Heat-Treatment of Colostrum: Effects on Colostrum Characteristics and on Passive Transfer and Health in Commercial Dairy Calves

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Dedication

This thesis is dedicated to my children,
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CHAPTER 1

A REVIEW OF THE LITERATURE ON COLOSTRUM

INTRODUCTION

Colostrum is the secretions produced by the mammary gland in the prepartum period.[1-3] While colostrum is nutritionally important for all species, it is also important for the passive immunological transfer of immunoglobulins (Ig), leukocytes, and other immune related components.[4-6] The syndesmochorial placenta of ruminants and the epitheliochorial placenta of pigs and horses, results in an agammaglobulinemia in newborns of these species, making them dependent on adequate and timely colostrum consumption to receive maternal Igs.[7, 8] Volume of colostrum fed, concentration of Igs in colostrum, and age at feeding of first colostrum, are established factors affecting the neonatal calf's serum Ig levels at 24 to 48 hours of age.[9-12] In recent years there has been a focus on microbial counts as a measure of hygienic quality of colostrum.[13-15] This literature review will assess the current literature and understanding of colostrum, the passive transfer of Igs, the relationship between colostral microbial counts and serum Ig levels, and the use of the heat-treatment of colostrum as a means to reduce microbial exposure to the neonatal calf and improve serum Ig levels.

PRODUCTION OF COLOSTRUM

Colostrogenesis is the transfer of maternal Igs from serum into mammary secretions. This process begins several weeks before calving and ceases abruptly just prior to or at parturition.[13] The increasing levels of estrogen and decreasing levels of

progesterone coincide with transfer of IgG_1 *via* receptors located on the mammary secretory cells.[16] Prolactin does not appear to have a role in colostrogenesis, but rather lactogenesis. The presence of prolactin has been shown to down regulate the IgG_1 receptor responsible for maternal Ig transfer to the mammary epithelium.[17]

Dry period length, nutrition, breed and vaccination programs are some factors investigated in dairy production medicine for their effects on colostrum production. Decreasing the length of the dry period from the traditional 55 days to 34 did not significantly change Ig concentration in colostrum.[18] Omitting the dry period altogether in both cattle and goats reduces colostral Ig concentrations.[19, 20] When cattle are fed by the NRC guidelines for nutrition, there are no improvements in colostral Ig concentrations by increased supplementation of various nutrients.[13, 21] Breed, parity, and seasonal differences are also reported to affect colostral Ig concentrations.[21-25] Vaccinating the dam improves the concentration of Igs in colostrum against specific pathogens, but does not significantly increase the total concentration of Igs in colostrum.[5, 26]

COLOSTRUM COMPOSITION

Colostrum is a complex mixture of both nutritional and immunological factors adapted for the neonatal calf. Total solids content has been reported to be at 23.9% and 27.6% in first milking colostrum.[1, 2] Colostrum is 6.7% fat and 14.9% protein in first milking colostrum and drops to lactational milk levels, 4.0% and 3.1%, respectively by the sixth milking.[1, 2].The energy supplied in colostrum by fat is important for

thermoregulation and gluconeogenesis for the neonatal calf.[21] The higher protein levels in colostrum are due partially to the high Ig levels, but also to casein, albumin, and other nutritional proteins which supply amino acids so the calf may begin its own protein synthesis and homeostasis.[21] The nutritional importance of fat and water soluble vitamins, minerals, insulin-like growth factors (IGFs) and transforming-growth factors (TGFs), epidermal-growth-factors (EGFs) in colostrum are well established.[1, 2, 6, 21]

Immunoglobulins, leukocytes, cytokines, lactoferrin, and other antimicrobial factors comprise the immunological portion of colostrum. The concentrations of Igs reported in colostrum are 35.0 mg/ml for IgG_1, 6.0 mg/ml for IgG_2, 1.7 mg/ml for IgA, and 4.3 mg/ml for IgM.[2] The concentrations of IgG_1 and IgG_2 are nearly equal in the plasma of the dam, but a receptor on the mammary epithelial cell is specific for IgG_1 and thus this Ig predominates in colostrum.[27-29]

Leukocytes are present in colostrum at approximately 1×10^6 cells/ml with macrophages making up 50%, T-lymphocytes 20%, B-lymphocytes $\leq$ 5%, and neutrophils 25% of leukocytes respectively.[30] Lymphocytes are demonstrated to traffic from the gut-associated-lymphoid-tissue (GALT) to the mammary gland.[4, 31] Although the same is not as expressively demonstrated for macrophages, the GALT is a likely source of origin as well as the circulatory system. Maternal lymphocytes are absorbed by the neonatal intestinal epithelium in pigs, lambs and calves by a mechanism that is not yet clearly elucidated, but is suggested to occur at the intercellular spaces in the epithelium.[32-34] In the case of pigs, the colostral lymphocytes will reach the mesenteric lymph nodes if the colostral leukocytes originate from the dam but not if the cells are

inactivated at 56°C for 20 minutes.[32] Using fluorescent labeling, lymphocytes are absorbed through the intestine *via* colsotrum and appear in the neonatal circulation.[35] Maternally transferred colostral leukocytes may enhance development of the neonatal immune system. An experimental study found an increased density of expression of Major Histocompatibility Complex (MHC) class I expression on neonatal lymphocytes in calves receiving whole colostrum versus those fed colostrum without maternal leukocytes. Additionally, in calves fed cell free colostrum there was an up-regulation of CD11a on the lymphocytes, which is an indicator of an ensuing immune response. A limitation of this study was a small sample size of five calves per group.[36]

In addition to the immunological properties of colostrum summarized above, colostrum contains several antimicrobial factors. Lactoferrin sequesters iron from the surrounding fluid making it less accessible to microbes. Lysozyme, lactoperoxiadase, and xanthine oxioreductase are enzymes known for the lytic and anti-microbial functions.[4, 6] Interleukin-1β (IL-1 β), interleuklin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interferon-γ (INF- -γ) are cytokines reported in colostrum.[37]

PASSIVE TRANSFER OF IMMUNITY

The most substantial transfer of Igs across the intestinal epithelium occurs in the distal jejunum and ileum of the microvillus by the process of micropinocytosis.[38-40] Clathrin, a protein which mediates the endocytosis of macromolecules, is a demonstrated mechanism of maternal Ig transport in the neonatal rat intestine, but its role is less clear in the bovine.[39, 41]Once the Ig molecule is absorbed, it moves *via* the apical tubular

system into the cell where it accumulates by coalescing into vesicles before being exocytosed into the lamina propria.[39, 42] From the lamina propria the Igs pass into the lymphatics and circulation. Colostral Igs are first detected in the lymphatic system one to two hours after feeding, and peak in the serum at thirty-two hours of age.[11, 39] The bovine neonatal intestine can absorb other macromolecules such as human gamma globulin, human albumin, and human serum protein demonstrating a non-specific uptake by the epithelium of the microvillus.[43] The process of vacuole excytosing its Ig contents at the basal laminar membrane does exhibit some specificity since bovine Ig molecules labeled with ferritin are observed in vacuoles near the basal cell membrane, but are not observed in the neonatal circulation.[40, 43] Gut closure is a process by which the neonatal intestine loses its permeability to absorb Igs. This process may begin as early as four hours of age and is complete at twenty-four hours of age.[11, 42] Successful passive transfer of maternal Igs occurs when the neonate's serum measured at 24-48 hours of age is greater than or equal to 10 mg/ml.[12, 44] Conversely, failure of passive transfer (FPT) occurs when the neonate's serum measures less than 10 mg/ml.

Calves with inadequate Ig levels are at greater risk for pre-weaning morbidity and mortality.[45, 46] Producers may accumulate additional losses with decreased weight gains and decreased mature equivalents in milk and fat production in the first lactation.[47-49] In 2007, 40.7% of herds in the United States have at least one calf experience FPT. Currently, the prevalence of failure of passive transfer is 19.2% at the calf level.[50] Radial immunodiffusion (RID) is the gold standard method for determining serum Ig levels. Turbidimetric immunoassay (TIA) is another laboratory method to measure Igs which is

automated and has a high correlation to RID.[8, 51] In dairy production medicine, FPT rates on a herd basis are commonly monitored *via* a hand held refractometer for serum total protein concentration (g/dl), an indirect measure of serum IgG concentration. Failure of passive transfer is not a concern at the herd level when 90% or greater of the animals tested have serum total protein values of 5.0 mg/dl or greater.[52] A serum total protein measurement of 5.0 or 5.2 mg/dl most accurately corresponds to a serum IgG value of 10 mg/ml cutpoint for FPT.[12, 53, 54]

COLOSTRUM MANAGEMENT AND FEEDING

The cutoff value for sufficient colostrum IgG concentration is greater than 50 g/L. The colostrum IgG concentration may be assessed on the farm by either hydrometer or refractometer. These methods each provided similar sensitivities of 0.75 and were superior to weighing the first milking. Weight of first milking is of such low sensitivity, its use cannot be recommended to predict colostrum IgG concentration.[12, 55-57] A commercially available immunoassay kit is available to screen colostrum Ig concentration with a sensitivity of 93% and specificity of 76%.[58]

Timing of the first colostrum feeding, volume of colostrum fed, concentration of Ig in colostrum, and method of feeding are the main established management factors to improve the successful transfer of Igs to the neonate. Since gut closure may begin as early as 12 hours of age, recommendations are to feed colostrum within the first two hours of life. The greatest rate of colostral Ig absorption occurs in the first four hours of

life and rapidly tapers after 12 hours of age.[10] Additionally, calves fed colostrum at greater than four hours of age have 2.65 greater odds for failure of passive transfer.[50]

The colostrum IgG concentration (g/L) and the volume (L) of colostrum fed together determine the total mass of Igs the calf is offered. Moreover, colostral Igs are more efficiently absorbed at higher concentrations as shown by varying the volume of colostrum fed by concentration.[59] In one study, when one liter of colostrum with an Ig concentration of 50 mg/ml was fed within two hours of birth the calf had a 90% probability of FPT. If the volume was increased to two or more liters the probability of FPT decreased to 17% using a more stringent cutoff value of for failure of passive transfer of 13.4 mg/ml ($\leq$ 5.5 g/dl TP).[60] If the concentration of colostrum is improved, then FPT rates are further reduced. It has been suggested that 150 to 200 grams of colostral Igs is the mass need to be fed to prevent FPT in a majority of calves.[60, 61]

Suckling the dam was conventionally believed to be the preferred method of feeding colostrum since the suckling reflex causes the closure of the esophageal groove thereby directing the colostrum or milk directly to the abomasum. However, high FPT rates have been reported in observational studies when calves were allowed to nurse the dam.[50, 62] In one observational study, after adjustments were made for colostral Ig concentrations, there was no difference between the FPT rates between calves fed by bottle versus esophageal feeder.[50] A recent experimental study demonstrated no difference in serum Ig concentrations between tube and bottle fed calves when three liters of a lacteal derived colostrum replacer were fed totaling 200 grams of IgG. In the same study however, if the volume of colostrum replacer product was reduced to 1.5 liters and

100 grams of IgG then there was a significant reduction in serum IgG in calves fed by tube *versus* a bottle.[63]

Pooling of colostrum is discouraged for several reasons. First, while the objective is to minimize the effects of colostrum with low Ig concentrations, it actually dilutes more highly concentrated colostrum.[12] Second, since pathogens such as *Mycobacterim avium* subsp. *paratuberculosis* and *Salmonella* are reported in milk, there is the potential to transmit these pathogens.[64, 65] Recently an observational study showed no significant difference in FPT rates in calves who received pooled colostrum.[50]

