Heat treatment of colostrum on commercial dairy farms decreases colostrum microbial counts while maintaining colostrum immunoglobulin G concentrations

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ABSTRACT

This study was conducted on 6 commercial dairy farms in Minnesota and Wisconsin to describe the effect of heat treatment (at 60°C for 60 min) on colostrum, on colostrum bacteria counts, and immunoglobulin G concentrations. First-milking colostrum was collected each day, pooled, divided into 2 aliquots, and then 1 aliquot was heat treated in a commercial batch pasteurizer at 60°C for 60 min. Frozen samples of pre- and post-heat-treated colostrum were submitted for standard microbial culture (total plate count and total coliform count, cfu/mL) and testing for immunoglobulin G concentrations (mg/mL). Data were analyzed from 266 unique batches of colostrum. Linear regression showed that heat treatment decreased colostrum total plate counts ($-2.25 \log_{10}$) and coliform counts ($-2.49 \log_{10}$), but, overall, did not affect colostrum IgG concentration. Though higher-quality batches of colostrum did experience a greater magnitude of loss of IgG as a result of heat treatment as compared with lower- or intermediate-quality batches of colostrum, the colostrum IgG concentrations in these batches remained high overall, and within acceptable limits for feeding. This study demonstrates that batch heat treatment of colostrum at 60°C for 60 min can be successfully conducted on commercial dairy farms by farm staff to decrease colostrum microbial counts while maintaining colostrum IgG concentrations.

Key words: colostrum, immunoglobulin, bacteria, heat treatment

INTRODUCTION

The neonatal calf relies on the passive absorption of colostrum Ig within the first few hours after birth to provide protection against infectious disease challenge early in life (Banks, 1993). Successful passive transfer in a calf is defined as having a serum IgG concentration ≥ 10 mg/mL at 24 to 48 h of age and is associated with such benefits as decreased risk for morbidity and mortality in the preweaning period, improved daily weight gain and feed efficiency, lower 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; Faber et al., 2005; Weaver et al., 2000).

Despite the aforementioned benefits, colostrum is a potential means of exposure to microbial pathogens for the newborn calf. Microorganisms can occur in colostrum by secretion from the mammary gland; contamination during milking, storage, or feeding; or by bacterial proliferation in stored colostrum (Fecteau et al., 2002; McGuirk and Collins, 2004; Johnson et al., 2007). *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., 1995; González and Wilson, 2003; CDC, 2008; Houser et al., 2008). Furthermore, some studies have reported that high concentrations of bacteria in colostrum may be associated with decreased Ig absorption, thereby potentially contributing to failure of passive transfer (James et al., 1981; Poulsen et al., 2002). As such, experts have recommended that a goal should be to collect and feed colostrum with fewer than 100,000 cfu/mL total plate count (McGuirk and Collins, 2004). However, 2 separate observational studies of commercial Midwest dairy herds reported that 82 and 92% of colostrum samples tested exceeded the 100,000 cfu/mL total plate count limit, indicating suboptimal microbial quality of much of the colostrum fed to dairy calves (Poulsen et al., 2002; Swan et al., 2007).

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Heat treatment may be one approach to decrease microbial contamination in colostrum. Ragsdale and Brody (1923) reported that colostrum can be safely heated at 60°C for up to 3 h 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 (PMO) pasteurization temperatures, ranging from 62.5 to 73°C, yielded unacceptable IgG denaturation and changes in viscosity (Meylan et al., 1996; Tyler et al., 2000; Godden et al., 2003). However, laboratory-based experiments showed that heat treatment at 60°C for 60 min did not negatively affect colostrum IgG concentration or viscosity, but significantly decreased or eliminated inoculated pathogens, including *Mycoplasma bovis*, *Listeria monocytogenes*, *E. coli*, *Salmonella enteritidis*, and MAP (Godden et al., 2006; McMartin et al., 2006). This heat-treatment protocol has since been validated in 2 university trials (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009). However, these trials were conducted by university personnel under tightly controlled conditions, in individual dairy herds, and with 25 or fewer unique batches of colostrum per study. Larger studies are lacking to determine if the same results can be achieved if the colostrum heat-treatment procedure is performed by herd staff on commercial dairy farms. The objective of the current study was to describe the effect of heat-treating colostrum at 60°C for 60 min on colostrum characteristics when using batch pasteurizers on commercial dairy farms.

