 paired colostrum samples heated to 60°C there was no decrease in titer when comparing the mean (±SD; range) $\log_2$(titer) for pre- (12.4 ± 1.8; 8.7 to 15.0) vs. post-heat-treated samples (13.9 ± 2.2; 11.0 to 18.0). Similarly, for the 10 paired colostrum samples heated to 63°C, there was no decrease in titer when comparing the mean (±SD; range) $\log_2$(titer) for pre- (12.4 ± 1.2; 11.0 to 14.0) vs. post-heat-treated samples (12.3 ± 1.6; 10.0 to 15.0). These results should be interpreted with extreme caution given the large proportion of samples with missing results.

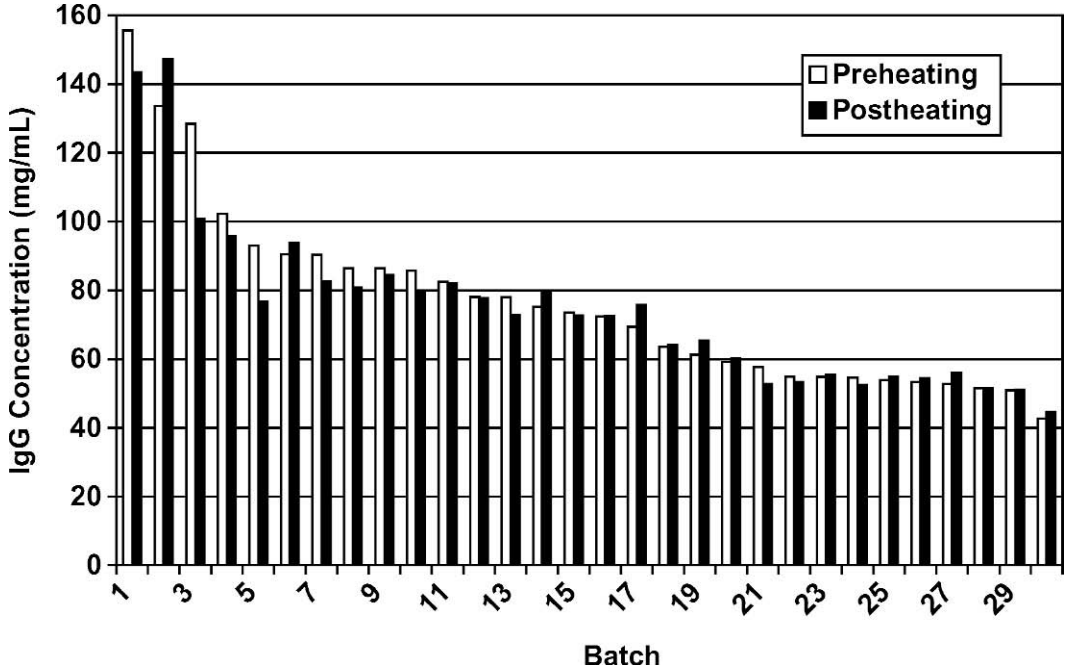

Figure 2. Immunoglobulin G concentrations in 30 pre- and post-heat-treated batches of colostrum when heating for 120 min at 60°C in a Rapid Visco Analyzer.

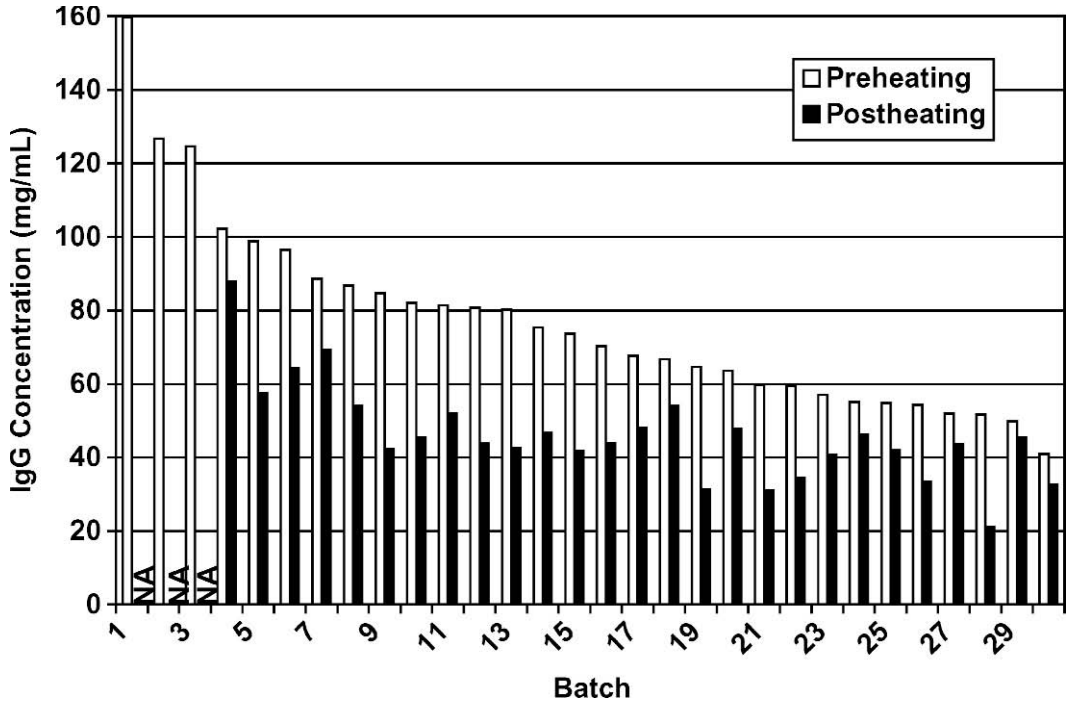

Figure 3. Immunoglobulin G concentrations in 30 pre- and post-heat-treated batches of colostrum when heating for 120 min at 63°C in a Rapid Visco Analyzer. NA = Results not available due to clumping of colostrum.

DISCUSSION

Pasteurization of bovine colostrum at the times and temperatures conventionally used for milk can successfully reduce or eliminate important bacterial pathogens including *Salmonella* spp., *Listeria monocytogenes*, *E. coli* O157 H7, *Staphylococcus aureus* (Green et al., 2002), and *Mycobacterium avium* ssp. *paratuberculosis* (Stabel et al., 2004). However, serious drawbacks to this practice include the denaturation of protective colostral antibodies and (sometimes) unacceptable increases in viscosity.

For example, in one experiment using an HTST approach, pasteurization of 5 unique 3.8-L batches of bovine colostrum at 72°C for 15 s using a large-scale, commercial, on-farm continuous flow pasteurizer (BetterMilk Inc., Winona, MN), resulted in an average 28.4% (± 30.9) loss of IgG, with pre- and postpasteurized IgG concentrations being 58.5 mg/mL (± 10.7) and 41.3 mg/mL (± 19.1), respectively (Green et al., 2003). Moreover, for all 5 batches, the colostrum congealed into a thick, pudding-like consistency during or immediately after the pasteurization process, creating a final product with unacceptable feeding and cleaning characteristics. In a separate experiment that heated colostrum at 71.7°C for 15 s, using a commercial on-farm HTST pasteurization system, Stabel et al. (2004) reported a 25% reduction in IgG concentration and gelling of the finished product in the bucket.

Conventional batch pasteurization of colostrum is slightly more successful, but still has problems. Meylan et al. (1995) reported that batch pasteurization of 5-mL aliquots from 18 colostrum samples at 63°C for 30 min resulted in a mean loss of only 12.3% (± 8.7) of IgG. However, given the small volumes studied, the external validity of these results is suspect. In a more recent laboratory study, pasteurization of 17 unique 3.8-L batches of bovine colostrum at 63°C for 30 min, using a small-scale commercial batch pasteurizer (Weck Inc., Luray, VA), resulted in only mild thickening of the colostrum, but an average 25.3% (± 26.0) loss of IgG, with mean pre- and postpasteurized IgG concentrations being 68.7 (± 17.8) and 48.9 (± 15.2) mg/mL, respectively (Green et al., 2003). In a second experiment, pasteurization of 10 unique 30-L batches of bovine colostrum at 63°C for 30 min, using a large-scale, commercial on-farm batch pasteurizer (DT-37, DairyTech Inc., Windsor, CO), resulted in only mild thickening, but an average 30.6% (± 19.3) loss of IgG, with mean pre- and postpasteurized IgG concentrations being 59.7 (± 13.5) and 40.0 (± 10.0) mg/mL, respectively (Green et al., 2003). Finally, in a controlled field study on a large commercial dairy in Colorado, an on-farm batch pasteurization system (DT-37,

Table 3. Least squares means (SE) from final linear regression analysis of the effect of temperature on IgG and viscosity changes in 30 batches of colostrum heated at 2 temperatures for 120 min in a Rapid Visco Analyzer

Dependent variable and model	Temperature		*P*-value
	Heated at 63°C	Heated at 60°C	
Absolute change in IgG concentration (mg/mL)			
Model 1: All batches of colostrum (n = 30)[1]	–24.86 (1.62)	–1.36 (1.54)	<0.0001
Model 2: High quality colostrum (≥73.0 mg/mL) (n = 15)[2]	–31.95 (2.61)	–4.62 (0.06)	<0.0001
Model 3: Moderate quality colostrum (<73.0 mg/mL) (n = 15)[2]	–18.11 (1.73)	0.79 (1.73)	<0.0001
Absolute change in viscosity [$\log_{10}$(cP)]			
Model 4: All batches of colostrum (n = 30)[1]	0.65 (0.04)	–0.0019 (0.04)	<0.0001
Model 5: High quality colostrum (≥73.0 mg/mL) (n = 15)[2]	0.85 (0.04)	0.11 (0.04)	<0.0001
Model 6: Moderate quality colostrum (<73.0 mg/mL) (n = 15)[2]	0.45 (0.05)	–0.11 (0.05)	<0.0001
Percentage change in IgG concentration (%)			
Model 7: All batches of colostrum (n = 30)[1]	–34.28 (1.98)	–1.23 (1.88)	<0.0001
Percentage change in viscosity (%)			
Model 8: All batches of colostrum (n = 30)[1]	33.30 (2.03)	0.07 (2.06)	<0.0001

[1]Models 1, 4, 7, and 8 control for initial colostrum quality (high vs. moderate) as a covariate in the model.