To avoid the potential spread of pathogens, or when maternal colostrum is unavailable, colostrum replacer products are commercially available. Colostrum replacers are a substitute for maternal colostrum and are labeled to aid in the prevention of FPT. Efficacy of colostrum replacer products to prevent FPT may vary by the specific product used and with the dose of IgG provided in the product.[61] Colostrum supplements provide additional Igs when colostral Ig concentrations are low.[66]

MANAGEMENT TO REDUCE MICROBIAL EXPOSURE

Traditionally, colostrum quality is understood to refer to the Ig concentration in colostrum. In recent years attention has also focused on the hygienic quality of colostrum and milk in terms of microbial counts and their potential effects on the neonatal calf. Microbial contamination may occur by secretion from the mammary gland, contamination from the teat skin and udder, contamination from inadequately sanitized milking equipment or storage containers, and improper storage. The microbial

contaminants in colostrum may include the normal commensal bacteria of bovine skin, environmental and fecal contaminants, and mammary pathogens.[15, 64, 67-70] *Mycobacterium avium* subsp. *paratuberculosis, Salmonella, Staphylococcus aureus, Escherichia coli,* and Bovine Leukemia Virus are some of the pathogens that may be found in colostrum, arriving either by secretion or by contamination during the milking or storage processes.[15, 64, 65, 68-71]

Timely collection of colostrum is important not only to prevent dilution of colostral Igs by the ensuing milk production, but also to prevent intramammary infections due to colostral leakage from the teats. Teat end cleanliness is essential in minimizing microbial contamination of raw milk and colostrum.[72, 73] In a study in which, milk samples were collected aseptically from the teat, bacterial total plate counts and coliform counts were well below the recommended cutpoint of 100,000 cfu/ml in a review on colostrum management.[13, 74] After harvesting from the mammary gland, milk collected in a bucket increased a little more than three $\log_{10}$ in both total plate count and coliform counts.[74] Thus, collecting the milk *via* the bucket or milking equipment can be a major source of bacterial contamination. Further increases in microbial counts can occur if milk is stored at ambient temperatures, with or without preservative, or with refrigeration. In one study, the lowest microbial counts accrued when colostrum was refrigerated with a preservative.[74] Freezing colostrum is the best option for overall preservation of nutritional content and Igs, without increases in microbial counts. However, colostral leukocytes are destroyed by freezing.[13, 75]

HEAT-TREATMENT OF COLOSTRUM

Heat-treatment of milk and colostrum is a method used to reduce microbial counts and thus provide a more hygienic product while preserving the desirable nutritional and immunological components. Heat-treatment of milk is relatively uncomplicated as compared to colostrum. Since colostral Igs are crucial for the neonatal calf, any treatment protocol must not significantly reduce the immunologic properties of colostrum. The first documented report on the heat-treatment of colostrum in 1923 investigated the time and temperature required to inactivate tuberculosis organisms without thickening the colostrum.[76] There are no further reports on heat-treatment of colostrum until 1981.[77] The next reported studies on colostrum pasteurization began as a result of observations that *Escherichia coli* were not able to penetrate the intestinal mucosa in the presence of colostrum.[78] However, there was still a concern about bacterial contamination of colostrum.[77, 79-8177, 81]

Escherichia coli have been observed to be pinocytosed and transported by vacuoles by the neonatal bovine intestine in colostrum deprived calves, adding further evidence to the idea of non-specific uptake of macromolecules by the neonatal intestine.[78] When *E. coli* organisms were administered at the same time as colostrum, the organisms reached the mesenteric lymph nodes but in lower numbers than colostrum deprived calves. Furthermore, the *E. coli* organisms do not reach the neonatal circulation as they do in colostrum deprived calves, thus illustrating a protective effect of colostrum.[78] Lastly, one study described the amount of Ig absorption was lowest for calves receiving indigenous duodenal microfloral *versus* calves receiving sterilized inoculate suggesting

that bacteria, even those inherent to the intestinal tract, interfere with Ig absorption and thus passive transfer.[82]

With this framework, the platform is set for heat-treatment of colostrum for the purpose of reducing pathogen exposure to the calf and to possibly improve neonatal serum IgG levels. In 1923, the first published report concluded that colostrum could be heat-treated at 60^{o}C (140^{o}F) for up to three hours to inactivate tuberculosis organisms in colostrum but also to prevent the thickened and heat coagulated product.[76] The study further described that calves fed the heat-treated colostrum appeared to be no different than calves fed fresh colostrum in weight gain.[76] The study did not adequately describe the weight gains for all the calves in the study, did not examine the passive transfer of IgG, and did not have a large enough sample size to examine morbidity and mortality events.[76] The next published report in 1981, enrolled forty-eight calves and treated the colostrum for 62.5^{o}C (145^{o} F) for 30 minutes. The study demonstrated the serum IgG concentration is significantly higher for calves fed heat-treated colostrum at twelve hours post feeding and higher, but less consistent at other time points, until reaching identical levels at thirty-six hours post feeding. This study had small sample sizes which make it difficult to make conclusions on the microbial counts in the colostrum pre or post treatment, the effect of treatment on the Ig concentration in colostrum, or problems of coagulation with the heat-treated colostrum product.[77]

A study in 1996 examined the effectiveness of heat-treating colostrum to reduce viable *Mycobacterium avium* subsp. *paratuberculosis* organisms under laboratory conditions simulating pasteurization. Six colostrum samples experimentally inoculated

with *Mycobacterium avium* subsp. *paratuberculosis* at three levels varying from 10^2-10^4 were heat treated at 63°C for thirty minutes. While the recovery of *Mycobacterium avium* subsp. *paratuberculosis* was significantly different between pasteurized and unpasteurized treatments, the organism was still recovered from two of the six slants at the highest inoculation level. The colostral Ig levels for twelve batches of colostrum showed a percent reduction from 0 to 24.9% in Ig concentrations with losses greatest in colostrum of the highest immunoglobulin concentration. The authors reported coagulation of the heat-treated colostrum in those samples with the highest Ig concentration.[83]

In 2000, another group used twenty calves and two different temperatures, 76°C and 63°C to heat-treat colostrum and compared the effect on serum Ig concentrations at twenty-four hours of age. The higher temperature was chosen since previous work showed *Mycobacterium avium* subsp. *paratuberculosis* is thermal tolerant at temperatures usually used for pasteurization. Calves receiving colostrum from the 76°C treatment had significantly lower serum colostral Ig levels whereas calves receiving heat-treated colostrum at 63°C had negligible differences in their serum Ig as compared to the untreated group. The viscosity of the heat-treated colostrum was an issue as the authors diluted the colostrum before heat-treatment and used a blender post-treatment so the heat-treated product could be administered *via* esophageal feeder. There were no reports on microbial counts pre or post heat-treatment in this study.[84]

Later in 2003, an on-farm batch pasteurizer is used at 63°C for 30 minutes to study the effects of pasteurization on colostral Igs as well as the passive transfer of the

colostral Igs to the calf. The authors report a mean 26.2% loss in colostral Igs due to the heat-treatment process. The percent loss in colostral Igs was apparently higher when larger batches, 95 L, of colostrum were used *versus* small batches of 57 L. It was hypothesized that the larger batches resulted in greater duration of exposure of the Igs to heat in coming up to pasteurization temperature and in the cooling phase. Issues with consistency of the pasteurized product were reported with this study, but it did not interfere with feeding the end product by bottle or esophageal feeder. Lastly, the authors observed a decrease in the passively transferred Ig levels in calves fed two liters of heat-treated colostrum *versus* those fed untreated colostrum. There was no difference between the treatment groups in serum Ig levels of calves fed four liters, however there were only twenty-eight calves total for that comparison.[85]

To follow on the previous study, two more studies were undertaken by the same group to determine the best parameters for heat-treatment of colostrum. The first was to ascertain the optimal temperature to minimize the Ig loss and the coagulation of the heat-treated colostrum using a Rapid Visco Analyzer. This study examined the effect of heat-treatment over five temperatures, 59^{o}C to 63^{o}C, for 120 minutes. It was reported that 60^{o}C for two hours resulted in non-significant loss of Ig of 1.6% as compared to 33.9% at 63^{o}C for two hours.[79] Likewise with viscosity, there were no significant changes in viscosity when heated at 60^{o}C for two hours.[79] An attempt was made to measure the function of the Igs by the serum neutralization assay for Bovine Viral Diarrhea (BVD) post-treatment, however a limited number of samples could be evaluated accurately due to coagulation issues in the colostrum that had been heated at 63^{o}C. A second study

evaluated the effect of duration of heat-treatment at 60°C on five pathogens, *Mycoplasma bovis, Listeria monocytogenes, Escherichia coli* O157:H7, *Salmonella enteriditidis,* and *Mycobacterium avium* subsp. *paratuberculosis.* In four replicate batches, no growth of *Mycoplasma bovis, Listeria monocytogenes, Escherichia coli* O157:H7, or *Salmonella enteriditidis* was detected after thirty minutes at 60°C. In eight replicate batches, it took an average of sixty minutes at 60°C before no growth of *Mycobacterium avium* subsp. *paratuberculosis* was observed. Heating colostrum for sixty minutes at 60°C resulted in no changes in the serum neutralization titer for Bovine Viral Diarrhea Virus. Thus, the current conditions recommended for heat-treatment colostrum are 60°C for 60 minutes to preserve Ig, destroy significant pathogens, and minimize issues with coagulation of the product.[79, 80]

Based on the results in the previous two studies, an on farm pilot study was undertaken to examine the effects of feeding heat-treated colostrum for sixty minutes at 60°C on serum Ig levels in the calf. The treatment process resulted in 2.5 $\log_{10}$ reduction in both total plate count and total coliform counts without significantly changing the colostral Ig concentration in the end product. The calves receiving the heat-treated colostrum showed significantly higher Ig levels and apparent efficacy of absorption as compared to calves fed fresh colostrum. The group showed no significant differences at twenty-four hours of age in any blood leukocyte counts or measurements of serum nutritional parameters.[14] More recently, another experimental university pilot study systematically assigned newborn calves to be fed either fresh or colostrum heat-treated for thirty minutes at 60°C. This study reported colostral microbial counts were reduced

by 2.5 $\log_{10}$, calf serum Ig levels were significantly higher starting at four hours of age until five weeks of age, and the apparent efficacy of absorption of IgG was higher at all measure time points from eight hours of age until forty-eight hours of age. However, these differences did not result in any significant differences in feed intake and weight gain. The authors reported no disease or death related to the treatment in this study, however the study lacked adequate sample size to investigate health differences.[67] A later study by the same authors, again found a greater apparent efficacy of IgG absorption and increased serum IgG levels for calves fed heat-treated colostrum.[86] The authors further concluded that high microbial levels did not interfere with IgG absorption since calves fed fresh colostrum with low microbial counts did not significantly differ in their serum IgG levels as compared to calves fed fresh colostrum with high microbial counts.[86] This study used a small sample size and the microbial levels reported here are relatively low as compared to other observational studies where the microbial levels are much higher.[87, 88]

CONCLUSION

The mechanism(s) of enhanced transfer of Igs associated with feeding heat-treated colostrum remains unknown. One hypothesis is the bacteria in colostrum bind to the microvillous membranes, competing or interfering with Ig molecules, thus reducing the absorptive capacity of the intestinal epithelium for Ig. *Escherichia* coli 055 have been demonstrated to cause exfoliation of microvilli.[78] With this, the cells capable of Ig absorption in the early neonatal period may be more quickly replaced by cells capable of macromolecular absorption but the pinocytosed vesicles fuse with lysozomes and are

digested.[89] If large numbers of bacteria are present or a bacterial species is overrepresented in the intestinal lumen, perhaps the pH is changed and thus the permeability of Igs to the intestinal epithelial cells is also changed. Immunoglobulins may be binding the bacteria within the intestinal lumen and thereby decreasing the amount available for absorption.[82, 90] Whatever the mechanism of bacterial interference with Ig absorption, this is an opportunity for further study. If there is clearer understanding of this relationship, more exact microbiological guidelines could be established for colostrum feeding programs. Lastly, another hypothesis for the improved apparent efficacy of absorption of IgG and improved serum IgG levels associated with calves fed heat-treated colostrum is that another macromolecule which competes with IgG for absorption across the neonatal intestine is altered during the heat-treatment process.