MATERIALS AND METHODS

Farm Enrollment

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 (approx. 250-km radius), willingness to adhere to study protocols, and having had at least 1 animal in the herd test positive for infection with MAP by fecal culture or serum ELISA within the previous 3 yr.

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. Daily, or on alternate days, refrigerated colostrum was pooled into 1 batch, thoroughly mixed, and divided into equal aliquots.

One aliquot was left fresh (**FR**), whereas the other was heat treated (**HT**) in a commercial batch pasteurizer at 60°C for 60 min (Dairy Tech 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 (1°F) during the 60-min holding phase. Colostrum was then automatically cooled to 15.6°C (60°F). 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 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. Fresh and HT colostrum was transferred to sanitized 3.8-L bottles and refrigerated for later feeding to newborn calves.

Colostrum Sample Analysis

University technicians visited study farms once per week to collect records and frozen colostrum samples. Colostrum samples were transported on ice to the Veterinary Diagnostic Laboratory, University of Minnesota (St. Paul). Frozen pre- and post-heat-treated colostrum samples were thawed and the IgG concentration (mg/mL) determined by turbidimetric immunoassay (Etzel et al., 1997). The duplicate colostrum samples were submitted to the Laboratory for Udder Health (University of Minnesota) for bacterial culture. Colostrum samples were thawed to 4°C, vortexed, and serially diluted 1:10 for 5 dilutions. Each dilution was plated on plate count agar for total plate count (**TPC**) and 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

All analyses were performed using SAS software (version 9.1; SAS Institute Inc., Cary, NC), with final significance declared at $P < 0.05$. Bacteriological data was not normally distributed so TPC and TCC measures were $\log_{10}$ transformed before analysis. Simple linear regression (PROC MIXED) was used to analyze the effect of heat treatment on the 3 outcome measures of interest; colostrum IgG concentration (mg/mL), TPC ($\log_{10}$ cfu/mL), and TCC ($\log_{10}$ cfu/mL). Farm name was forced as a fixed effect into all models to control for the clustering of batches within farm and to examine if a farm effect existed. Model fit was assessed by exam-

ining the residual log-likelihood value. The statistical model used was

$$Y = \beta_0 + \beta_1 T + \beta_2 F + \beta_3 T \times F + \varepsilon,$$

where Y is the outcome variable [colostrum IgG, $\log_{10}(\text{TPC})$, or $\log_{10}(\text{TCC})$], β_0 is the intercept, β_{1-3} are the parameter estimates for the explanatory variables offered, T is the colostrum-treatment group (HT or FR), F is the farm name (1 through 6), $T \times F$ is the interaction term for colostrum treatment and farm, and ε is the error term of the general liner model. Because a significant treatment by farm interaction was found for the models predicting the outcomes TPC and TCC, the data were stratified and analyzed by farm.

The aforementioned analysis showed that, overall, colostrum IgG concentrations were not different in HT versus FR samples. However, when the authors calculated the magnitude of change in IgG concentration after heat treatment for each batch (Change = FR – HT, mg/mL), a positive correlation was observed between the initial IgG concentration and the magnitude of change in IgG concentration after heat treatment ($R^2 = 0.36$, $P < 0.001$), suggesting the presence of an interaction between initial IgG concentration and treatment when looking at the effect of heat treatment on IgG loss. To investigate this relationship further, the MIXED procedure in SAS was used to evaluate the relationship between initial colostrum IgG concentration (explanatory variable) and magnitude of change of IgG after heat treatment (dependent variable), where the magnitude of IgG change was expressed either as an absolute difference (mg/mL) or as a percentage difference (%), when initial IgG concentration was included in the model statement as a categorical variable after being classified into 1 of 5 categories: 1) ≥ 80 mg/mL, 2) 70 to 79.9 mg/mL, 3) 60 to 69.9 mg/mL, 4) 50 to 59.9 mg/mL, and 5) < 50 mg/mL of IgG. Category 4 (IgG 50 to 59.95 mg/mL) was used as the referent category because previous studies have reported this to be a cut point for differentiating high versus low quality colostrum (Maunsell et al., 1999). Herd was included in the model as a fixed effect to control for clustering of batches within herd.