[2]Because a significant interaction between colostrum quality and temperature was detected in models 1 and 4, models 2, 3, 5, and 6 were developed after stratifying the data by colostrum quality.

DairyTech, Inc.) was used to pasteurize 25 unique 57-L (15-gallon) batches of fresh bovine colostrum to 63°C for 30 min (Godden et al., 2003). In this study, viscosity was only mildly increased. However, there was a mean loss of 23.6% (± 12.9) of IgG, with mean pre- and post-pasteurized IgG concentrations being 58.7 (± 12.5) and 44.1 (± 9.2) mg/mL, respectively. Furthermore, calves fed pasteurized colostrum had acceptable but numerically lower serum IgG concentrations (13.5 ± 2.2 mg/mL) than calves fed fresh colostrum (16.1 ± 2.4 mg/mL; Godden et al., 2003).

The results of the current study agree with earlier studies (Godden et al., 2003; Green et al., 2003) in that heating bovine colostrum in a batch system to industry standard temperatures (63°C) resulted in significant denaturation of IgG (least squares mean = –34% reduction in IgG concentration) and a significant increase in viscosity (least squares mean = +33%). This experiment also detected that, when heated to 63°C, high quality colostrum suffered from greater losses in IgG concentration and greater changes in viscosity than did moderate quality colostrum. This observation was also reported by Meylan et al. (1995) and Godden et al. (2003). Previous researchers (Lindstrom et al., 1994) utilized differential scanning calorimetry to determine that the denaturation temperature of IgG ranges from 62.6 to 67.6, depending on the pH. Consequently, when colostrum is heated above approximately 63°C, thermally induced unfolding and subsequent aggregation of IgG can occur as a result of the exposure of hydrophobic or sulfur-containing amino acids. This explains both the increase in viscosity and the measurable decrease in IgG concentration that are reported in this and other studies when colostrum is heated to temperatures approaching or exceeding 63°C.

Overall in this study, heating colostrum to 60°C resulted in no change in total IgG concentration (mg/mL) or viscosity when heating 50-mL volumes in an RVA for up to 120 min. Progressively greater reductions in IgG concentration and progressively greater increases in viscosity were observed for colostrum samples heated to 61, 62, and 63°C, respectively. After stratifying the data by colostrum quality, there were no changes in IgG concentration or viscosity when heating moderate quality colostrum to 60°C. However, when heating high quality colostrum to 60°C, there were some minor losses in IgG (–4.62 mg/mL or –1.23%) and some minor increases in viscosity [0.11 $\log_{10}$(cP) or 0.07%]. Although of academic interest, these minor changes are not expected to be of practical importance because the end product should still be of very high quality with excellent feeding characteristics.

One of the objectives of this study was to evaluate the effect of temperature on antibody activity by use of a SN assay. Although the partial results reported would suggest that activity was not affected, readers should interpret these results with extreme caution given the dramatic loss of data (missing results) from this analysis attributed to congealing, particularly for the set of samples heated to 63°C. Specifically, the SN assay results reported are likely to be highly biased because the omitted samples that congealed would most likely represent samples in which antibody activity would have been impaired. As such, the authors suggest that the partial SN results produced in this study should be interpreted as inconclusive.

The results of this research suggest that bovine colostrum can be successfully heated to 60°C for up to 120 min without affecting viscosity or reducing IgG

concentration. However, there is a great deal more research to be completed before the practice of heat-treating colostrum can be widely recommended for adoption by dairy producers. For example, the authors need to verify the external validity of this study's findings. That is, can the same results be achieved when heat-treating large volumes of colostrum in a commercial on-farm batch pasteurization system? Another obvious step would be to determine the duration of heating necessary, at this lower temperature, to kill important pathogens in colostrum such as *E. coli*, *Salmonella* spp., *Listeria* spp., *Mycoplasma* spp., and *Mycobacterium avium* ssp. *paratuberculosis*. Current industry standards recommend that batch pasteurization of milk be carried out at 63°C for 30 min. It is expected that, by lowering the temperature to 60°C for colostrum, the duration of heating will need to be extended to achieve the same pathogen kill. The results of studies addressing these questions will be reported in a future companion paper.

Eventually, a controlled field study will be required to demonstrate that calf serum IgG concentrations, growth, and health are at least unaffected, if not improved, when calves are fed heat-treated colostrum. Ultimately, significant health, performance, and economic benefits must be described to justify feeding heat-treated colostrum to calves on commercial dairy farms.

CONCLUSIONS

Fifty-milliliter volumes of bovine colostrum can be heated at 60°C in an RVA for at least 120 min without affecting IgG concentration or viscosity. Additional research is needed to determine if these study findings can be successfully replicated when using a large-scale commercial on-farm batch pasteurization system, and to determine the duration of heating required at 60°C to effectively destroy important pathogens in colostrum.

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Effect of feeding heat-treated and unheated colostrum on immunoglobulin G absorption, health and performance of neonatal Holstein dairy calves

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ABSTRACT. First colostrum is an important source of nutrients and immune factors which are necessary for calves in the first weeks of life. Despite these benefits, colostrum can also represent one of the earliest potential exposures of dairy calves to infectious agents which these patogens can act directly on growth and cause diseases such as scours or septicemia. With recent increased interest in pasteurized milk feeding systems, producers have been curious to learn if there may also be benefits from feeding pasteurized colostrum. This study was realized to determine the effects of feeding heat-treated colostrum or unheated colostrum on passive transfer of immunity, immunoglobulin G (IgG) concentration, total plate count, health and performance of neonatal dairy calves. First-milking colostrum was collected from Holstein cows and frozen at -20°C to accumulate a large batch. Pooled batches of colostrum were mixed and divided equally: One half was fed unheated colostrum; whereas the other half was fed after heat treatment at 60°C for 30 min. Forty newborn male Holstein dairy calves were fed either unheated (n = 20) and heat-treated colostrum (n = 20), 10% of their birth weight. Calves received 4 L within 1 to 2h after birth and residuals was fed 6h after birth. Serum samples collected from calves and were assayed for serum total protein (STP) and IgG. Feed intake recorded weekly and body weight and skeletal growth measures recorded at d 3 and d 63 (weaning). Every day, calves clinically diagnosed either as being healthy or suffering from respiratory disease and neonatal calf diarrhea. Heat-treated colostrum resulted in lower colostrum bacterial concentration (2.01 vs. 3.96 cfu mL^{-1}). Calves fed heat treated colostrum had greater STP in 24, 72h and 23d, IgG concentrations at 24 and 72h plus unheated colostrum. Also weaning weight and average daily gain were greater in calves feed heated colostrum. There were no differences in starter intake and feed efficiency between two groups. Calves fed heat-treated colostrum had lower fecal scores, diarrhea and pneumonia incidence. There were not differences in skeletal growth measurements except body barrel. These results shows that feeding heated colostrum can provide better growth and health in neonatal calves.

Keywords: immunoglobulin G; colostrum; health; serum total protein; weaning.

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Introduction

Calves are agammaglobulinemic at birth because syndesmochorial structure of placenta so depend on ingestion and absorption of colostral immunoglobulins, especially immunoglobulin G (IgG) across the intestinal epithelium during the first 24h of life to establish a protective serum IgG concentration (Elizondo-Salazar & Heinrichs, 2008). Successful passive transfer of IgG in a calf is defined as having a serum IgG concentration ⩾10 mg ML^{-1} at 24 to 48h of age and is associated with such benefits as decreased risk for morbidity and mortality in the preweaning period and improved improved milk production later in life (DeNise, Robison, Stott, & Armstrong, 1989; Donovan, Dohoo, Montgomery, & Bennett, 1998). Despite aforementioned benefits, colostrum can be a potential early source of exposure to microbial pathogens. Contamination of colostrum with pathogen may result to the multiple sources, including secretion from mammary gland, during milking, or proliferation during storage feeding or storage (Stewart et al., 2005). In any case, failure of passive transfer can occur and the incidence of respiratory or digestive disease may increase in these animals (Godden et al., 2006; Pardon et al., 2015).