Previous studies have evaluated the treatment effect in small university pilot studies. However, further research is needed to determine if similar results may be achieved on commercial dairy farms. Furthermore, large scale studies are needed to measure if there are any short or long-term health and economic benefits that may be realized when feeding heat-treated colostrum on commercial dairy farms. If the practice of heat-treating colostrum is to be adopted on commercial farms, there needs to be a clinical justification such as improved calf health and growth. The objective of this thesis and research is to evaluate the effect of heat-treatment of colostrum on colostral Ig concentration and microbial counts, and to evaluate the effect of feeding heat-treated

colostrum to calves on the passive transfer of the Igs, average daily gain and the morbidity and mortality in the pre-weaning period.

CHAPTER 2

The Effect of Heat-Treatment of Colostrum on Colostrum Characteristics on Commercial Dairy Farms: Microbial Counts and Immunoglobulin G Concentration

CHAPTER OVERVIEW

Timely consumption of a sufficient quantity of high quality colostrum is essential for the newborn dairy calf for the prevention of failure-of-passive-transfer (FPT). In recent years, research has investigated heat-treatment of colostrum as one technique to improve the hygienic quality of colostrum and reduce pathogen exposure. While pilot studies have shown promise, large scale studies have been lacking to describe the effects of adopting this management tool in commercial dairy herds. The objective of this study was to describe the effects of heat-treatment of colostrum on total plate count (TPC), total coliform count (TCC), and colostral immunoglobulin G (IgG) concentrations when implemented under field conditions on six commercial dairy farms. During the summer of 2007, 266 matched pairs of fresh unheated and heat-treated colostrum batches were created on the six farms, and the colostrum samples frozen for later testing of microbial analysis and determination of IgG concentrations. All farms saw a reduction in microbial counts although the magnitude of the reduction varied by the farm. Overall there was 2.25 $\log_{10}$ reduction in TPC and a 2.49 $\log_{10}$ reduction in TCC in heat-treated versus fresh colostrum (p-value < 0.0001). There was no effect of treatment on colostral IgG concentration, with the mean IgG for fresh, untreated batches and heat-treated colostrum being 60.7 mg/ml and 59.2 mg/ml, respectively (p-value = 0.38). Though the heat-

treatment process had no overall effect on IgG concentration, batches of colostrum where the IgG concentration was greater than or equal to 70 mg/ml experienced greater loss of IgG in mg/ml as a result of the heat-treatment process than did batches of colostrum where the IgG concentration was less than 50 mg/ml.

INTRODUCTION

Colostrum is the first secretions harvested from the mammary gland after parturition. Colostrum contains high levels of nutrients, immunoglobulins, maternal leukocytes, and anti-microbial factors such as lactoferrin, lysozyme, and cytokines.[2, 4, 6, 30, 37] Ruminants have a syndesmochorial placenta that does not allow the transfer of maternal immunoglobulins (Ig) across the placenta.[7] Thus, the neonatal calf must consume a volume of colostrum approximately equal to 10% of its birth weight of sufficient IgG concentration, greater than or equal to 50 mg/ml, within the first few hours after birth, while the intestine is capable of absorbing macromolecules for successful passive transfer of Ig to occur. Successful passive transfer is defined as serum IgG concentration greater than or equal to 10 mg/ml at 24 hours of age, and is associated with improved daily weight gains, reduced risk for morbidity and mortality in the preweaning period as well as improvements in lactational performance later in life.[12, 47, 49, 91-93]

Despite colostrum's nutritional and immunological importance, colostrum is potentially the first means of exposure to pathogens at the critical time point when the neonatal intestine is open and permeable for absorbing IgG macromolecules. Recent surveys have described a variety of microbial species and counts in colostrum that is fed

to calves.[15, 64, 65] *Salmonella, Escherchia coli* O157:H7, *Mycobacterium avium* subsp. *paratuberculosis, Mycoplasma*, and Bovine Leukemia Virus are a few of the pathogens that may be isolated from colostrum.[64, 65, 68, 94, 95] Microorganisms arrive in colostrum by either secretion from the mammary gland, by contamination during the milking, storage, and feeding processes, or through bacterial proliferation in stored colostrum.[13-15,17] If pathogenic microorganisms arrive in colostrum, they have the potential to cause disease. Furthermore, bacteria have been demonstrated to be non-specifically pinocytosed by the neonatal intestine in the early perinatal period.[78] One experimental study reported an association between bacteria and decreased IgG absorption for calves that received indigenous duodenal microflora versus calves that received the same indigenous inoculate that had been autoclaved.[82] Although the nature of this relationship between the presence of microbes in colostrum and their effect on the absorption of colostral Igs is not understood and requires further study, this early research suggested that there may be benefits to reducing levels of microbial contamination in colostrum.

From these early observations, several management strategies have been investigated with the purpose of reducing microbial contamination of colostrum.[74, 96] One approach is the heat-treatment of colostrum. Early experiments on heat-treatment of colostrum used temperatures ranging from 62.5 to 73^{o}C and had unacceptable results with IgG losses as high as 33.9% and undesirable changes in viscosity.[83-85] However, more recent studies determined that heat-treatment at 60 ºC for 60 minutes resulted in significant pathogen reduction or elimination without negatively affecting colostral IgG concentration or feeding characteristics.[14, 67, 79, 80] Furthermore, two recent clinical trials,

each conducted in a single herd, reported that calves which were fed pasteurized colostrum had significantly higher serum IgG levels and a greater apparent efficacy of IgG absorption as compared to calves fed the untreated, fresh colostrum.[14, 67] After these promising early findings, a larger prospective experimental study commenced to describe the effect of heat-treating colostrum on colostrum characteristics and on preweaning and adult animal health and performance, when this management tool is implemented under field conditions on commercial dairy farms. The primary objective of this manuscript was to describe the effect of heat-treating colostrum at 60°C for 60 minutes on colostrum characteristics, microbial counts and colostral IgG concentrations, when using an on-farm batch pasteurizer under the working conditions of six commercial dairy farms. A secondary objective was to describe the relationship between colostrum quality and magnitude of IgG loss when colostrum is heat-treated at 60°C for 60 minutes.

MATERIALS AND METHODS

Farm Enrollment

Six commercial dairy farms in Minnesota and Wisconsin were enrolled in the study the summer of 2007. The farms were selected due to the convenience of their proximity to the University of Minnesota and willingness to adhere to the study protocol. This study was approved by the University of Minnesota Institional Animal Care and Use Committee (IACUC). Criteria for enrollment in the study were participation in DHIA or some record management program and the herd have at least one animal test positive for *Mycobacterium avium* subsp. *paratuberculosis* (MAP), by either fecal culture or serum

ELISA within the last three years. All six herds used freestall housing with predominantly Holstein cows, although a few crossbreds were represented. Each farm used group maternity pens except one farm which used an individual maternity pen. The colostrum feeding calf enrollment period for this study ran between June and August of 2007 with a target sample size of sufficient power for the purpose of a longer term study to evaluate the risk for infection with MAP in calves fed heat-treated versus fresh colostrum. In that sample size calculation, greater than 500 calves were needed for each group treatment. Each farm was assigned a unique number for the purposes of labeling samples, collecting data and data entry. The REFLECT statement guidelines for reporting methods and processes in clinical trials in livestock and food safety were followed in writing this paper (Appendix).[97]

Colostrum Preparation

All handling of colostrum, pooling, heat-treating and sampling was performed by personnel on each of the six farms. First milking colostrum was collected within two hours post calving and refrigerated. On each farm, daily or on alternate days, this stored, refrigerated colostrum was pooled into one batch. After thoroughly mixing a pooled batch of colostrum, the batch was split into two halves. One half of each batch was left untreated or fresh, while the remaining half was heat-treated using an on-farm batch pasteurizer (Dairy Tech., Inc.; Windsor, CO) at 60 ^{o}C for 60 minutes. Two 50 ml aliquots of colostrum were aseptically collected from each unique batch of colostrum and for each treatment, fresh or heat-treated, and frozen at -20 ^{o}C for later analysis. The remaining colostrum was dispensed into sanitized feeding bottles, uniquely labeled by

date, farm number, unique batch number, and treatment, before being refrigerated for later feeding to calves enrolled in the study.

Colostrum Sample Analysis

Enrollment information and the frozen colostrum aliquots were collected weekly from each farm by research technicians and transported on ice to the Laboratory for Udder Health, College of Veterinary Medicine, University of Minnesota (St. Paul, MN). The laboratory technicians were blinded to number coding applied to farm but not to the treatment, fresh or heat-treated. The authors do not believe this introduced bias into the study as the Laboratory for Udder Health technicians were only minimally involved in reading and recording the plate results for this study and not involved in the study in any other matter. The frozen colostrum samples were thawed at 4^{o}C, vortexed and serially diluted 1:10 for a total of five dilutions. Each dilution, from each batch and colostrum treatment was plated on Plate Count Agar for Total Plate Count (TPC) and MacConkey Agar for Total Coliform Count (TCC). Plates were incubated for forty-eight hours at 37^{o}C and results recorded in colony forming units/ml (cfu/ml). After microbiological culture, colostrum samples were submitted to determine IgG concentration (mg/ml) using turbidimetric immunoassay (TIA). TIA is a rapid, automated and accurate method for determining IgG levels which correlates highly with radioimmunodiffusion (RID), the gold-standard of IgG measurement.[51]

Statistical Analysis

Descriptive statistics were generated to describe the colostrum bacteriological and IgG concentration characteristics of interest overall and by farm. Since the

bacteriological data was not normally distributed, TPC and TCC measures were $\log_{10}$ transformed for the purposes of analysis. Only batches of colostrum where both the fresh and heat-treated sample, a matched pair, was available were included in the analysis. Of the total 276 possible pairs, 266 were matched pairs and included in the analysis. On one farm, four unique batches of colostrum were flagged by the farm personal because it was suspected that, due to a short-term pasteurizer malfunction, the colostrum may have been overheated during the heat-treatment. However, in the analysis, removing these batches did not change the results for the analysis of the differences in IgG concentrations between treatments. Therefore, these four batches were maintained in the analysis. The unit of analysis was each unique batch of colostrum.