RESULTS

Farm Demographics

Mean (SD; range) herd size, rolling herd average milk yield, 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 with predominantly Holstein cows, although a few crossbreeds were represented. Five farms used group maternity pens, whereas 1 farm used individual maternity pens.

Effect of Heat Treatment on Colostrum Characteristics

Of a total of 276 unique batches of colostrum were processed by personnel on study farms. Complete (pair-matched) data were available from 266 unique batches for inclusion in the statistical analysis. Mean IgG, $\log_{10}\text{TPC}$, and $\log_{10}\text{TCC}$ measures in FR colostrum were 60.7 mg/mL, and 5.4 and 4.4 cfu/mL, respectively (Table 1). Heat treatment significantly decreased TPC and TCC (Table 1). This effect was generally consistent across all herds, except that a numeric, though nonsignificant, decrease in TCC occurred for farm 3.

Heat treatment did not change colostrum IgG concentration overall; the mean IgG change after heat treatment was -1.5 mg/mL; $P = 0.38$; Table 1). This was consistent across all farms. However, further analysis showed that higher-quality batches of colostrum did experience greater IgG loss as compared with low- or intermediate-quality batches. For example, when IgG change was expressed as an absolute change (mg/mL), batches of colostrum with ≥ 70 to 79.9 mg/mL initial IgG concentration experienced a 6.7 mg/mL or 8.8% loss of IgG after heat treatment, and batches of colostrum with ≥ 80 mg/mL initial IgG concentration experienced a 9.8 mg/mL or 9.8% loss of IgG after heat treatment. These changes in IgG were significantly different than for batches of colostrum with average initial IgG concentrations (50 to 59.9 mg/mL; -1.0 mg/mL or -1.9% loss; Table 2). Only 5.6% ($n = 15$) of batches assembled contained > 100 mg of IgG/mL.

DISCUSSION

Laboratory studies have reported that batch heat treatment of colostrum at 60°C for 60 min preserved colostrum IgG concentrations and feeding characteristics while significantly decreasing or eliminating bacterial pathogens (Godden et al., 2006; McMartin et al., 2006). These findings were verified in 2 separate clinical trials, each conducted on individual dairies by university personnel (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009). The current 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 TPC by 2.25 $\log_{10}$ and decreased TCC by 2.49 $\log_{10}$, without decreasing overall colostrum IgG con-