Recent researches on heat treatment of colostrum have shown great advantage in reducing number of bacteria (Donahue et al., 2012; Godden et al., 2006; Johnson, Godden, Molitor, Ames, & Hagman, 2007) and it has been known that feeding heat-treated colostrum may increase IgG absorption and as result increase serum IgG concentration (Elizondo-Salazar & Heinrichs, 2009a; Johnson et al., 2007). This mechanism is unknown, but Johnson et al. (2007) hypothesized that because antibodies in colostrum may bind pathogens present in gut before absorption, by reducing the contamination in heat-treated colostrum, and consequently the number of pathogen in the gut, more antibodies are potentially free for absorption. Corley, Staley, Bush, and Jones (1977) reported microbes in colostrum might also act competitively by occupying binding sites on the apical plasma membrane of the epithelial cell. They observed microbial attachment, exfoliation of microvilli and intracellular penetration of ileal epithelial cells when *E. coli* O55 was administered to colostrum-deprived calves. Furthermore, some studies have reported that high concentrations of bacteria in colostrum may be associated with decreasing IgG absorption, thereby potentially contributing to failure of passive transfer (Elizondo-Salazar & Heinrichs, 2009a, 2009b). National US statistics estimate that the proportions of preweaned calves with failure of passive transfer, treated for scours or other digestive problems, and treated for respiratory disease are 19.2, 17.9, and 11.4%, respectively (USDA-APHIS, 2007). As a consequence, cost for treatment of bovine respiratory disease (BRD) and neonatal calf diarrhea (NCD) and death rates due to both diseases may increase during the first 21 d of life (Meganck, Hoflack, & Opsomer, 2014; Windeyer et al., 2014). So, a good colostrum feeding protocol should also avoid bacterial contamination (Meganck et al., 2014).

Heat treatment may be one approach to reduce microbial contamination in colostrum. The history of developing a technique to heat-treat colostrum has been reviewed elsewhere (Donahue et al., 2012). Ragsdale and Brody (1923) reported that colostrum can be safely heated at 60°C for up to 3h without denaturing Ig or creating an undesirable, heat-coagulated product while inactivating tuberculosis organisms. The recent introduction of commercial on-farm pasteurizers has since created interest in feeding heat-treated colostrum to dairy calves. Experiments to pasteurize colostrum using traditional Pasteurized Milk Ordinance pasteurization temperatures, ranging from 62.5 to 73°C, yielded unacceptable IgG denaturation and changes in viscosity (Godden et al., 2003; Meylan et al., 1996; Tyler et al., 2000). In most situations, heating colostrum at 60°C for 30 or 60 min should be sufficient to maintain IgG concentrations and fluid characteristics while eliminating or significantly reducing important pathogens including Listeria monocytogenes, *E. coli* O157:H7, Salmonella enteritidis, and M. avium ssp. paratuberculosis (Godden et al., 2006; McMartin et al., 2006). There is lack of researches to indicate effects of heat-treated colostrum on health and growth parameters.

The first objective of this study was to compare the effects of heat-treated colostrum on total plate count and IgG absorption. The second objective of this study was to compare effects of heat-treated colostrum on health and diarrhea and pneumonia incidence.

Material and methods

Colostrum collection and heat treatment

First milking colostrum was collected from Holstein cows into 1.5 L plastic bottles and frozen immediately at −20°C to inhibit bacterial growth. At the start of the experiment, colostrum samples were thawed at 4°C and then thoroughly mixed for 10 min. Thawed colostrum divided into equal aliquots. One aliquot was left unheated whereas the other was heat-treated at 60°C for 30 min by on farm colostrum pasteurization system (V4, SHIRMAK GROUP). Fresh and heat treated colostrum were transferred to sanitized 1.5 L bottles and refrigerated at -20°C for later feeding to calves enrolled in the study.

Immediately before administration, an additional 10 mL aliquot of colostrum from heated and unheated colostrum were collected and frozen at -20°C for later bacterial culture to assess how much bacterial growth had occurred and colostrum IgG measurements. Colostrum samples were thawed to 4°C, mixed by vortex, and serially diluted 1:10 for 5 dilutions. Each dilution was plated on plate count agar for total plate count and plates were incubated for 48h at 37°C and the number of colonies recorded log10 (cfu mL^{-1}).

Calf enrollment

The protocol for animal experiments was approved by University of Agricultural Sciences and Natural Resources, Gorgan, Iran. Calves were removed from the dam within 20 to 30 min of birth before suckling

could occur. To be eligible for enrollment, calves had to be singletons, weight from 40 to 45 kg, similar median calving ease score 3 (1 to 5 scale), and have similar median parity of dam. Forty male newborn Holstein calves were fed either unheated (n = 20) and heat-treated colostrum (n = 20), 10% of their birth weight. During first 24h, calves received colostrum 4 L, within 1 to 2h after birth and residual was fed 6h after birth. Systematic, rather than random, assignment to treatment groups was used to ensure that the age (duration of storage) of heat-treated and unheated colostrum was approximately equal at the time of feeding, because the storage period will allow for bacterial growth in colostrum. After assignment to a colostrum feeding group, the appropriate bottle of colostrum (heat-treated or unheated) was removed from refrigerator and warmed approximately to 39°C. During the days 2 and 3, calves were fed pasteurized milk 2 L at 9 AM and 2 L at 6 PM. Milk feeding program during days 4 to 63 (9 weeks) was 4, 4, 5, 7, 7, 7, 5, 2 and 1 litter per each day which except week 9, calves were fed just 1 L in the morning and half of milk was received in the morning and residual was fed in afternoon. The composition of the diets is presented in Table 1. Solid feed (starter) was offered ad libitum to all calves after d 3. During the research all the calves receive same amount of milk in the milk feeding program.

Table 1. Ingredient composition (% of DM) of calf starter diets and chemical compositions of starters (% of DM)

Ingredients	Diet
Ground corn	45
Ground barley	11
Soybean meal	34
Calcium carbonate	1.4
Salt	0.2
Sodium bicarbonate	1.5
Dicalcium phosphate	0.2
Milk replacement	5
Vitamins[1]	1
Minerals[2]	0.7
Chemical composition	
DM (%)	90.07
ME (Mcal kg^{-1})	2.6
NEm (Mcal kg^{-1})	2.06
NEg (Mcal kg^{-1})	1.57
CP (%)	20.2
Ether extract (%)	3.00

[1]Minerals contained (mg kg-1): Ca (800), P (80), Mg (100), Cu (10), Mn (18), Zn (35.2), Co (0.12), I (0.28), and Se (0.17) on a DM basis. [2]Vitamins contained: vitamin A (1280000 IU kg^{-1}) , vitamin D3 (72000 IU kg^{-1}),vitamin E (4000 IU kg^{-1}) and 1.25 g of butylated hydroxytoluene kg^{-1} as a synthetic antioxidant. on a DM basis.

Blood sample analyses for Ig and serum total protein measures in calves

Precolostrum (0h) and postcolostrum (24, 72h and 23, 43 and 63d) blood samples collected from jugular vein from all calves into serum vacutainer tubes, centrifuged and the serum separated from the clot within 1 h of collection. STP concentrations were determined using spectrophotometer (Pars Azmoon kit, Iran). Sera were then stored at -20°C until it could be analyzed for serum IgG concentration (mg mL^{-1}). Serum IgG concentration were determined by immunoprecipitation using single radial immunodiffusion (SRID; Sera vet, Spain). Serum samples (3 µL) were applied to serial RID plates containing agarose gel with anti-bovine IgG. Plates were left undisturbed for 24h at room temperature after adding samples. Resulting ring diameters were measured with a monocular comparator, and IgG content of samples was calculated by regression analysis. A standard curve was generated with reference sera supplied by the manufacturer.

Growth factor and health analyses

Intake of starter was recorded weekly throughout the experiment. Overall average daily gains and feed efficiency (feed efficiency = kg of body weight gain kg^{-1} of total DMI) were determined during 63 d of life. Body length (distance between the points of the shoulder and rump), heart girth (circumference of the chest), withers height (distance from base of the front feet to the withers), hip height (distance from the base of the rear feet to the hip bone), body barrel (circumference of the belly before feeding), and hip width (distance between the left and right trochanter major) measurements of calves were recorded at d 3 and d 63 (weaning). Fecal scoring was performed daily, as follows: 1 = normal; 2 = soft to loose; 3 = loose to watery; 4 = watery, mucous, and slightly bloody; and 5 = watery, mucous, and bloody.

Statistical analysis

Repeated measure design using PROC MIXED of Statistical Analysis System software (SAS, 2004) was used for body weight, average daily gain, skeletal growth, feed intake, feed efficiency, STP and IgG data analysis. The statistical model used for analyses was yijklm = μ + ti + dij + pk + (t × p)ik + β(body weight) + eijklm, where yijklm = observations or dependent variables, μ = overall mean, ti = effect of treatment i, dij = animal random effect with mean 0 and variance that is equal to the covariance between repeated measurements within animals, pk = the effect of period k, (t × p)ik = the effect of interaction between treatment i and period k, β = regression coefficient of observations on birth weight as a covariate considered only for growth traits (starter intake, average daily gain, feed efficiency, body weight, and other body measurements) and eijklm = random residual effect. Health and fecal scores were tested by performing $\chi 2$ tests using the frequency procedure of SAS. All the statistical tests were carried out at 5 percent probability level ($p < 0.05$).