Linear regression (proc mixed, SAS version 9.1, Cary, NC) was used to describe the effect of treatment, fresh or heat-treated, on the outcomes of interest; TPC, TCC and colostrum IgG concentration. Farm was investigated as an additional covariate of interest and found to be significant in all models. Farm was forced in all models as a fixed effect to control for the clustering of batches within farm. The statistical model used for the analysis was:

$$Y = \beta_0 + \beta_1 T + \beta_2 F + \beta_3 T*F + \varepsilon.$$

Where β_0 is the intercept, $\beta_{1\text{-}3}$ are the parameter estimates for the explanatory variables, T is the treatment of colostrum, heat-treated or fresh, and F is the farm number, 1, 2, 3, 4, 5, or 6, T*F is the interaction term for colostrum treatment and farm, and ε is the error term of the general liner model. Since a significant interaction between treatment and farm was determined to be present for the bacteriological results, the data was stratified and

analyzed by farm. An additional dichotomous covariate was created to describe the original fresh colostrum as either of high quality, greater than or equal to 70 mg/ml, or low quality, less than 70 mg/ml, and offered into the model. This covariate describing colostrum concentration was not significant and did not interact with the variable describing treatment and so was subsequently removed from all models. Final statistical significance was declared at a p-value < 0.05.

A secondary objective of this study was to describe the relationship between colostrum quality and magnitude of IgG loss when colostrum was heat-treated at 60^{o}C for 60 minutes. For this analysis, the IgG concentration of the original fresh colostrum was categorized into one of five categories or quintiles. The first category, 1, was defined as IgG concentration ≥ 80 mg/ml, category 2 as IgG 70 to 79.9 mg/ml IgG, category 3 as IgG 60 to 69.9 mg/ml IgG, category 4 as IgG 50 to 59.9 mg/ml, category 5 as IgG < 50 mg/ml. Colostrum IgG loss (mg/ml) was calculated by transposing the data set in SAS, and subtracting the heat-treated colostral IgG concentration from the fresh colostral IgG concentration for each unique batch of colostrum. This difference, or colostrum IgG loss in mg/ml, was then divided by the fresh IgG colostrum concentration for that unique batch of colostrum and multiplied by one hundred to obtain the percent of IgG lost due to the heat-treatment process. Linear regression (proc mixed, SAS version 9.1, Cary, NC) was then used to describe the relationship between IgG concentration of the original untreated colostrum sample expressed in categories and colostral IgG loss after heating, expressed as either mg/ml loss or percent loss. The category of less than 50 mg/ml was selected as the lowest category and referent since 50 mg/ml is the current industry

cutpoint for acceptable IgG concentration for good immunologic quality of colostrum. Contrast analysis comparing IgG loss during heat-treatment among all five colostrum quality categories was also conducted using Proc Mixed to determine which categories of colostrum IgG concentration were different from each other. Farm was forced into the models as a fixed effect to control for the clustering of batches within farm. Final statistical significance was declared at a p-value < 0.05.

RESULTS

The demographics for the herds involved in this study are summarized in Table 1. Overall, in both measures of microbial counts, TPC and TCC, were significantly lower for colostrum heated at 60^{o}C for 60 minutes than for the fresh, untreated colostrum (Tables 2 and 3). The mean $\log_{10}$ TPC and TCC measures for fresh colostrum were 5.4 and 4.4, respectively while the mean $\log_{10}$ TPC and TCC measures for heat-treated colostrum were 3.6 and 2.3, respectively. In the final models with treatment and farm included as covariates, there was 2.25 $\log_{10}$ reduction in the mean for TPC and a 2.49 $\log_{10}$ reduction in the geometric mean for TCC associated with the heat-treatment of colostrum (p-value < 0.0001). All farms saw reductions in microbial counts in heat-treated colostrum, although the magnitude of the reduction varied by the farm. When the results were stratified by farm, all farms experienced significant reductions in both TPC and TCC except for farm three where heat-treated colostrum had a numerical, but not statistically significant, reduction in the $\log_{10}$ TCC.

Results of the analysis for the effect of heat-treatment on colostral IgG concentrations are presented in Table 4. In this field trial there was no significant reduction in colostral IgG concentrations when colostrum was heat-treated at 60^{o}C for 60 minutes using an on farm batch pasteurizer. The mean colostral IgG concentration for fresh and heat-treated colostrum was 60.7 and 59.2 mg/ml, respectively (p-value = 0.38).

While there was no significant loss of colostral IgG concentration overall as a result of heat-treatment, secondary analysis showed that individual batches of colostrum of higher IgG concentrations experienced a greater magnitudes of IgG loss as compared to batches of lower IgG colostrum concentration. The results for colostrum IgG loss in mg/ml by colostrum quality category are reported in Tables 5 and 6, respectively. When IgG loss after heat-treatment was expressed as mg/ml loss, batches of colostrum with IgG concentration greater than or equal to 70 mg/ml, experienced significantly greater loss of IgG (mg/ml) after heat-treatment as compared to the referent category of less than 50 mg/ml (Table 5; Graph 1). When IgG loss was expressed as percent loss, colostrum batches with IgG concentrations greater than or equal to 60 mg/ml, experienced significantly greater percent loss of IgG after heat-treatment as compared to the referent category, where colostral IgG concentrations were less than 50 mg/ml (Table 6; Graph 2).

DISCUSSION

Early studies in laboratories and on individual farms have demonstrated that heat-treatment of colostrum at 60 ºC can preserve colostral IgG concentration and feeding characteristics while significantly reducing bacterial concentrations. A pilot study

conducted on one commercial dairy reported that heat-treating colostrum at 60ºC for 60 minutes resulted in a 2.5 $\log_{10}$ reduction in TPC and TCC without significant losses in colostral IgG concentration (mg/ml).[14] A second university study on a single dairy also reported that reported that heat-treating colostrum at 60 ºC for 30 minutes resulted in a 2.5 $\log_{10}$ reduction in TPC and TCC without significant losses in colostral IgG concentration (mg/ml). Both studies reported that calves which were fed heat-treated colostrum experienced significantly enhanced efficiency of IgG absorption and greater serum IgG levels, as compared to calves fed untreated fresh colostrum.[14, 67] The mechanism to explain the improved efficiency of absorption when feeding heat-treated colostrum has not yet been described.

The current study is the first large scale field study conducted on multiple commercial dairy farms, designed to describe the effect of on-farm heat-treatment of colostrum on TPC, TCC, and colostral IgG concentrations under field conditions. While the commercial dairies enrolled into this study are progressive and well managed, it is reasonable to expect that similar farms would experience similar results to those found in this study. In this study the direction and magnitude of the reduction in microbial counts in colostrum, resulting from the heat-treatment process, were similar to the reductions published in previous single-herd university studies. Furthermore, there was no significant effect of heat-treatment on colostrum IgG concentration. These results are consistent with previous research results demonstrating that the adverse effects of heat-treatment of colostrum on IgG denaturation can be avoided by using a longer time and lower temperature approach.[14, 67, 80]

While heat-treatment did not have a negative effect on IgG concentration overall, there was variation in the amount of IgG lost in mg/ml or percent loss among the 266 batches studied. Secondary analysis showed that the IgG concentration of the colostrum being heat-treated is one factor associated with the magnitude of loss. Greater IgG loss, expressed in mg/ml, occurred in colostrum batches where the initial colostrum IgG concentration was greater than or equal to 70 mg/ml as compared to colostrum batches where the concentration was less than 50 mg/ml. Similarly, greater IgG loss, expressed as a percentage, occurred in higher quality colostrum batches where IgG concentrations were greater than or equal to 70 mg/ml as compared to colostrum batches of less than 50 mg/ml IgG concentration. These results are consistent with the findings of an earlier laboratory study where heat-treated colostrum experienced a greater IgG loss, 4.62 mg/ml, for colostrum with original IgG concentrations greater than 73.0 mg/ml.[79]The exact mechanism by which IgG is denatured and unfolds when heating colostrum has not been described. However, it is understood that when proteins in general are heat-treated they lose their primary three-dimensional functional conformation, unfold and then may aggregate with other whey proteins or casein either by disulfide bond linkage, calcium-linked complexes, or hydrophobic interactions.[98] A study which examined the heat stability of IgG as an isolated protein and then in the presence of colostrum found that components within the colostral milieu further increase both the heat and pressure stability of the IgG molecule.[99] Two studies further describe that the unfolding between the two distinct regions of the IgG molecule, F_c (fraction constant) and F_{ab} (fraction antigen-binding) occur independently of each other.[99, 100] Another study demonstrated

that as the protein concentration increased in milk the rate of denaturation of whey proteins also increased.[98] Nevertheless, from the practical viewpoint of a dairy producer, the fact that colostrum batches of higher IgG concentration may experience greater IgG loss than batches of colostrum with lower IgG concentrations is unimportant, as the IgG concentrations in the final product after heat-treatment will still be satisfactory enough to achieve a high likelihood of passive transfer of maternal IgG. Thus, after controlling for the volume and timing of colostrum feeding, a greater total mass of IgG will still be presented to the calf if a colostrum of higher IgG concentration is fed.

While these results suggest that on-farm heat-treatment of colostrum can be successfully implemented on commercial Midwest dairy farms, this study and others have, thus far, focused primarily on describing the effects on major colostrum characteristics of importance, namely bacterial concentrations and IgG concentrations. Two studies have evaluated the effect of heat-treatment on the major nutritional constituents and found no difference between heated and unheated colostrum.[67, 86] Additional studies are needed to investigate the effects of heat-treatment on the nonspecific immune factors and colostral leukocytes, as these constituents may also play a role in calf health and development. Lastly, the short and long term health and performance and the economics of feeding heat-treated colostrum to commercial dairy calves should be evaluated in future studies.

CONCLUSIONS

Heat-treatment of colostrum significantly reduced both TPC and TCC in a field trial on six commercial dairy farms. Each of the farms saw reductions in microbial counts in heat-treated colostrum, though the magnitude of the reduction in microbial counts varied by the farm. The heat-treatment process did not cause a significant reduction in colostral IgG overall, although higher quality colostrum experienced greater losses of IgG as compared to lower quality colostrum. An accompanying manuscript will describe the effect of feeding heat-treated colostrum on preweaning health and performance in newborn calves on these six commercial dairy farms.

ACKNOWLEDGEMENTS

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Table 1: Study Herd Demographics

Farm #	State	Milking Herd Size	RHA kilograms	SCC cells/ml	Dry Period (days)
1	MN	1,500	13,892	205,000	45
2	MN	1,600	14,512	149,900	48
3	WI	1,500	12,956	275,000	45
4	WI	2,500	12,345	350,000	37
5	MN	1,200	12,363	276,000	45
6	MN	1,400	11,280	430,000	44

Table 2: Effect of Heat-Treatment of Colostrum on Total Plate Count

			Total Plate Count – Log_{10}, cfu/ml		
	n	Treatment	Mean* (se, range)	Difference** (se)	p-value
All Farms	266	Heat-treated	3.6 (0.07, 1.0 - 8.4)	-2.25 (0.23)	< 0.0001
	266	Fresh	5.4 (0.06, 2.6 - 9.1)	referent	.
Farm 1	56	Heat-treated	4.0 (0.15, 1.1 - 6.4)	-1.31 (0.19)	< 0.0001
	56	Fresh	5.3 (0.13, 2.6 - 6.8)	referent	.
Farm 2	44	Heat-treated	3.1 (0.12, 1.2 - 5.4)	-1.87 (0.18)	< 0.0001
	44	Fresh	5.0 (0.13, 3.2 - 6.7)	referent	.
Farm 3	73	Heat-treated	4.3 (0.13, 1.9 - 8.4)	-1.27 (0.17)	< 0.0001
	73	Fresh	5.6 (0.10, 3.0 - 9.1)	referent	.
Farm 4	35	Heat-treated	2.7 (0.12, 1.2 - 5.4)	-2.38 (0.18)	< 0.0001
	35	Fresh	5.1 (0.14, 3.6 - 6.3)	referent	.
Farm 5	25	Heat-treated	3.1 (0.18, 2.1 - 5.2)	-2.83 (0.25)	< 0.0001
	25	Fresh	5.9 (0.18, 3.8 - 7.3)	referent	.
Farm 6	33	Heat-treated	3.3 (0.17, 1.5 - 6.0)	-2.25 (0.22)	< 0.0001
	33	Fresh	5.6 (0.14, 4.0 - 6.8)	referent	.