Table 1. Effect of heat treatment on colostrum characteristics

Parameter	No. of batches	Fresh ¹	Heat-treated ¹	Difference ²	P-value
Colostrum IgG (mg/mL)					
All farms	266	60.7 (1.3, 9.8–139.9)	59.2 (1.3, 12.1–139.5)	–1.5 (1.7)	0.38
Farm 1	56	53.6 (3.0, 18.2–131.1)	52.2 (2.9, 14.9–103.6)	–1.4 (4.2)	0.73
Farm 2	44	56.8 (2.6, 23.8–108.1)	55.7 (2.5, 21.9–117.8)	–1.0 (3.6)	0.77
Farm 3	73	54.0 (2.4, 9.8–100.5)	53.7 (2.3, 12.1–115.9)	–0.3 (3.3)	0.93
Farm 4	35	77.6 (4.2, 28.2–139.9)	74.6 (3.8, 31.3–139.5)	–3.0 (5.7)	0.60
Farm 5	25	66.2 (3.3, 39.3–107.8)	63.5 (2.9, 36.4–99.4)	–2.7 (4.4)	0.54
Farm 6	33	70.5 (2.5, 38.9–101.9)	68.1 (2.9, 35.7–107.2)	–2.4 (3.8)	0.54
Total plate count (log ₁₀ cfu/mL)					
All farms	266	5.4 (0.06, 2.6–9.1)	3.6 (0.07, 1.0–8.4)	–2.25 (0.23)	<0.0001
Farm 1	56	5.3 (0.13, 2.6–6.8)	4.0 (0.15, 1.1–6.4)	–1.31 (0.19)	<0.0001
Farm 2	44	5.0 (0.13, 3.2–6.7)	3.1 (0.12, 1.2–5.4)	–1.87 (0.18)	<0.0001
Farm 3	73	5.6 (0.10, 3.0–9.1)	4.3 (0.13, 1.9–8.4)	–1.27 (0.17)	<0.0001
Farm 4	35	5.1 (0.14, 3.6–6.3)	2.7 (0.12, 1.2–5.4)	–2.38 (0.18)	<0.0001
Farm 5	25	5.9 (0.18, 3.8–7.3)	3.1 (0.18, 2.1–5.2)	–2.83 (0.25)	<0.0001
Farm 6	33	5.6 (0.14, 4.0–6.8)	3.3 (0.17, 1.5–6.0)	–2.25 (0.22)	<0.0001
Total coliform count (log ₁₀ cfu/mL)					
All farms	266	4.4 (0.08, 0–6.8)	2.3 (0.10, 0–7.0)	–2.49 (0.31)	<0.0001
Farm 1	56	4.9 (0.15, 2.0–3.4)	3.4 (0.19, 0–6.6)	–1.52 (0.24)	<0.0001
Farm 2	44	4.1 (0.17, 1.9–6.8)	0.9 (0.16, 0–3.6)	–3.21 (0.23)	<0.0001
Farm 3	73	3.7 (0.19, 0–6.3)	3.4 (0.17, 0–7.0)	–0.30 (0.26)	0.25
Farm 4	35	4.6 (0.15, 2.8–6.1)	0.6 (0.16, 0–3.4)	–3.95 (0.22)	<0.0001
Farm 5	25	5.3 (0.26, 2.5–6.8)	2.2 (0.18, 0–3.9)	–3.18 (0.32)	<0.0001
Farm 6	33	4.3 (0.18, 2.4–6.4)	1.8 (0.21, 0–5.0)	–2.49 (0.27)	<0.0001

¹Crude means (SE, range) reported.²Difference of least squares means (SE).

centration (mg/mL). In theory, decreasing bacterial exposure to calves through colostrum while maintaining colostrum quality (IgG concentrations) should result in a healthier calf. This hypothesis will be examined in an accompanying manuscript.

A novel finding of the current study was the positive relationship between colostrum quality and magnitude of IgG loss when heat treating colostrum. Specifically, when expressed either as an absolute change (mg/mL) 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). Previous studies may have been too small

(i.e., too few batches of colostrum) or lacked sufficient variation in initial IgG concentrations among colostrum batches to be able to investigate and detect this relationship. Despite the presence of this relationship, no dairies reported excessive thickening or congealing of colostrum batches after heat treatment. Furthermore, despite the IgG decrease of 6.7 to 9.8 mg/mL or 8.8 to 9.8% that was observed in high and very high quality batches, respectively, the colostrum IgG concentrations in these batches remained high overall, and certainly within acceptable limits.

An explanation for why very high quality batches of colostrum experienced a greater magnitude of IgG loss when heat-treated is not presently available. It is un-

Table 2. Relationship between initial colostrum quality and magnitude of change in IgG concentrations associated with heat treatment of colostrum