Results and discussion

Effect of heat treatment on colostrum bacteria counts and immunoglobulin G concentration.

There was no difference in the mean colostral IgG concentration for heat-treated vs. unheated colostrum (57.6 mg mL^{-1} vs. 60.6 mg mL^{-}) ($p = 0.22$) (Table 2). Early studies with colostrum pasteurization using the same times and temperatures recommended for milk found that heating denatured 12 to 30% of colostral IgG and increased viscosity (Meylan et al., 1996). Elizondo-Salazar, Jayarao, and Heinrichs (2010) reported when colostrum was heated at 60°C there was a reduction in IgG, especially in IgG1, even when colostrum was heated for just 30 min. The greatest reduction in IgG concentration was observed when colostrum was heated at 63°C. Previous laboratory studies have also reported success in reducing or eliminating pathogens when colostrum has been heat-treated (Godden et al., 2006; Johnson et al., 2007). In our study, heating temperature was monitored automatically and temperature fluctuations during heating were +0.1 to −0.1°C , so heating denatured 5% of the colostral IgG. Mean total plate count was lower ($p < 0.0002$) in heat-treated colostrum (2.01 cfu mL^{-1}) vs. unheated colostrum (3.96 cfu mL^{-1}). Calf birth weight, median parity of the dam and median calving ease score did not vary among treatments (Table 2). Researches have shown that colostral quality continues to increase with parity after the second calving, and older cows generally have the best quality colostrum, while dystocia would reduce colostrum IgG absorption (Donovan, Badinga, Collier, Wilcox, & Braun, 1986; Morrill et al., 2012). Therefore, to reduce the test error in this study, parity of the dam and calving ease score were considered among the two groups.

In our study heating colostrum at 60°C for 30 min was tend to reduction of colostrum pathogens. Despite the fact that Johnson et al. (2007) reported heat treated colostrum at 60°C for 60 min was tend to significant reduction in standard plate count and coliform count and it appears that 60°C for 30 min is adequate for achieving a goal of bacterial reduction in heat-treated colostrum. An additional 30 min at 60°C would probably eliminate more pathogenic bacteria; however, a different response in calf IgG concentration or apparent efficiency of absoption would not be expected (Elizondo-Salazar & Heinrichs, 2009b). Donahue et al. (2012) in multi-herd study confirms the results of these previous smaller, single-herd trials. Heat treatment of colostrum at 60°C for 60 min, as performed by farm staff on 6 commercial dairies, decreased total coliform count by 2.25 log10 and decreased total plate count by 2.49 log10, without decreasing overall colostral IgG centration.

Table 2. Description of calf and colostrum parameter.

Parameter	Treatment group		SEM	P < value
	Raw[1]	Heat-Treated[2]		
Colostrum characteristics				
IgG concentration (mg mL^{-1})	60.59	57.56	0.42	0.22
Total plate count (log10 cfu mL^{-1})	3.96 [a]	2.01[b]	0.38	0.0002
Calf characteristics				
Mean birth weight (kg)	43 – (40 to 45)	43.15 – (40 to 45)	0.27	0.78
Median parity of dam	2 – (1 to 5)	2 – (1 to 5)	0.22	0.71
Median calving ease score	2 – (1 to 3)	1 – (1 to 3)	0.93	0.14

[1]Raw = Unheated colostrum. [2]Heat-treated = Heated colostrum at 60°C for 30 min. a,b Least squares means within a row with different superscript letters differ at $p < 0.05$ for treatment effect.

In theory, decreasing bacterial exposure to calves through colostrum while maintaining colostrum quality (IgG concentrations) should result in a healthier calf. Also, Donahue et al. (2012) reported there was a positive relationship between colostrum quality and magnitude of IgG loss when heat treating colostrum. Specifically, when expressed either as an absolute change in pre-versus post-heat-treated IgG concentrations, batches of high quality colostrum experienced a significantly greater magnitude of change in IgG, as compared with the referent category (50 – 59.9 mg of IgG mL^{-1}).

Effect of feeding heat-treated colostrum on serum total protein, serum immunoglobulin G concentrations and apparent efficiency of absorption.

According the results in Table 3, there was no difference in STP at 0h (precolostrum feeding) between two groups. However, STP at 24 and 72h and 23d were greater for calves fed heat-treated colostrum (6.80, 6.61 and 6.01) vs. calves fed unheated colostrum (6.31, 6.21 and 5.72). Also there was no difference in STP at 43 and 63 d between two groups. Serum IgG concentrations at birth (0h) were below detectable concentrations of the assay and did not produce ring. IgG concentrations at 24 and 72h of life were greater for calves fed heat-treated colostrum (15.37 and 12.95) vs. calves fed unheated colostrum (12.53, 11.37) (Table 3). A value of 50 g L^{-1} for STP at 24h of age is establishing the cutoff point for successful passive transfer (Donovan et al., 1998). Tyler et al. (1996) compared the performance of commonly used tests for passive transfer, demonstrated STP concentration of 52 g L^{-1} was equivalent to an IgG concentration of 10 g L^{-1}. Elizondo-Salazar and Heinrichs (2009a) reported calves received heat-treated colostrum showed greater STP concentrations at 8, 12, 16, and 20h than calves fed unheated colostrum and it was due to absorption of colostral IgG. The concentrations of STP and IgG in serum at 24h have been shown to be positively correlated (Elizondo-Salazar & Heinrichs, 2009a). In Our research STP and serum IgG concentration were significantly greater in calves fed heated colostrum and results are consistent with aforementioned researches. In a research on 1,071 new born calf demonstrated colostral total plate count and total coliform count had negative association with serum IgG (Godden et al., 2012). Microbes might also act competitively by occupying binding sites on the apical plasma membrane of the epithelial cell. Evidence of interaction of intestinal microflora with the absorptive surface of intestinal epithelial cells was demonstrated by Corley et al. (1977). Johnson et al. (2007) in a research hypothesized that because 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 consequently the number of pathogens in the gut, more antibodies are potentially free for absorption.

Apparent efficiency of absoption (AEA, %) of IgG at 24 and 72h of life was also greater for calves fed heat-treated colostrum (23.94 and 20.19%) vs. calves fed unheated colostrum (19.29 and 17.50%) (Table 3). 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 equation described by Quigley III and Drewry (1998), assuming a plasma volume of 8.5% of birth weight. By increasing serum IgG concentration in calves fed heat treated colostrum and reduction in total plate count during this process, AEA in calves fed heated colostrum had been increased and it was due to rising binding sites for absorbing IgG in gut and it was approved by authors hypothesis.

Table 3. Serum total protein (g dL^{-1}), immunoglobulin G concentration (mg mL^{-1}) and apparent efficiency of absorption (%) in calves receiving unheated or heat-treated colostrum.

Parameter	Treatment group		SEM	P- values
	Raw[1]	Heat-Treated[2]		
STP[3] (g dL^{-1})				
0h	4.16	4.19	0.93	0.83
24h	6.31[b]	6.80[a]	0.06	0.017
72h	6.21[b]	6.61[a]	0.06	0.03
23d	5.72[b]	6.01[a]	0.07	0.04
43d	5.90	6.03	0.11	0.06
63d	6.57	6.68	0.16	0.80
IgG[4] (mg mL^{-1})				
0h	0	0	0	0
24h	12.53[b]	15.37[a]	0.93	0.01
72h	11.37[b]	12.95[a]	0.93	0.03
AEA[5] for IgG (%)				
24h	19.29[b]	23.94[a]	0.93	0.003
72h	17.50[b]	20.19[a]	0.93	0.003

[1]Raw = Unheated colostrum. [2]Heat-treated = Heated colostrum at 60°C for 30 min. [3]STP = Serum total protein. [4]IgG = immunoglobulin G. [5]AEA = Apparent efficiency of absorption. a,b Least squares means within a row with different superscript letters differ at $p < 0.05$ for treatment effect.

Effect of feeding heat-treated colostrum on fecal score, diarrhea and pneumonia incidence

Calves fed unheated colostrums had greater fecal score vs. calves fed heat treated colostrum (1.61 vs. 1.22; $p < 0.0001$) (Table 4). The percent of diarrhea incidence during 63 d was greater significantly for calves received unheated colostrum vs. calves fed heated colostrum (12.16 vs. 3.8 %; $p = 0.002$). Rate of incidence to pneumonia during 63 day of lives between treatments shows that calves fed unheated colostrum had greater rate (18 incidences) than calves fed unheated colostrum (5 incidences) ($\chi 2 = 0.03$) (Figure 1).

Table 4. Fecal score and diarrhea incidence between calves fed heat-treated and unheated colostrum.