*crude means are reported

**Difference of the lsmeans between the two treatment groups

Table 3: The Effect of Heat-Treatment of Colostrum on Total Coliform Counts

			Total Coliform Count – Log_{10}, cfu/ml		
	n	Treatment	Mean* (se, range)	Difference** (se)	p-value
All Farms	266	Heat-treated	2.3 (0.10, 0 - 7.0)	-2.49 (0.31)	< 0.0001
	266	Fresh	4.4 (0.08, 0 - 6.8)	referent	.
Farm 1	56	Heat-treated	3.4 (0.19, 0 - 6.6)	-1.52 (0.24)	< 0.0001
	56	Fresh	4.9 (0.15, 2.0 - 3.4)	referent	.
Farm 2	44	Heat-treated	0.9 (0.16, 0 - 3.6)	-3.21 (0.23)	< 0.0001
	44	Fresh	4.1 (0.17, 1.9 - 6.8)	referent	.
Farm 3	73	Heat-treated	3.4 (0.17, 0 - 7.0)	-0.30 (0.26)	0.25
	73	Fresh	3.7 (0.19, 0 - 6.3)	referent	.
Farm 4	35	Heat-treated	0.6 (0.16, 0 - 3.4)	-3.95 (0.22)	< 0.0001
	35	Fresh	4.6 (0.15, 2.8 - 6.1)	referent	.
Farm 5	25	Heat-treated	2.2 (0.18, 0 - 3.9)	-3.18 (0.32)	< 0.0001
	25	Fresh	5.3 (0.26, 2.5 - 6.8)	referent	.
Farm 6	33	Heat-treated	1.8 (0.21, 0 - 5.0)	-2.49 (0.27)	< 0.0001
	33	Fresh	4.3 (0.18, 2.4 - 6.4)	referent	.

*crude means are reported

**Difference of the lsmeans between the two treatment groups

Table 4: The Effect of Heat-Treatment on Colostrum IgG Concentration (mg/ml)

			Colostrum IgG, mg/ml		
	n	Treatment	Mean* (se, range)	Difference** (se)	p-value
All Farms	266	Heat-treated	59.2 (1.27, 12.1 - 139.5)	-1.5 (1.7)	0.38
	266	Fresh	60.7 (1.33, 9.8 - 139.9)	referent	.
Farm 1	56	Heat-treated	52.2 (2.9, 14.9 - 103.6)	-1.4 (4.2)	0.73
	56	Fresh	53.6 (3.0, 18.2 - 131.1)	referent	.
Farm 2	44	Heat-treated	55.7 (2.5, 21.9 - 117.8)	-1.0 (3.6)	0.77
	44	Fresh	56.8 (2.6, 23.8 - 108.1)	referent	.
Farm 3	73	Heat-treated	53.7 (2.3, 12.1 - 115.9)	-0.3 (3.3)	0.93
	73	Fresh	54.0 (2.4, 9.8 - 100.5)	referent	.
Farm 4	35	Heat-treated	74.6 (3..8, 31.3 - 139.5)	-3.0 (5.7)	0.60
	35	Fresh	77.6 (4.2, 28.2 - 139.9)	referent	.
Farm 5	25	Heat-treated	63.5 (2.9, 36.4 - 99.4)	-2.7 (4.4)	0.54
	25	Fresh	66.2 (3.3, 39.3 - 107.8)	referent	.
Farm 6	33	Heat-treated	68.1 (2.9, 35.7 - 107.2)	-2.4 (3.8)	0.54
	33	Fresh	70.5 (2.5, 38.9 - 101.9)	referent	.

*crude means are reported

**Difference of the lsmeans between the two treatment groups

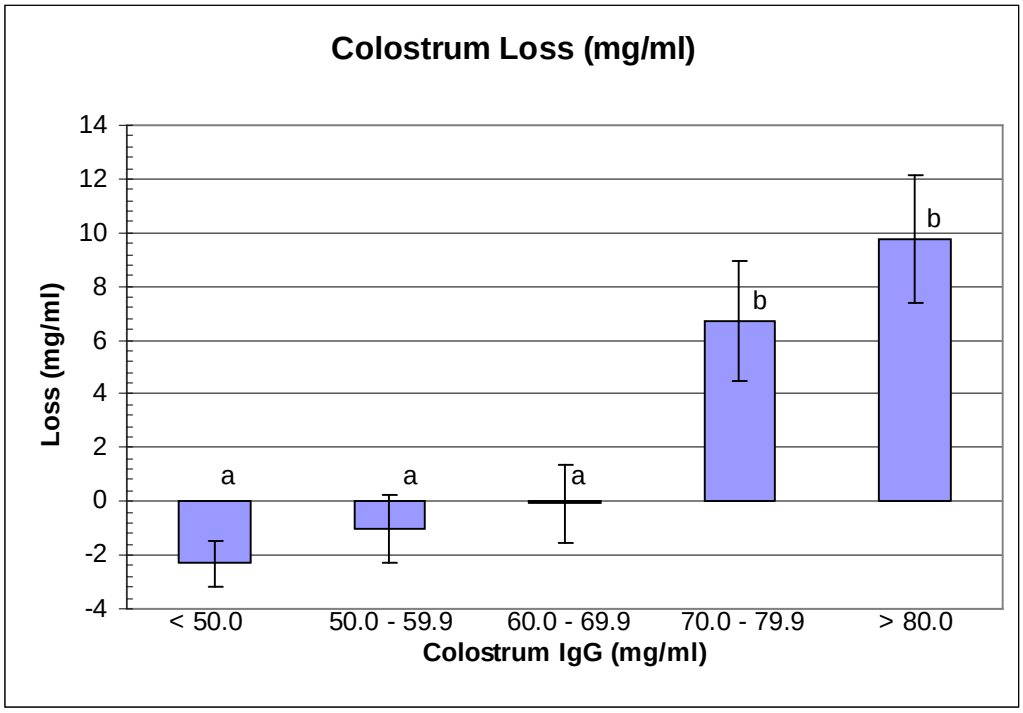
Table 5: Colostrum IgG Loss from Heat-Treatment by Quintiles

			Colostrum IgG Loss, mg/ml		
	n		Mean* (se, range)	Difference** (se)	p-value
Quintile 1	39	≥ 80 mg/ml	9.8 (2.4, -9.1 - 47.5)	12.7 (2.3)	< 0.0001
Quintile 2	40	≥ 70 to < 80 mg/ml	6.7 (2.2, -20.5 - 57.5)	9.9 (2.3)	< 0.0001
Quintile 3	44	≥ 60 to < 70 mg/ml	-0.09 (1.4, -46.9 - 12.9)	2.9 (2.2)	0.19
Quintile 4	67	≥ 50 to < 60 mg/ml	-1.0 (1.3, -47.5 - 25.6)	1.7 (1.9)	0.37
Quintile 5	76	< 50 mg/ml	-2.3 (0.9, -28.5 - 14.7)	referent	.

*crude means are reported

**Difference in means between groups compared to the referent category, < 50 mg/ml

Graph 1: IgG Loss (mg/ml) by colostrum concentration of IgG (mg/ml) in quintiles



Means that differ significantly from each other (p-value $\leq$ 0.05) have a different letter. Bars represent the standard error.

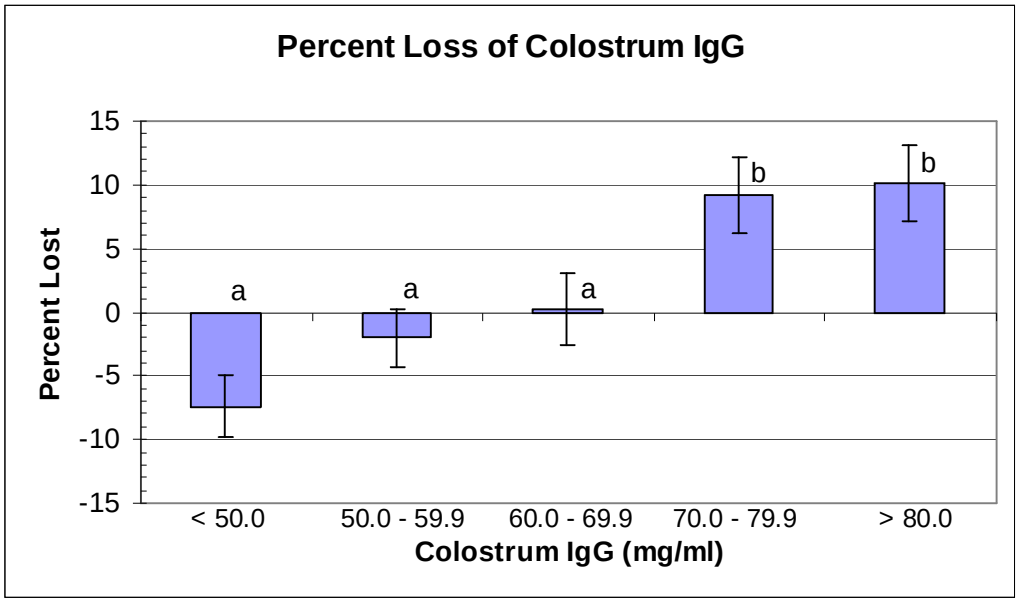
Table 6: Effect of Heat-Treatment on IgG Percent Lost

			Colostrum IgG Percent Lost		
	n		Mean* (se, range)	Difference** (se)	p-value
Quintile 1	39	≥ 80 mg/ml	9.8 (2.3, -8.4 - 42.6)	17.5 (3.9)	< 0.0001
Quintile 2	40	≥ 70 to < 80 mg/ml	8.8 (2.9, -26.9 - 76.4)	16.6 (3.9)	< 0.0001
Quintile 3	44	≥ 60 to < 70 mg/ml	-0.04 (2.2, -68.0 - 21.2)	7.6 (3.7)	0.04
Quintile 4	67	≥ 50 to < 60 mg/ml	-1.9 (2.2, -84.4 - 50.7)	5.4 (3.2)	0.09
Quintile 5	76	< 50 mg/ml	-7.3 (2.5, -72.6 - 31.0)	referent	.

*crude means are reported

**Difference in means between groups compared to the referent category, < 50 mg/ml

Graph 2: Percent IgG lost by colostrum concentration of IgG (mg/ml) in quintiles



Means that differ significantly (p-value $\leq$ 0.05) have a different letter.
Bars represent the standard error.