Item	No. (%) of batches	Fresh colostrum IgG ¹ (mg/mL)	Heat-treated colostrum IgG ¹ (mg/mL)	Change in IgG (mg/mL)	Change in IgG (%)
All batches	266 (100%)	60.7 (21.8)	59.2 (20.7)	1.5 (0.7)	0.2 (1.2)
Categorized by initial IgG concentration					
1) ≥80 mg/mL	39 (14.7%)	97.4 (14.8)	87.7 (18.6)	9.8 (14.8)**	9.8 (14.5)*
2) ≥70 to <80 mg/mL	40 (15.0%)	75.5 (3.0)	68.8 (14.1)	6.7 (14.4)**	8.8 (18.5)*
3) ≥60 to <70 mg/mL	44 (16.5%)	64.5 (3.1)	64.6 (10.5)	–0.09 (9.6)	–0.04 (14.3)
4) ≥50 to <60 mg/mL ²	67 (25.2%)	55.4 (3.1)	56.4 (10.4)	–1.0 (10.2)	–1.9 (18.3)
5) <50 mg/mL	76 (28.6%)	36.4 (9.9)	38.7 (12.5)	–2.3 (7.6)	–7.3 (21.8)

¹Mean (SD) reported.²Referent category.**P* < 0.01; ***P* < 0.001: significantly different from referent category within column (category 4).

derstood that when proteins in general are heat-treated they lose their primary 3-dimensional functional conformation, unfold, and then may aggregate with other whey proteins or casein by disulfide bond linkage, calcium-linked complexes, or hydrophobic interactions (Law and Leaver, 1999). A study that examined the heat stability of IgG as an isolated protein and then in the presence of colostrum found that components within the colostrum milieu further increase both the heat and pressure stability of the IgG molecule (Indyk et al., 2008). Two studies further describe that the unfolding between the 2 distinct regions of the IgG molecule, fraction constant (F_c) and fraction antigen-binding (F_{ab}), occur independently of each other (Vermeer and Norde, 2000; Indyk et al., 2008). Vermeer et al. (2000) reported that the F_{ab} fragment and the F_c fragment of the IgG protein fragmented when heated to 61 and 71°C, respectively. Hartmann et al. (2004) reported that different IgG molecules of murine origin (IgG2a and IgG1) showed different susceptibility to heat. Although the aforementioned studies help to explain some of the IgG denaturation observed when colostrum is heated at high temperatures, these studies offer no obvious explanation for why batches of bovine colostrum with higher initial IgG concentrations experienced a greater magnitude of IgG loss when heat treated as compared with colostrum with low or intermediate initial IgG concentrations in the current study. Clearly, the underlying mechanism to explain this relationship requires further study.

Although this study had several strengths, including the participation of multiple commercial dairy farms and staff, the use of commercial pasteurization equipment, and testing of a relatively large number of batches of colostrum, it had some limitations. Due to budget constraints, colostrum samples were only tested for IgG concentrations, TPC, and TCC, but not tested for other constituents, including specific bacterial pathogens, various nutrients, or immune factors, including leukocyte viability. Elizondo-Salazar and Heinrichs (2009) reported that heat treatment at 60°C for 30 min resulted in some minor changes in nutritional composition, but that final composition was in accordance with values reported for normal colostrum. In a pilot study, Koewler et al. (2010) reported that heat treatment of colostrum using a prototype pasteurizer model resulted in a significant decrease in colostrum leukocyte viability. However, that research was not completed using commercially available on-farm pasteurization equipment. Further research is needed to investigate the full effect of heat treatment on colostrum leukocytes, as well as to investigate the role and clinical significance of these cells to the neonatal calf.

Successful heat treatment of colostrum requires adherence to strict protocols to avoid excessive IgG loss or severe thickening. Important factors include precise temperature control, preventing temperatures from fluctuating more than 1° above the target of 60°C, rapid active heating and cooling of colostrum before and after the holding phase of 60°C, and constant agitation during the heating-up, holding, and cooling-down phases of the process.

CONCLUSIONS

When performed by farm staff on 6 commercial dairy farms, heat treatment of colostrum at 60°C for 60 min significantly decreased both TPC and TCC, but, overall, did not negatively affect colostrum IgG concentration. Though higher quality batches of colostrum did experience a greater magnitude of loss of IgG as a result of heat treatment compared with lower or intermediate quality batches of colostrum, the colostrum IgG concentrations in these batches remained high overall, and within acceptable limits for feeding. Heat-treatment of colostrum at 60°C for 60 min can be successfully conducted on commercial dairy farms to decrease colostrum microbial counts while maintaining colostrum IgG concentrations.

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