Parameter	Treatment group		SEM	P- values
	Raw[1]	Heat-Treated[2]		
Fecal score	1.66[a]	1.22[b]	0.01	<0.0001
Diarrhea incidence (%)	12.16[a]	3.8[b]	1.4	0.002

[1]Raw = Unheated colostrum. [2] Heat-treated = Heated colostrum at 60°C for 30 min. a,b Least squares means within a row with different superscript letters differ at $p < 0.05$ for treatment effect.

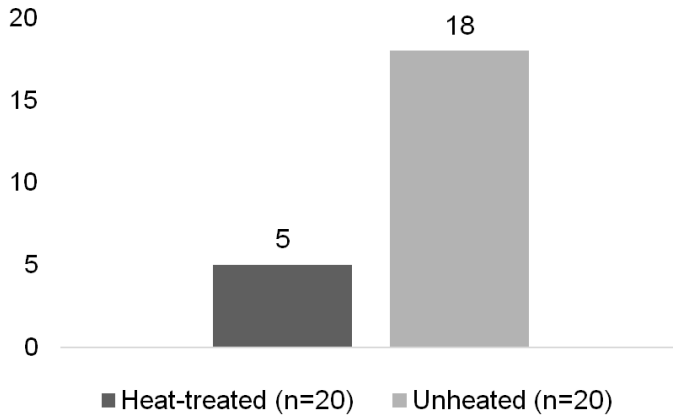

Figure 1. Rate of incidence to Pneumonia between Holstein dairy calves fed heat-treated (n=20) and unheated (n = 20) colostrum ($\chi^2 = 0.03$).

Neonatal calf diseases are multifactorial (Al Mawly et al., 2015; Lorenz et al., 2011), a proper quantity of good-quality colostrum and milk intake is essential to control them (Meganck et al., 2014). Other factors that could alter the prevalence of neonatal calf diseases are individual farm management practices and the preventive medicine programs applied to each farm. Godden et al. (2012) in a research on 1,071 calves confirmed unheated colostrum had a positive relationship with total plate count, total coliform count, and risk for any illness and scours. Several clinical studies had been demonstrated that colostrum or blood plasma immunoglobulins can provide local immunity against several pathogens associated with calf diarrheal disease such as *Escherichia coli* and corona virus (Acres, 1985; Crouch et al., 2000). Gelsinger, Jones, and Heinrichs (2015) reported calves that received heated and low-bacteria colostrum nearly tended to experience a fecal score ⩽2 more often than calves that received unheated or low-bacteria colostrum. In the present study calves with higher serum IgG concentration had less diarrhea incidence and fecal score (Table 4).

Armengol and Fraile (2016) in a research on 287 calf stated a reduction in terms of morbidity (9.8%) and mortality (3.7%) in calves fed heated (60°C for 60 min) colostrum and milk in comparison with animals receiving unheated colostrum and milk during the first 21 d of life.

Effect of feeding heat-treated colostrum on performance of neonatal calves.

According to the results (Table 5), weaning weight (63d) was greater for calves were fed heated colostrum vs. unheated colostrum (90.94 vs. 87.10 kg; $p = 0.01$). Average daily gain was significantly greater for calves fed heat-treated colostrum vs. unheated colostrum (0.77 vs. 0.71 kg d^{-1}; $p = 0.01$) (Table 5). Whereas there were no differences in starter intake and feed efficiency between two groups (Table 5). Colditz (2004) reported calves with failure of passive transfer also had lesser weight gains likely because of decreased nutrient utilization and reduced feed intake associated with more disease in these calves compared with calves with adequate passive transfer. Berge, Besser, Moore, and Sischo (2009) demonstrated calves with greater serum IgG concentration had a significantly greater average daily gain to 28d of age. In an other

research, Yang, Zou, Wu, Li, and Cao (2015) shows calves that receive colostrum and According to aforementioned researches and ours research results, calves with heated colostrum by having more serum IgG concentration have more average daily gain and body weight versus calves fed unheated colostrum. In contrast, Elizondo-Salazar and Heinrichs (2009a) reported feeding heated colostrum in 60°C for 30 min had no differences among treatments in body weight and feed intake from birth until 8 weeks. In the present study calves birth weight, median ease score and median parity of dam were same between heated and unheated groups (Table 2). In addition, the average daily gain and weaning weight in the heat–treated clostrum were greater, while feed intake was not significantly different between the two groups. It seems that increasing the performance in heat-treated calves is due to an increase in the immune system and a reduction in the mortality (Tables 3, 4).

Table 5. weaning weight, total intake of starter, average daily gain, and feed efficiency Holstein calves fed heat-treated and unheated colostrum.

Parameter	Treatment group		SEM	P- values
	Raw[1]	Heat-Treated[2]		
Weaning weight (kg)	87.10[b]	90.94[a]	1.01	0.01
Starter intake (kg)	40.56	45.70	0.27	0. 1
Average daily gain (kg d^{-1})	0.71[b]	0.77[a]	0.01	0.01
Feed efficiency	0.63	0.64	0.93	0.62

[1]Raw = Unheated colostrum. [2]Heat-treated = Heated colostrum at 60°C for 30 min. a,b Least squares means within a row with different superscript letters differ at $p < 0.05$ for treatment effect.

Effect of feeding heat-treated colostrum on body skeletal growth measurements of neonatal calves

Skeletal growth measurement results have shown in Table 6 and there was no significantly different in skeletal body measurements between two treatments except body barrel that was greater for calves fed heat-treated colostrums.

Overall all the calves fed heating colostrum had greater skeletal growth than other group. Elizondo-Salazar and Heinrichs (2009a) reported there was no different in colostrum minerals composition between heated and unheated colostrums. Also in this research there was no significant difference between treatments in starter intake (Table 3).

In our research as it was expected, there was no difference in skeletal growth except body barrel between calves received heated and unheated colostrum during 63 days (Table 6). This different seems to be because of bloat in calves that increased body barrel diameter in changing diets from milk to starter.

Table 6. Skeletal growth measurements between calves fed heat-treated and unheated colostrum

Parameter	Treatment group		SEM	P- values
	Raw[1]	Heat-Treated[2]		
Body length, cm	74.43	74.92	0.73	0.67
Hip height, cm	85.71	87.08	0.65	0.48
Hip width, cm	16.08	16.48	0.24	0.25
Heart girth, cm	96.53	96.80	0.99	0.85
Body barrel, cm	93.44[b]	96.11[a]	0.91	0.04
Withers height, cm	85.71	87.08	0.59	0.11

[1]Raw = Unheated colostrum. [2]Heat-treated = Heated colostrum at 60°C for 30 min. a,b Least squares means within a row with different superscript letters differ at $p < 0.05$ for treatment effect.

Conclusion

Feeding heat-treated colostrum at 60°C for 30 min reduced bacterial concentration while preserving the colostral IgG concentration. AEA of IgG and calf serum IgG was greater at 24 and 48h of age for calves fed heat-treated versus unheated. STP concentration at 24, 72h and 23d were greater for calves fed heated colostrum. The average daily gain and weaning weight in the heat–treated clostrum were greater, while feed intake was not significantly different between the two groups. It seems that increasing the performance in heat-treated calves is due to an increase in the immune system and a reduction in the mortality. Moreover, fecal score, percent of diarrhea incidence and Pneumonia incidence during 63 were greater for calves fed unheated colostrum. Body skeletal growth measurements of neonatal calves between treatments were similar. Future studies will also need to investigate the effects of heating colostrum in colostral GH and IGF1 hormones and intestinal development.

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Preventing Bacterial Contamination and Proliferation During the Harvest, Storage, and Feeding of Fresh Bovine Colostrum

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ABSTRACT

The objectives of this study were to identify control points for bacterial contamination of bovine colostrum during the harvesting and feeding processes, and to describe the effects of refrigeration and use of potassium sorbate preservative on bacteria counts in stored fresh colostrum. For objective 1, first-milking colostrum samples were collected aseptically directly from the mammary glands of 39 cows, from the milking bucket, and from the esophageal feeder tube. For objective 2, 15-mL aliquots of colostrum were collected from the milking bucket and allocated to 1 of 4 treatment groups: 1) refrigeration, 2) ambient temperature, 3) refrigeration with potassium sorbate preservative, and 4) ambient temperature with potassium sorbate preservative. Subsamples from each treatment group were collected after 24, 48, and 96 h of storage. All samples underwent bacteriological culture for total plate count and coliform count. Bacteria counts were generally low or zero in colostrum collected directly from the gland [mean (SD) $\log_{10}$ cfu/mL$_{udder}$ = 1.44 (1.45)]. However, significant bacterial contamination occurred during the harvest process [mean (SD) $\log_{10}$ cfu/mL$_{bucket}$ = 4.99 (1.95)]. No additional bacterial contamination occurred between the bucket and the esophageal feeder tube. Storing colostrum at warm ambient temperatures resulted in the most rapid increase in bacteria counts, followed by intermediate rates of growth in nonpreserved refrigerated samples or preserved samples stored at ambient temperature. The most effective treatment studied was the use of potassium sorbate preservative in refrigerated samples, for which total plate count and total coliform counts dropped significantly and then remained constant during the 96-h storage period.
(**Key words:** colostrum, bacterial contamination, storage, preservative)

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Abbreviation key: **TCC** = total coliform count, **TPC** = total plate count.