Table 7: REFLECT checklist for Effect of Heat-Treatment on Colostrum Characteristics

REFLECT Checklist			
Paper section and topic	Item	Descriptor	Reported on Page #:
Title and Abstract	1	How study units are allocated, clearly state if the intervention was natural exposure or deliberate challenge	18
Introduction, Background	2	Scientific background and rationale	19-21
Methods, Participants	3	Eligibility criteria and study units	19
Interventions	4	The level the intervention was allocated	22
	4b	Precise details of the agent and challenge model	22
Objectives	5	Clearly state primary and secondary objectives	21
Outcomes	6	Clearly defined primary and secondary outcome measures, level of measurement	23-25
Sample Size	7	How sample size was determined	21-22
Randomization – sequence generation	8	Method used to generate the random allocation sequence at the relevant level	22
Randomization – allocation concealment	9	Method used and concealment of sequence	22-23
Randomization – implementation	10	Who generated the sequence, enrolled study units, and assigned study units to group	22
Blinding (masking)	11	Were participants blinded to the intervention of justification if no blinding used	22-23
Statistical Methods	12	Methods used to compare outcomes and clearly state the level of analysis	23-25
Results, Study Flow	13	Study Units through each stage for each level of the organization	23-25
Recruitment	14	Dates defining the recruitment	21
Baseline Data	15	Baseline demographic and clinical data	Table 1
Numbers analyzed	16	Number of study units, and state “intention to treat”	23-24
Outcomes and estimation	17	A summary of results for each group, accounting for relevant structure	25-27 Tables 2 – 6 Graphs 1 & 2
Ancillary analyses	18	Address multiplicity of analysis preformed	23-25
Adverse events	19	All important adverse events	23-24
Discussion, Interpretation	20	Interpretation of results, bias or imprecision, herd immunity if relevant and the relevance of the disease challenge	27-30
Generalizability	21	Generalizability (external validity) of the findings	27-28
Overall Evidence	22	General interpretation of the results in the context of the current evidence	27-30

CHAPTER 3

Effect of Feeding Heat-Treated Colostrum on Passive Transfer, Health and Growth of Preweaned Dairy Calves

CHAPTER OVERVIEW

One of the areas of contemporary colostrum research is the heat-treatment of colostrum to improve the hygienic quality of colostrum by inactivating pathogens and by reducing microbial exposure. Earlier smaller studies have described improved passive transfer of immunoglobulin G (IgG) to calves fed heat-treated colostrum. Larger clinical trials are needed to describe the effect of feeding heat-treated colostrum on the passive transfer of maternal immunity and any impact on health or performance in the pre-weaning period on commercial dairy farms. The objective of this study was to describe the effect of feeding heat-treated colostrum in neonatal dairy calves on serum IgG (mg/ml), serum total protein (g/dl), weight gain parameters (kg), and the risk for morbidity and mortality events in the pre-weaning period on commercial dairy farms. In the summer of 2007, 1099 newborn calves enrolled in this study where 576 calves were fed heat-treated colostrum and the remaining 523 were fed fresh colostrum on six commercial dairy farms in Wisconsin and Minnesota. Technicians visited the farm weekly to collect enrollment information, collect blood to determine serum IgG and serum total protein (STP) levels in the first week of life, and to collect records of treatment and death events. Birth and weaning weights were recorded for a subset of calves in three farms.

The mean serum IgG was 18.11 mg/ml for calves fed heat-treated colostrum as compared to 15.48 mg/ml for calves fed fresh colostrum ($p < 0.0001$). There was no difference in any of the weight variables between the two colostrum treatment groups where the mean average daily gain was 0.62 kg in both groups of claves. The treatment rate and morbidity rate was 32.5% and 2.5%, respectively, for calves fed heat-treated colostrum as compared to 36.3% and 1.7%, respectively, for calves fed fresh colostrum which was not statistically significant. The hazard rate for a treatment event was 0.88 (95% CI = 0.72, 1.08) and the hazard rate for death event was 1.49 (95% C.I. = 0.64, 3.48), for calves fed heat-treated colostrum as compared to calves fed fresh, untreated colostrum. These hazard rates were not statistically significant. Although feeding heat-treated colostrum resulted in a statistically significant improvement in serum IgG levels between one and eight days of age, in this study, treatment had no meaningful or statistically significant effect on weight gain, risk for treatment, or risk for mortality during the pre-weaning period.

INTRODUCTION

The passive transfer of maternal immunity from colostrum to neonates is abundantly described in the literature for a variety of species. The consumption of colostral immunoglobulins (Ig) is particularly important to the ruminant, equine, and porcine species where multiple maternal and fetal layers obstruct the transfer of Igs to the fetus in utero. The neonatal calf is born agammaglobulinemic because of the

syndesmochorial placenta. In order to absorb an adequate mass of Ig, it is recommended that a newborn calf consume 10% of its birth weight of high immunologic quality colostrum (colostrum IgG ≥ 50 mg/ml) within the first couple hours after birth before gut closure occurs. Gut closure refers to the cessation of macromolecular permeability of the neonatal intestine which begins to ensue as early as four hours of life and reaches completion by twenty four hours of age.[11, 42] Calves with adequate passive transfer status have achieved serum IgG concentrations greater than or equal to 10 mg/ml at twenty-four to forty-eight hours of age.[12, 44]

Failure-of-Passive Transfer (FPT) is associated with increased risk for morbidity, mortality, decreases in daily weight gains as well as reductions in future lactation performance.[12, 47, 49, 49, 91, 92] Research and extension education has helped to decrease of FPT rates of calves by improving the recommendations made in harvesting colostrum, feeding methods, the timing, quantity, and the immunological quality of the colostrum fed.[101] The National Animal Health Monitoring System recently reported 19.2% of calves in the Dairy 2007 study had FPT status, a decrease from the 41.0% reported in the 1991-1992 National Dairy Heifer Evaluation Project.[101] However, opportunities remain to improve the health of calves in the early neonatal and pre-weaning period. Contemporary research has focused on the hygienic quality of colostrum in terms of microbial counts and its relationship with the passive transfer of immunity in calves. This research is also directed at reducing the risk of exposure to pathogens which can be isolated from milk and colostrum.

Heat-treatment of colostrum is one of the management strategies currently being investigated for the purpose of reducing pathogen exposure and microbial contamination of colostrum. Observational studies have described a wide variety of microbial species may be present in colostrum which is fed to calves including, *Salmonella*, *Escherchia coli* O157:H7, *Mycobacterium avium* subsp. *paratuberculosis*, and *Mycobacterium bovis* and Bovine Leukemia Virus.[15, 64, 65, 68, 102] These microbes can be present in colostrum either by direct secretion from the mammary gland, introduced into colostrum by contamination during milking, handling, or bacterial proliferation during the storage process.[15, 64, 65, 69, 70, 74] Clinical trials have described that when calves are fed colostrum heat-treated at 60^{o}C for 60 or 30 minutes, these calves had a greater efficacy of IgG absorption which resulted in significant improvements of serum IgG concentrations as compared to calves fed fresh untreated colostrum.[14, 67] However, it has not been determined if there are decreased risks for pre-weaning morbidity and mortality or increased weight gains in the pre-weaning period for calves fed heat-treated colostrum as compared to those fed fresh colostrum. There is a need for larger clinical trials to determine the effect of feeding heat-treated colostrum in dairy calves on relevant health and performance parameters. The primary objective of this study was to describe the passive transfer of IgG, preweaning weight gain, and preweaning risk for treatment and mortality for calves fed colostrum heat-treated at 60^{o}C for 60 minutes, as compared to calves fed fresh untreated colostrum, when managed under the routine conditions of commercial dairy farms. Further studies will need to examine the long term health and

production performance of animals which received heat-treated colostrum at birth.

MATERIALS AND METHODS

Farm Enrollment

This study protocol was approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC). The REFLECT statement guidelines for reporting methods and processes for clinical trials in livestock and food safety were followed in reporting the results from this study (Appendix).[97] In the summer of 2007, between June and August, six commercial dairy farms in Wisconsin and Minnesota were enrolled in this study due to their proximity to the University of Minnesota and their willingness to comply and adhere with the study protocol. Additional criteria for herd enrollment in this study included participation in DHIA or some record management program and have at least one adult cow test positive for *Mycobacterium avium* subsp. *paratuberculosis* (MAP) either by fecal culture or by serum ELISA within the past three years.

The herds consisted predominately of Holstein cows although a few crossbreds were represented. One farm used individual maternity pens while the remaining five farms used group maternity pens. All farms used freestall housing for lactating cows. Each farm was assigned a unique number for the purposes of labeling samples, collecting data, and data entry.

Colostrum Preparation

All colostrum handling was performed by personnel on each of the six farms. Within two hours of calving, the colostrum was harvested and refrigerated. Daily or on alternate days, each farm processed a batch of colostrum by pooling the available refrigerated colostrum and mixing. After thorough mixing, the unique batch of colostrum was divided into half. The first half of each batch was left untreated or fresh, while the remaining half was heat-treated at 60^{o}C for 60 minutes using an on-farm batch pasteurizer (Dairy Tech., Inc.; Windsor, CO). For each unique batch and treatment of colostrum, two 50 ml aliquots of colostrum were aseptically collected and frozen at -20^{o}C for later analysis of IgG concentration (mg/ml) and bacterial culture as reported in the proceeding chapter (chapter 2) of this thesis. The remaining colostrum was dispensed into clean, sanitized feeding bottles and uniquely labeled by date, farm number, unique batch number of colostrum and colostrum treatment, untreated or heat-treated, before being refrigerated for a later feeding as calves were enrolled in the study.

Calf Enrollment and Feeding

This study is part of a larger study where the sample size was originally determined for the purpose of determining if feeding heat-treated colostrum at birth reduced the risk for testing positive MAP later in life with a goal of enrolling 600 calves per treatment group. Calves were separated from their dams at birth and moved to an individual housing unit. The calves were alternately assigned by birth order to a colostrum treatment feeding of either heat-treated (n = 576 calves), or fresh colostrum, (n = 523 calves), and fed a volume of 3.8 L as soon as possible after birth. Five farms fed

the entire volume of colostrum by esophageal tube while one farm fed colostrum by bottle and any remaining volume of colostrum via esophageal feeder. One farm fed a second feeding of 1.9 L of colostrum whereas the remaining five farms fed only a single colostrum feeding. Enrollment information collected include farm number, dam identification, birth date and time, calf identification, time of colostrum feeding, colostrum batch number fed, colostrum treatment, and for three farms, birth and weaning date and weights were recorded for a subset of calves. The calves were subsequently handled under each farm's regular feeding and management protocols. No attempt was made to blind the personnel to the calf's assignment to treatment. The farm staff which enrolled the calves was different than the staff which managed the calves until weaning, hence the staff managing the calves from day one through weaning were blinded to treatment group. University technicians visited each farm weekly to collect enrollment information, frozen colostrum samples, and collect whole blood from all calves between the ages of 24 hours and eight days post colostrum feeding. For a subset of animals in three farms, birth weights were recorded by electronic scale and weaning weights recorded by tape measurement. All treatment and mortality events were recorded by farm personnel.

All samples were transported back to the University of Minnesota Dairy Production Medicine Laboratory in ice packed coolers. Whole blood was centrifuged, serum separated, collected, and dispensed into two equal aliquots for analysis. Serum total protein (STP) was recorded by a hand held refractometer (VET 360, Reichert, Inc. Depew, NY) and then frozen at -20^{o}C for later analysis. The colostrum and serum

samples were later analyzed to determine IgG concentration (mg/ml) by Turbidimetric Immunoassay (TIA). TIA is an automated and validated method to determine IgG levels and highly correlates with radioimmunodiffusion (RID), the gold-standard of IgG measurement.[51] The mass of colostral IgG fed (g) was calculated by multiplying the colostral IgG concentration (g/L) by the volume of colostrum fed (L), and the necessary conversion factors to obtain grams.

Statistical Analysis

All analyses were performed using SAS (version 9.1, SAS Institute, Cary, N.C.) Descriptive statistics were generated to describe the bacteriological and IgG concentration for each colostrum sample, serum IgG (mg/ml), STP (g/dl), and number of treatment and morbidity events by treatment group and farm. Only colostrum batches that were fed to a calf were included in the analysis. The unique colostrum batch fed was not properly recorded for nine calves. For an additional 17 calves, the colostrum sample was not saved and thus not available for IgG analysis. Of the 1099 calves enrolled in the study, serum samples were not available for ten calves. The serum samples were not collected in eight calves because the calf either died or was sold before a blood sample could be collected. Two samples were either lost or broken in lab. The calves with missing values for serum IgG were equally distributed between the two colostrum feeding groups. The unit of analysis was the individual calf.

Linear regression (proc mixed) was used to describe to describe the effect of colostrum treatment on STP, serum IgG, and weaning weights. Univariate models were explored for the following covariates, treatment, farm, IgG fed (g), and unique batch of

colostrum. Multivariate models were investigated by a forward step-wise approach evaluating the type-3 effects for each predictor and evaluating over model fit using -2 Log Likelihoods. When significant interaction terms were found with farm, the analysis was stratified by farm. Farm was forced in all models as a fixed effect to control for the clustering of calves within a farm. The effect of clustering for each unique batch of colostrum was explored as a random effect in multivariate models. The simplest and most parsimonious model was selected to describe the effect of heat-treatment on the outcomes of interest. Final significance was declared at p-value < 0.05.

A treatment event was used a proxy for a morbidity event. Logistic regression (proc genmod) was first used to explore the effect of colostrum treatment for the risk of a treatment for sick event or death event in the pre-weaning period. First, univariate models were explored for the following predictors, treatment, farm, IgG fed (g), serum IgG (mg/ml), failure-of-passive-transfer status, and unique batch of colostrum. Then multivariate models used a step-wise forward approach for these covariates evaluating the type-3 effects and the Log Likelihood with significance declared if the 95% confidence interval did not contain one. Farm was again forced into the model as a fixed effect to control for clustering of calves within the farm. Clustering of calves within batches of colostrum was examined as a random effect in the multivariate models. The most parsimonious model was selected to describe the effect of treatment on the risk for a treatment and death event.

Cox regression (proc TPHreg) was used to describe the effect of colostrum treatment on the hazards for a treatment for sick event or a death event in the preweaning

period. The same procedures used in linear and logistic regression univariate and multivariate model building described above were also used in this portion of analysis. Farm was forced into the models as a fixed effect to control for clustering of calves within a farm. Calves were censored at 60 days if they did not experience a treatment for sickness or death event. Unique batch of colostrum was examined as a random effect. Proc lifetest was used to generate survival graphs for treatment and death events.

RESULTS

The demographics for the herds enrolled in this study are summarized in Table 1. A summary of the calves enrolled in each treatment group overall, by farm and gender is shown in Table 2. There was a significant increase in serum IgG (mg/ml) in the calves fed heat-treated colostrum versus calves fed fresh colostrum. The adjusted means for serum IgG were 18.11 mg/ml and 15.48 mg/ml for the calves fed heat-treated versus fresh colostrum, respectively (Table 3). The full model included treatment, farm, and colostral IgG fed (g) as described below:

$$Y = \beta_0 + \beta_1 T + \beta_2 F + \beta_3 G + \varepsilon.$$

Where β_0 is the intercept, $\beta_{1\text{-}3}$ are the parameter estimates for the explanatory variables, T is the treatment of colostrum (heat-treated or fresh), F is the farm number (1, 2, 3, 4, 5, or 6), G is the mass of IgG (g) fed in colostrum and ε is the error term of the general liner model. All farms experienced a numeric improvement in serum IgG as a result of feeding the heat-treated colostrum although the magnitude of the improvement varied by the farm. The increased serum IgG levels were significant for all but two of the farms.

Including batch as a random effect in the model did not significantly change the estimate of the effect of feeding heat-treated colostrum on the serum IgG levels and so this term was removed from the final model. The coefficient (SE) for the effect of mass of colostral IgG (g) fed on serum IgG was a 0.031 (0.003) mg/ml increase for each one gram increase of colostrum IgG fed (p-value < 0.0001). The final, overall estimate (SE) of the effect of feeding heat-treated colostrum on serum IgG was a significant increase of 2.63 (0.34) mg/ml (p-value < 0.0001).

The effect of feeding heat-treated colostrum on STP (g/dl) mirrored those for serum IgG (mg/ml) (Table 4). When STP (g/dl) was measured between one and eight days of age, calves fed heat-treated colostrum had statistically significantly increased measurements versus calves fed fresh colostrum. The full model with farm, treatment, and grams of colostral IgG fed as described below:

$$Y = \beta_0 + \beta_1 T + \beta_2 F + \beta_3 G + \varepsilon.$$

Where β_0 is the intercept, $\beta_{1\text{-}3}$ are the parameter estimates for the explanatory variables, T is the treatment of colostrum (heat-treated or fresh), F is the farm number (1, 2, 3, 4, 5, or 6), G is the mass of IgG (g) fed in colostrum and ε is the error term of the general liner model. The effect of treatment resulted in an adjusted mean of 5.92 (g/dl) for calves fed heat-treated colostrum versus 5.77 (g/dl) for calves fed fresh colostrum (p-value < 0.001, Table 4). Similar to serum IgG, the magnitude of the improvement in STP levels varied by the farm. Half of the farms detected a statistically significant increase in STP associated with feeding the heat-treated colostrum. Controlling for the effect of clustering with each unique batch of colostrum as a random effect did not significantly

change the estimate for the average difference for the treatment effect. The coefficient (SE) for the effect of mass of colostral IgG (g) on STP was 0.0016 (0.0002) g/dl increase for each additional one gram increase of colostral IgG fed (p-value < 0.0001).

Results for the logistic regression analyses investigating the effect of treatment on treatment and mortality risk are summarized in Table 5. The most parsimonious model included treatment and farm for both the risk for either a treatment or death event. Colostral IgG fed (g) was a significant predictor and improved the fit of the model for the analysis for the risk of a death event but not for the risk of treatment for a sick event. The following equations describe the models used in this portion of the analysis:

$\text{logit}(p)_{\text{treatment for sick event}} = \beta_0 + \beta_1 T + \beta_2 F$

and $\text{logit}(p)_{\text{death event}} = \beta_0 + \beta_1 T + \beta_2 F + \beta_3 G.$

Where p describes the probability of the event of interest, β_0 is the intercept, $\beta_{1\text{-}3}$ are the parameter estimates for the explanatory variables, T is the treatment of colostrum (heat-treated or fresh), F is the farm number (1, 2, 3, 4, 5, or 6), G is the mass of IgG (g) fed in colostrum. There was not a significant interaction between colostrum treatment and farm. Including unique batch of colostrum as a random effect did not change the estimates for the treatment effect and so this term was removed from the final models. For the calves fed heat-treated colostrum, 32.5% experience a treatment event and 2.5% a death event. For the calves fed fresh colostrum, 36.3% experienced a treatment event and 1.7% a death event (Table 5). The odds ratio was 0.84 and 1.51 for the risk of a preweaning treatment and death event respectively. The coefficient (SE) for the effect of colostral IgG fed (g) was 0.006 (0.003) for the model for the risk of a death event (p-

value = 0.05). The 95% confidence interval for the odds ratios for both of these risks included one and therefore was not statistically significant.

The survival analysis models included both colostrum treatment and farm as covariates for both models for treatment and death events but colostral IgG (g) fed was significant only in the model for a death event. These models are described below:

$h(t) = h_0(t)e^{\beta X}$

Where $\beta X_{\text{treatment for a sick event}} = \beta_1 T + \beta_2 F$ and

$\beta X_{\text{death event}} = \beta_1 T + \beta_2 F + \beta_3 G$.

$\beta_{1\text{-}3}$ are the parameter estimates for the explanatory variables, T is the treatment of colostrum (heat-treated or fresh), F is the farm number (1, 2, 3, 4, 5, or 6), G is the mass of IgG (g) fed in colostrum. There was no difference associated with colostrum treatment for a sick event since the hazard rate was 0.88 (C.I. = 0.72, 1.08, p-value = 0.22) (Table 6, Figure 1). There was no difference associated with colostrum treatment for a death event since the hazard rate was 1.49 (C.I. = 0.64, 3.48, p-value = 0.35) (Table 6, Figure 2). The coefficient (SE) for the effect of colostral IgG fed (g) was 0.006 (0.003) for the model for the hazard of a death event (p-value = 0.04).

Weight variables collected from a subset of calves 278 calves fed heat-treated colostrum and 272 calves fed fresh colostrum on three farms. There was no significant difference in birth weight (kg), weaning weight (kg), weight gained between birth and weaning (kg), or average daily gain (kg) between the groups of calves fed either heat-treated or fresh colostrum (Table 7). The simplest most parsimonious model for all the pre-weaning weight outcomes contained the covariates treatment and farm.

DISCUSSION

The primary motivation for heat-treatment of both milk and colostrum fed to dairy calves on commercial dairy farms is reducing the exposure to microorganisms in general, but also to specific pathogens such as *Escherichia coli, Salmonella, Mycoplasma, Mycobacterium avium* subsp. *paratuberculosis* (MAP), and Bovine Leukemia Virus which are a concern in the contemporary dairy industry.[64, 65, 68, 71, 94, 102] One of the earliest laboratory studies on heat-treating colostrum, reported in 1923, was for the purpose of evaluating the time and temperature required to inactivate tuberculosis organisms without damaging the necessary Ig required for passive transfer of maternal immunity.[76] This early study determined colostrum can be safely heated at 60^{o}C for up to three hours without denaturing Igs and creating an undesirable, heat-coagulated product which cannot be fed[76] The most recent research which supports the time and temperature recommendations of 60^{o}C for 60 minutes for heat-treating colostrum were designed to avoid a heat-coagulated product, preserve Ig function, but also to inactivate MAP.[79, 80]

The current study is the first large clinical trial to evaluate the effect of feeding heat-treated colostrum on passive transfer of immunity, health and growth in calves raised under the conditions of six commercial dairy farms. In this study there was a statistically significant improvement in serum IgG (mg/ml) and STP (g/dl) in calves fed heat-treated colostrum. Other studies have also described improved serum IgG in calves fed heat-treated versus fresh colostrum.[14, 67, 77, 86, 103] It has been hypothesized that this effect may be explained by the fact that bacteria, inactivated by heat-treatment process,

are no longer present to interfere with the passive absorption of Ig across the neonatal intestine, or to be neutralized by maternal Ig, thereby making more colostral Ig available for absorption.[14,82] However, this hypothesis is not yet proven.

A couple of observational studies have reported large variations in the types and concentrations of bacterial organisms in fresh colostrum.[15, 64] There are conflicting studies describing the relationship between bacterial levels in colostrum and the passive transfer of Ig.[14, 86, 87] One previous observational study of commercial herds in Wisconsin described high levels of bacterial contamination in fresh colostrum: 82% of the colostral samples had bacterial counts greater than 100,000 cfu/ml and many were greater than 1,000,000 cfu/ml.[87] That study reported that, for 101 calves tested, the level of bacterial contamination of colostrum had a negative relationship with passive transfer of immunity.