INTRODUCTION

The 1993, 1996, and 2002 National Animal Health Monitoring System dairy studies report that an unacceptably high mortality rate (8.4 to 10.7%) exists among preweaned heifers on US dairy farms (NAHMS, 1993, 1996, 2002). Failure of passive transfer, resulting from suboptimal colostrum management, has been identified as a key risk factor contributing to these high mortality rates (Wells et al., 1996). Experts have identified that the hallmarks of a successful colostrum management program consider the quality of the colostrum fed (Ig concentration), the quantity provided at first feeding, and how quickly the first feeding is offered (Davis and Drackley, 1996). Successive National Animal Health Monitoring System dairy studies over the past decade have reported a steady slow increase in the adoption of recommended colostrum management practices, such as hand-feeding the calf, and feeding larger volumes at first feeding (NAHMS, 1996, 2002). However, the continuation of unacceptably high preweaning mortality rates in dairy heifers indicates that there is still much room for improvement in our colostrum and calf management programs. Colostrum cleanliness may represent one important area of opportunity.

Although colostral immune factors are essential for calf health, bacterial contamination of colostrum may negate some of these benefits. Bacterial pathogens that may be transmitted in colostrum and milk, either by direct shedding from the mammary gland or from postharvest contamination, include *Mycobacterium avium* spp. *paratuberculosis*, *Salmonella* spp., *Mycoplasma* spp., *Listeria monocytogenes*, *Campylobacter* spp., *Mycobacterium bovis*, and *Escherichia coli* (Lovett et al., 1983; Farber et al., 1988; McEwen et al., 1988; Clarke et al., 1989; Giles et al., 1989; Streeter et al., 1995; Grant et al., 1996; Steele et al., 1997; Walz et al., 1997). These infectious agents may act directly to cause diseases such as enteritis or septicemia. 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. Possible mechanisms for this effect could include competition between microbes and Ig molecules for common receptors on the intestinal epithelial cell, or physical binding of colostral Ig by microbes within the intestinal lumen, thus decreasing the availability of transportable Ig (James and Polan, 1978; James et al., 1981; Staley and Bush, 1985). Although field studies describing the significance of this effect in calves are extremely limited, one recent study reported that a negative association exists between bacteria counts in colostrum and Ig absorption (Poulsen et al., 2002). This same study reported that 82% of colostrum samples collected exceeded the industry standard of 100,000 cfu/mL total plate count (**TPC**). Although actual numbers were not published, the study reported that total bacterial and fecal coliform counts in colostrum were positively correlated with abnormal fecal consistency (Poulsen et al., 2002).

Clearly, more field studies are necessary to investigate the relationship between bacterial counts in colostrum and both the efficiency of absorption of colostral antibodies and calf health. However, if a negative relationship does exist, then we must develop and validate practical recommendations on how to prevent contamination of colostrum, either during the harvest or storage processes. The first objective of this study was to describe critical control points for bacterial contamination in the colostrum collection and feeding processes. The second objective was to describe the effect of refrigeration (vs. ambient temperature) and the use of potassium sorbate preservative on bacteria counts in fresh bovine colostrum stored for up to 96 h.

MATERIALS AND METHODS

Housing and Management of Study Animals

Research activities were completed in August, 2003, in a commercial 400-cow sand-bedded, freestall facility that houses transition cows from entry into the close-up period (~45 d before calving), through the calving process, and until approximately 10 to 14 d in milk, after which time the lactating cows are returned to 1 of 2 large commercial dairy operations to complete their lactation. First-milking colostrum is routinely collected into a floor bucket within 1 h of calving. Newborn calves are fed 3.8 L of the dam's first-milking colostrum, using an esophageal tube feeder, usually within 15 to 30 min of milking the dam.

The standard procedure for sanitation of all equipment used in collection and feeding of the first colostrum (including the floor bucket, milking equipment, and esophageal tubes) was as follows: 1) disassembly of all equipment, 2) rinsing with lukewarm water to remove all visual soil and milk, 3) placing in hot water containing detergent, 4) scrubbing all exterior and interior surfaces with a brush, 5) rinsing with hot water containing acid sanitizer, and 6) allowing all equipment to drain completely and air dry.

Sample Collection and Storage Procedures

To address the first objective, 3 distinct samples of first-milking colostrum were obtained from each of 41 just-fresh cows. Following routine udder-preparation, which included fore-stripping and predipping with a 0.5% iodine-based teat dip, and drying the teat ends with a clean cloth towel, all teat ends were scrubbed with an alcohol-soaked gauze pad. The first sample was then aseptically stripped directly into a sterile 20-mL plastic sampling vial with approximately equal amounts of milk collected from the 4 quarters, and then frozen at –20°C. The milking unit was attached and the cow milked immediately after collecting this first sample (group 1 in Table 1). The second sample of colostrum was aseptically collected from the floor bucket into a sterile 240-mL plastic sampling vial within 15 to 20 min of milking the cow (group 3 in Table 1). A 15-mL aliquot was taken from the 240-mL sample and frozen. This 240-mL sample was also the source for aliquots described in the next paragraph for objective 2. Finally, a third sample was aseptically collected directly from the esophageal feeder tube into a sterile 20-mL plastic sampling vial, within 15 to 30 min of milking the cow (group 2 in Table 1), and also frozen.

To address the second objective, a 240-mL first-milking colostrum sample was aseptically collected directly from the floor bucket within 15 to 30 min of milking the cow. As described in the previous paragraph, a 15-mL aliquot from the 240 mL was taken and immediately frozen for use under objective 1. The remaining large volume was then divided into 12 identical aliquots of approximately 15 mL each. These aliquots were then randomly allocated to 1 of 4 different treatment groups:

Group 4. Samples 4a, 4b, 4c: Stored in refrigerator (4°C).
Group 5. Samples 5a, 5b, 5c: Stored at ambient temperature.
Group 6. Samples 6a, 6b, 6c: Potassium sorbate (0.5% wt/vol) and refrigerated (4°C).
Group 7. Samples 7a, 7b, 7c: Potassium sorbate (0.5% wt/vol) and stored at ambient temperature.

For samples in groups 6 and 7, a 50% potassium sorbate solution was mixed with colostrum immediately after sample collection to create a 0.5% solution (wt/

vol). For all 4 treatment groups, subsamples a, b, and c were frozen after 24, 48, and 96 h of storage, respectively. Local weather service data reported that the average daily temperature for the study period was 23°C (range: 19.6 to 26.8°C).

Laboratory Analyses

All frozen colostrum samples were submitted to the Laboratory for Udder Health, University of Minnesota (St. Paul, MN) where they underwent microbiological culture procedures to determine TPC, total coliform count (**TCC**), and testing for sample pH. After the colostrum samples were thawed and thoroughly mixed, serial 10-fold dilutions of the colostrum were made in sterile brain-heart infusion broth. Two hundred microliters of colostrum from each dilution was then pipetted onto MacConkey agar and spread over the entire surface. The plates were incubated at 37°C for 24 h, and all pink colonies (lactose fermenters) were counted. Colonies were confirmed as coliforms using the API 20E coliform identification test (bioMerieux, Inc., Hazelwood, MO). The same serial 10-fold dilutions of colostrum in brain-heart infusion broth were used for TPC determination. Two hundred microliters of each dilution was placed on the surface of plate count agar plates and spread as above. The plates were incubated at 32°C for 48 h and all colonies counted. An Orion pH meter (Orion Research, Cambridge, MA) was used to perform pH testing of colostrum samples.

Statistical Analyses

Final analysis was completed for 39 of the 41 cows sampled. All samples from 2 cows were omitted from analysis because there were significantly higher coliform counts in samples collected directly from the udder (1.0×10^7 cfu/mL) than in subsequent samples collected from the milking bucket (2.6×10^6 cfu/mL), leading to concerns about gross contamination of the first sample during or after the sample collection process.

Critical Contamination Points During Colostrum Harvest and Feeding

Descriptive statistics were produced to describing the mean, SD, and range of the $\log_{10}$ transformed values for the TPC and TCC in the colostrum samples from the udder, the floor bucket, and the esophageal tube feeder (Table 1). An ANOVA was used (Proc Mixed; SAS Institute, 2000) to describe the relationship between the explanatory variable of interest, sample source (udder, bucket, or esophageal feeder) and the 2 dependent variables of interest, $\log_{10}$(TPC, cfu/mL) and $\log_{10}$(TCC, cfu/mL). A variable describing "cow" was included as a random effect in the model to control for multiple samples clustered within cow. Statistical significance was declared at $P < 0.05$.

Effect of Refrigeration and Preservative on Bacterial Counts in Stored Fresh Colostrum

Descriptive statistics were produced to describe the mean, SD, and range of the $\log_{10}$ transformed values for the TPC and TCC, plus mean, SD, and range of the sample pH measures, for the 4 treatment groups after 24, 48, and 96 h of storage (Tables 1 and 2). Multivariate ANOVA was then used (Proc Mixed; SAS Institute, 2000) to describe the relationship between the explanatory variable of interest, storage treatment group (4 = refrigeration, 5 = ambient temperature, 6 = refrigeration plus preservative, 7 = ambient temperature plus preservative), and the 3 dependent variables of interest: $\log_{10}$ TPC (cfu/mL), $\log_{10}$ TCC (cfu/mL), and sample pH, and controlling for sampling time (24, 48, and 96 h) as a covariate in the model. The presence of an interaction between treatment and time was investigated. A variable describing "cow" was included as a random effect in these models to control for multiple samples clustered within each cow. Statistical significance was declared at $P < 0.05$.

RESULTS

Critical Contamination Points During Colostrum Harvest and Feeding

Bacteria counts were very low in samples collected directly from the udder, with 100% of samples collected meeting the goal for the maximum TPC to be fed to calves at <100,000 cfu/mL. However, there was a significant increase in bacteria counts in samples collected directly from the floor bucket (Table 1, Figure 1). There was no difference in TPC or TCC between samples collected from the floor bucket vs. the esophageal feeder tube ($P > 0.05$). Only 64% (23/39) of samples collected from the esophageal feeder tube had a TPC less than 100,000 cfu/mL.

Effect of Refrigeration and Preservative on Bacterial Counts in Stored Fresh Colostrum

Multivariate analysis indicated the presence of a significant interaction between storage method (treatment groups 4, 5, 6, or 7) and storage period (24, 48, or 96 h). Thus, the data was subsequently stratified by storage period and reanalyzed to describe the effect of treatment. When examining TPC in untreated samples stored at ambient temperature (group 5), bacteria

Table 1. Description of bacterial counts in colostrum samples by treatment group and storage conditions.

Treatment group	Source	Total plate count, $\log_{10}$ (cfu/mL)			Total coliform count, $\log_{10}$ (cfu/mL)		
		Mean	SD	Range	Mean	SD	Range
1	Udder	1.44	1.45	0–4.78	0.90	1.20	0–4.42
2	Esophageal feeding tube	4.66	1.89	2.0–8.75	4.45	2.03	0.70–8.56
3	Floor bucket	4.99	1.95	2.0–9.25	4.71	2.17	0–9.16
4a	Refrigerated, 24 h	5.75	1.93	2.0–9.01	5.50	2.06	2.18–8.91
4b	Refrigerated, 48 h	6.03	1.76	2.70–9.36	5.55	2.02	0–8.94
4c	Refrigerated, 96 h	6.17	1.80	3.0–9.57	5.91	1.80	2.95–9.01
5a	Ambient temperature, 24 h	7.26	0.80	6.09–9.19	6.39	1.30	3.34–9.10
5b	Ambient temperature, 48 h	6.62	0.83	4.54–8.62	4.96	1.84	0–8.60
5c	Ambient temperature, 96 h	6.63	0.86	4.05–8.26	4.48	1.80	0–7.89
6a	Refrigerated + preservative, 24 h	3.60	1.63	0–8.24	3.14	1.72	0–7.78
6b	Refrigerated + preservative, 48 h	3.64	1.45	1.40–7.67	3.39	1.64	0–7.71
6c	Refrigerated + preservative, 96 h	3.55	1.64	0–7.59	3.39	1.77	0–7.72
7a	Ambient + preservative, 24 h	5.43	1.35	3.08–9.25	4.71	1.11	1.85–6.70
7b	Ambient + preservative, 24 h	6.48	0.80	4.60–7.88	5.02	0.98	1.70–6.55
7c	Ambient + preservative, 24 h	6.54	0.92	4.06–8.84	2.87	1.13	0–5.20

counts increased most rapidly during the first 24 h, followed by a slight decrease and then leveling off by 96 h (Table 1, Figure 2). Moderate and similar increases in bacteria counts over the entire 96-h period were observed for untreated samples stored using refrigeration (group 4), and for preserved samples stored at ambient temperature (group 7). After 96 h of storage, there was no difference in TPC among treatment groups 4, 5, and 7 (Figure 2). By contrast, the TPC in samples stored using potassium sorbate preservative and refrigeration had dropped by 24 h and stayed low and constant over the duration of the 96-h study period (group 6). The TPC in this treatment group was lower than in any other treatment group at 24, 48, and 96 h of storage (Table 1, Figure 2).

When examining the TCC in untreated samples stored at ambient temperature, counts increased most rapidly during the first 24 h followed by a significant drop after 48 and 96 h (group 5) (Table 1, Figure 3). Total coliform counts rose moderately during the first 24 h in untreated refrigerated samples and continued to rise very slowly until 96 h (group 4). Total coliform counts in preserved samples stored at ambient temperature remained relatively unchanged during the first 48 h, but then dropped by 96 h (group 7). Total coliform counts in preserved refrigerated samples (group 6) dropped significantly by 24 h and stayed low and constant over the duration of the 96-h study period (group 6). Coliform counts in the latter treatment group were lower than for any other treatment group at 24, 48, and 96 h of storage, with the exception of samples in treatment group 7 at 96 h (Table 1, Figure 3).

The mean (SD) pH in the bucket sample (0 h) was 5.59 (0.39) (Table 2, Figure 4). The pH in samples stored using potassium sorbate preservative and refrigeration rose slightly after 24 h and then remained constant (group 6), whereas the pH levels remained relatively unchanged in untreated refrigerated samples throughout the entire 96-h study period (group 4) (Table 2, Figure 4). By contrast, sample pH fell significantly in any samples stored at ambient temperature; after 24 h of storage for untreated samples stored at ambient temperature (group 5), and after 48 h of storage for preserved samples stored at ambient temperature (group 7) (Table 2, Figure 4).

DISCUSSION

Critical Contamination Points During Colostrum Harvest and Feeding

If feeding clean colostrum is an important factor influencing calf health, then the first step in achieving this goal must be to prevent contamination during the harvest and feeding processes. Although this study was limited to evaluating the practices of only one farm, it is the first, to our knowledge, to identify the critical control points for bacterial contamination during the colostrum harvest and feeding processes, and to quantify the degree of contamination occurring. The study demonstrated that most colostrum samples collected aseptically directly from the gland have extremely low numbers of bacteria present, and frequently no growth. However, the results showed a significant increase in TPC and TCC after harvesting of that colostrum into a milking bucket. This identifies the harvesting process as a significant control point for contamination of colostrum. Possible sources of such contamination could include the teat skin, milking cup liners, hoses, or floor bucket. Such results indicated that an improvement in the udder preparation routine or improvement in the sanitation procedure for the milking equipment might

Table 2. Description of mean, SD, and range of pH for colostrum samples by treatment group and storage conditions.

		Storage period							
		0 h		24 h		48 h		96 h	
Group	Storage	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
4	Refrigerated	5.59 (0.39)	4.14–6.08	5.46 (0.44)	3.87–6.08	5.52 (0.48)	4.00–6.09	5.51 (0.48)	3.95–6.09
5	Ambient temperature	5.59 (0.39)	4.14–6.08	5.14 (0.52)	3.95–5.87	4.93 (0.41)	4.17–6.11	4.40 (0.45)	3.97–6.10
6	Refrigerated + preservative	5.59 (0.39)	4.14–6.08	5.79 (0.22)	4.98–6.16	5.81 (0.16)	5.32–6.14	5.79 (0.19)	5.13–6.13
7	Ambient temperature + preservative	5.59 (0.39)	4.14–6.08	5.64 (0.32)	4.64–6.09	5.43 (0.31)	4.60–5.86	4.96 (0.25)	4.50–5.40

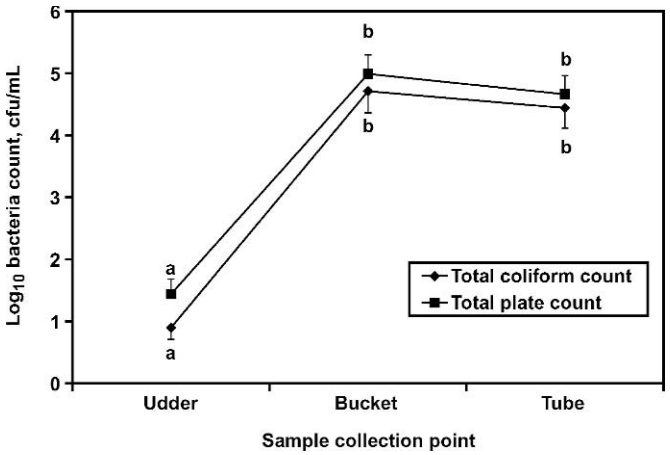

Figure 1. Mean $\log_{10}$ total plate count and mean $\log_{10}$ total coliform count for colostrum samples collected from the udder, the milking bucket, and the esophageal feeder tube. [a,b]Different subscripts differ within bacteria type group, $P < 0.05$.

be required on the study farm. The current study did not show additional contamination occurring during the feeding process (e.g., from the esophageal feeder tube), although it is possible that this could serve as an additional source of contamination on dairy farms if feeding instruments are not properly sanitized between uses. The information from this study has provided a first step in identifying the important control points that researchers and producers should address. Further research is needed to describe the amount and variation in the degree of bacterial contamination that occurs on a large sample of commercial dairy farms, to establish realistic and scientifically based targets for maximum levels of bacterial contamination occurring during the

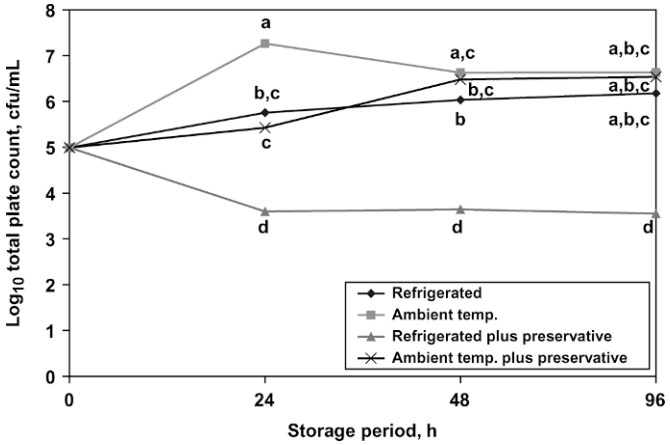

Figure 2. Effect of storage method over time on mean $\log_{10}$ total plate count in fresh bovine colostrum. [a,b,c,d]Different subscripts differ within given storage period, $P < 0.05$.

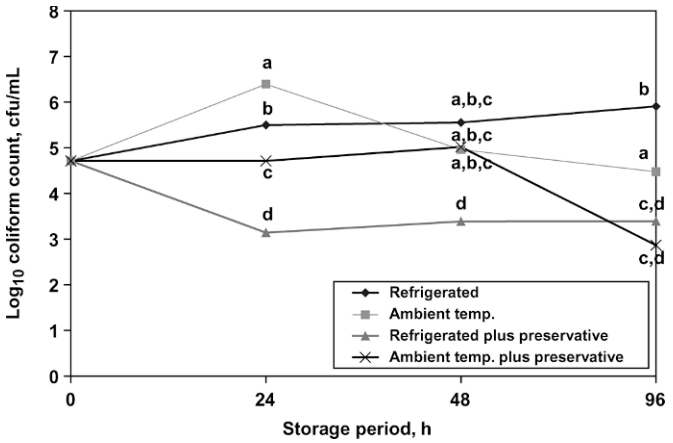

Figure 3. Effect of storage method over time on mean $\log_{10}$ total coliform count in fresh bovine colostrum. [a,b,c,d]Different subscripts differ within given storage period, $P < 0.05$.

harvest and feeding processes, and to develop and validate a set of best management practices that producers can implement to prevent bacterial contamination during these processes.

Storage at Ambient Temperature vs. Refrigeration

The results of the current study are comparable to previous findings in that the total microbial population in untreated colostrum stored at warm ambient temperatures multiplied rapidly early on, and then either leveled off or diminished. In a previous study of naturally fermented bovine colostrum stored at room temperature for 10 d, the TPC was found to increase over the first 4 d of storage (Sukumaran and Subrahmanyam, 1980). Palmer and Mudd (1972) reported an increase in the TPC during the first 3 d of storage, followed by a leveling off or decrease in number.

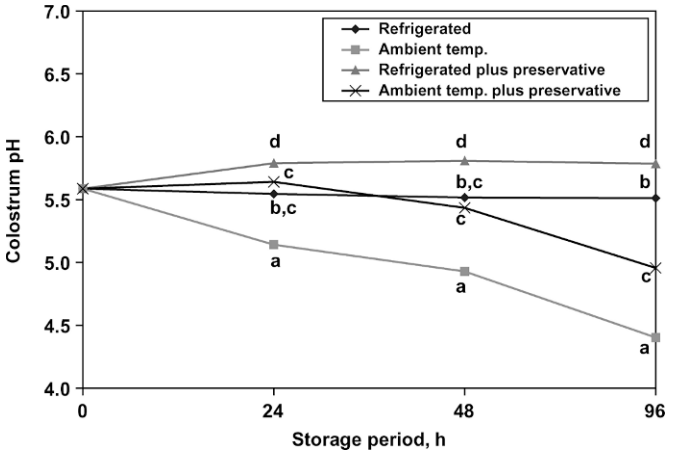

Figure 4. Effect of storage method over time on pH in fresh bovine colostrum. [a,b,c,d]Different subscripts differ within given storage period, $P < 0.05$.

In the current study, the TCC in the samples stored at warm ambient temperatures also rose significantly during the first 24 h and then declined, with concentrations at the end of the 96 h storage period being similar to the initial counts at time zero. This was similar to other studies that reported an initial increase in coliforms, followed by a marked decrease after 3 to 5 d of storage (Roy, 1964; Palmer and Mudd, 1972; Sukumaran and Subrahmanyam, 1980).

The current study showed that bacterial growth for both TPC and TCC was significantly delayed in untreated samples stored in the refrigerator (vs. warm ambient temperature). However, by 48 and 96 h of storage, there was no significant difference in the TPC between these 2 treatment groups. By contrast, the TCC in untreated samples stored at ambient temperature were lower after 96 h of storage compared with TCC in untreated samples stored in the refrigerator. These results show that refrigeration is effective at retarding pathogen growth. However, this benefit was short-lived. As such, it may be possible that producers storing untreated colostrum in the refrigerator should still strive to feed it as quickly as possible (e.g., within 1 to 2 d), as compared with the 1-wk guideline commonly followed in the industry. More studies are needed to further investigate this hypothesis and its relationship to calf health.

In this study the drop in TCC observed in untreated samples stored at ambient temperature (group 5) was correlated with the significant drop in pH that ensued by 24 h, due to onset of the fermentation process. It appeared that initiation of fermentation was delayed slightly, but still occurred, in preserved samples stored at ambient temperature (group 7), as evidenced by a significant drop in pH by 48 h of storage in this group of samples. Many earlier studies have reported a similar initial rapid growth period followed by a decrease in coliform bacteria counts associated with the increased acidity of fermented colostrum (Palmer and Mudd, 1972; Thompson and Marth, 1976). Some readers might interpret these results to infer that producers should intentionally allow fresh colostrum to ferment, before feeding to newborn calves, to reduce coliform exposure. However, the authors suggest that producers may want to avoid fermentation of stored fresh colostrum for the following 3 reasons: First, the current study showed that, even after fermentation of untreated colostrum stored at ambient temperature for 96 h, coliform counts remained relatively high [mean (SD) $\log_{10}TCC_{96h} = 4.48$ (1.80) cfu/mL]. Thompson and Marth (1976) also reported that coliform counts were still relatively high

(10^4 to 10^6 cfu/mL) even after 21 d of storage. Second, it has been documented that counts of some pathogens other than coliforms may remain high (e.g., *Salmonella typhimurium*) or even increase (e.g., *Salmonella dublin*) after 5 d in acidic fermented bovine colostrum (Palmer and Mudd, 1974). Finally, some studies have reported that newborn calves absorbed smaller amounts of Ig from fermented colostrum than from fresh colostrum or colostrum stored by freezing (Snyder et al., 1974; Foley et al., 1978).

Use of Potassium Sorbate Preservative

In this study, treatment of colostrum with potassium sorbate preservative slowed the rate of bacterial growth and prevented or delayed the fermentation process in treated samples stored at ambient temperature (group 7). However, by far the most successful treatment tested was treatment with potassium sorbate preservative combined with refrigeration. Total coliform counts and TPC in these samples were significantly reduced by 24 h, and stayed stable throughout the 96-h study period. Sample pH measures were highest, and remained constant over 96 h, in these samples. These results agree with several previous studies reporting that chemical treatment with such additives as formaldehyde, propionic acid, potassium sorbate, sorbitol, or sodium benzoate, will retard or prevent bacterial and coliform growth in fermented colostrum (Palmer and Mudd, 1972; Muller and Smallcomb, 1977; Rindsig et al., 1977). In a comprehensive review of the literature on the long-term storage of fermented colostrum, Foley and Otterby (1978) recommended the use of chemical preservatives if fermented colostrum was to be stored at warm temperatures. However, the results of this study would suggest that there are additive benefits to combining the use of a potassium sorbate preservative with refrigeration of fresh colostrum to be stored for short periods of time (up to 96 h).

CONCLUSIONS

This study demonstrated that the colostrum harvest process is a significant critical control point for bacterial contamination of first-milking colostrum. Storing untreated colostrum at warm ambient temperatures resulted in the most rapid increase in bacterial counts, followed by intermediate rates of growth in untreated samples stored using refrigeration, or samples treated with potassium sorbate preservative and stored at ambient temperature. The most effective treatment was the use of potassium sorbate preservative combined with refrigeration, in which TPC and TCC dropped significantly and then remained constant during the 96-h storage period. Future studies will be necessary to describe the relationship between bacterial counts in colostrum and subsequent calf health and to define science-based recommendations for bacteria levels in fresh colostrum fed to calves. Moreover, studies are needed to compare the effectiveness of different types and doses of preservative agents, to describe the shelf life of preserved and refrigerated fresh colostrum, and to describe the cost-benefit of using potassium sorbate preservative in stored fresh colostrum.

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