Conversely, a recent study using a small number of calves in a university herd found that, although there was a positive association with feeding heat-treated colostrum and increased serum IgG levels overall.[67] The same authors reported in a latter paper that the improvement in passive transfer may not have been related to microbial counts since calves fed fresh colostrum with higher microbial counts, 5.61 $\log_{10}$ (cfu/ml) for TPC, had similar IgG levels as those fed fresh colostrum with low microbial counts, 3.97 $\log_{10}$ (cfu/ml) for TPC.[86] However these microbial counts were moderate to low as compared to colostrum bacteria levels found in commercial dairy herds. For example, one previous study of 12 commercial herds in MN and WI reported mean microbial levels in colostrum of 8.21 $\log_{10}$ (cfu/ml) for TPC and 6.44 $\log_{10}$ (cfu/ml) for TCC.[88]

While the nature of the relationship between bacterial contamination in colostrum and passive transfer obviously needs more study, readers should consider other possibilities as well. It has been hypothesized that perhaps another macromolecule, not as yet described, is altered during the heat-treatment process which allows for a greater efficacy of IgG absorption when the heat-treated product is fed.[86] An experimental study reported that adding bovine serum albumin to colostral whey decreased the absorption of IgG suggesting both a competition and a limited capacity for macromolecular absorption by the neonatal intestine.[104] Thus, it may be possible that the mechanism to explain improved absorption of Ig fed heat-treated colostrum may lay with the denaturation of some other unidentified protein or factor in colostrum which usually competes with the absorption of IgG in the neonatal intestine. Perhaps there is a protein or other factor which is altered by heat-treatment that has a role in gut closure and allows for more efficient absorption of colostral IgG. Clearly, the mechanism to explain the improved passive transfer of IgG in calves fed heat-treated colostrum is not understood and requires further study.

It was expected that calves fed heat-treated colostrum would be healthier and grow better, due both to improved serum IgG levels and decreased microbial exposure through colostrum (reported in previous chapter). In this study however, calves fed heat-treated colostrum did not experience any advantage in weight gain or health during the pre-weaning period. This is similar to results reported in two small studies which showed no differences in calves fed heat-treated colostrum versus calves otherwise consuming an

adequate amount of fresh colostrum.[67, 76] However, the latter two studies lacked sufficient sample size to investigate the effects of treatment on health outcomes.

One possible explanation for this lack of observed treatment on health outcomes could relate to the baseline level of passive transfer in the control group. The mean serum IgGs for the calves in both colostrum treatment groups were greater than 15.0 mg/ml, well above the FPT level of 10.0 mg/ml, which is associated with decreased weight gains, and increased risks for morbidity and mortality. Previous observational studies described that mortality 31% to 39% was attributed to the FPT status.[45, 91]The overall baseline mortality rate in the present study was only 2.0% which is well below the baseline mortality rates of 8.2% and 7.8% reported in previous observational studies.[45, 105, 106] So it is likely that, in this study, no difference could be detected in the mortality rates between the two treatment groups because of the low number of events. A future study could further explore this outcome by conducting the study using a larger number of calves in a larger number of herds with greater variation in baseline mortality rates. Treatment for a sick event, the proxy used for a morbidity event in this study, was moderate, 34% overall, whereas the most recent NAHMS report indicates morbidity of 23.9% for gastrointestinal illness and 17.9% for respiratory illness.[106] If there were significant differences in risk for treatment or morbidity, this study was adequately powered to detect these differences if they existed. Thus in this study while feeding heat-treated colostrum resulted in statistically significant improvements in serum IgG (mg/ml) these improvements did not result in meaningful differences clinically.

A second possible explanation for the observed lack of effect of treatment on health outcomes considers a relatively low-to-moderate level of microbial challenge in colostrum fed to control calves. In the present study, the mean for the Total Plate Count (TPC) was 5.4 $\log_{10}$ (cfu/ml) and the Total Coliform Count (TCC) was 4.4 $\log_{10}$ (cfu/ml) for the calves fed fresh colostrum as compared to 3.6 $\log_{10}$ (cfu/ml) and 2.3 $\log_{10}$ (cfu/ml) for the same bacterial counts respectively for calves fed heat-treated colostrum (reported previously in a separate chapter). It may be in the current study that no differences were detected between the treatment groups in the preweaning period because the microbial counts in the colostrum fed to control calves did not present a great enough challenge to have significant negative effects on weight gain, morbidity, or mortality outcomes studied here. Alternately, it is possible that the difference in colostrum bacteria counts between treated and control colostrum fed was not enough to cause a measureable difference in the growth or health parameters measured. Future studies should investigate the relationship between colostrum bacteria counts and health in calves.

A third possible explanation for the observed lack of effect of treatment on health outcomes considers colostral leukocytes or other immune factors that may be present in colostrum but which were not described in this study. Colostral leukocytes have been demonstrated to be absorbed by the neonatal intestine and reach the neonate's lymphoid tissue and circulation.[33, 35]It is suggested these colostral leukocytes may play a role in enhancing the development of the neonatal immune system.[36, 107, 108] Previous studies with feeding heat-treated colostrum found no difference in leukocyte counts or their phenotypes between calves fed heat-treated or fresh colostrum.[14] Recently, it has been

demonstrated that the heat-treatment process significantly reduces the viability of these colostral leukocytes.[109] Thus, while colostral bacteria were inactivated and calf serum IgG levels improved, perhaps these beneficial effects were negated by lack of transfer of viable colostral leukocytes and account for no observable significant differences in this study. This hypothesis requires further investigation.

Finally, a recent study has documented that while the heat-treatment process causes some minor changes in nutrients, these compositional changes are in agreement with the range of nutrient values reported in previous studies for fresh colostrum.[1, 2, 67]This same study found no difference in feed intakes and no difference in weight gains between calves fed heat-treated and fresh colostrum.[67] The other pilot study which explored the effect of the passive transfer of immunity for calves fed heat-treated colostrum found no difference in serum vitamin levels and serum neutralization titers for type 1 bovine viral diarrhea virus.[14] Thus, it appears when calves are similarly managed and all known factors are controlled, there do not appear to be any negative effects associated with feeding heat-treated colostrum.

CONCLUSION

This is the first large clinical trial on commercial dairies to study the effects of feeding heat-treated colostrum on passive transfer, health, and growth during the preweaning period. Calves fed colostrum heat-treated at 60^{o}C for 60 minutes, experienced an increase of 2.63 mg/ml in serum IgG versus calves fed fresh colostrum which was statistically significant. Serum total protein, likewise was statistically

significantly increased overall in the calves fed the heat-treated colostrum as compared to calves fed fresh colostrum. There was no effect of treatment on preweaning weight gain, and no difference in the risk or for having a treatment or death event in the preweaning period. Further study is needed to describe if these results are repeatable in dairy herds with varying levels of management, health, and colostrum feeding practices. This study is ongoing and will follow the animals enrolled to evaluate if feeding heat-treated colostrum at birth results in a decreased risk for testing positive for MAP and other pathogens in future lactations and its effect on lactation production parameters and longevity. Future research should seek to elucidate the mechanisms and explain why there is improved passive transfer of IgG associated with feeding heat-treated colostrum.

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Table 1: Study Herd Demographics

Farm #	State	Milking Herd Size	RHA kilograms	SCC cells/ml	Dry Period (days)
1	MN	1,500	13,892	205,000	45
2	MN	1,600	14,512	149,900	48
3	WI	1,500	12,956	275,000	45
4	WI	2,500	12,345	350,000	37
5	MN	1,200	12,363	276,000	45
6	MN	1,400	11,280	430,000	44

Table 2: Summary of Calves Enrolled

		Heat-Treated Colostrum			Fresh Colostrum		
Number of Calves	Total	Male	Female	Total	Male	Female	Total
All Farms	1099	69	507	576	87	436	523
Farm 1	177	1	101	102	1	74	75
Farm 2	181	0	103	103	0	78	78
Farm 3	149	0	75	75	0	74	74
Farm 4	114	0	58	58	0	56	56
Farm 5	302	68	82	150	86	66	152
Farm 6	176	0	88	88	0	88	88

Table 3: Effect of Feeding Heat-Treated Colostrum on Serum IgG

			Serum IgG (mg/ml)		
	n	Treatment	Mean*(se)	Difference**(se)	p-value
All Farms	549	Heat-Treated	18.11 (0.24)	2.63 (0.34)	< 0.0001
	515	Fresh	15.48 (0.25)	referent	.
Farm 1	100	Heat-Treated	18.73 (0.61)	3.62 (0.95)	0.0002
	73	Fresh	15.11 (0.72)	referent	.
Farm 2	100	Heat-Treated	18.05 (0.55)	3.72 (0.83)	< 0.0001
	77	Fresh	14.33 (0.63)	referent	.
Farm 3	72	Heat-Treated	15.59 (0.65)	1.16 (0.92)	0.21
	72	Fresh	14.43 (0.65)	referent	.
Farm 4	58	Heat-Treated	26.41 (0.79)	6.07 (1.12)	< 0.0001
	56	Fresh	20.33 (0.80)	referent	.
Farm 5	133	Heat-Treated	11.26 (0.38)	1.79 (0.53)	0.0008
	150	Fresh	9.47 (0.36)	referent	.
Farm 6	86	Heat-Treated	19.19 (0.67)	0.63 (0.95)	0.51
	87	Fresh	18.56 (0.67)	referent	.

*lsmeans reported

**Difference of the lsmeans between the two treatment groups

Table 4: Effect of Feeding Heat-Treated Colostrum on Serum Total Protein

Serum Total Protein (g/dl)

	n	Treatment	Mean*(se)	Difference**(se)	p-value
All Farms	550	Heat-Treated	5.92 (0.02)	0.15 (0.03)	< 0.0001
	515	Fresh	5.77 (0.02)	referent	.
Farm 1	100	Heat-Treated	5.91 (0.05)	0.21 (0.08)	0.005
	73	Fresh	5.70 (0.06)	referent	.
Farm 2	101	Heat-Treated	5.92 (0.04)	0.19 (0.06)	0.003
	77	Fresh	5.73 (0.05)	referent	.
Farm 3	72	Heat-Treated	5.94 (0.06)	0.04 (0.08)	0.62
	72	Fresh	5.90 (0.06)	referent	.
Farm 4	58	Heat-Treated	6.43 (0.07)	0.52 (0.10)	< 0.0001
	56	Fresh	5.91 (0.07)	referent	.
Farm 5	133	Heat-Treated	5.39 (0.04)	0.09 (0.06)	0.13
	150	Fresh	5.31 (0.04)	referent	.
Farm 6	86	Heat-Treated	6.01 (0.06)	0.0089 (0.0839)	0.92
	87	Fresh	6.00 (0.06)	referent	.

*lsmeans reported

**Difference of the lsmeans between the two treatment groups

Table 5: Logistic Regression Analysis for the Effect of Feeding Heat-Treated Colostrum on the Risk of Treatment for a Sick and Death Event in the Preweaning Period

Treatment for a Sick Event					
	n	%	O.R.	95% C.I.	p-value
Heat-Treated	187 / 576	32.5	0.84	0.65, 1.10	0.20
Fresh	190 / 523	36.3	referent	.	.

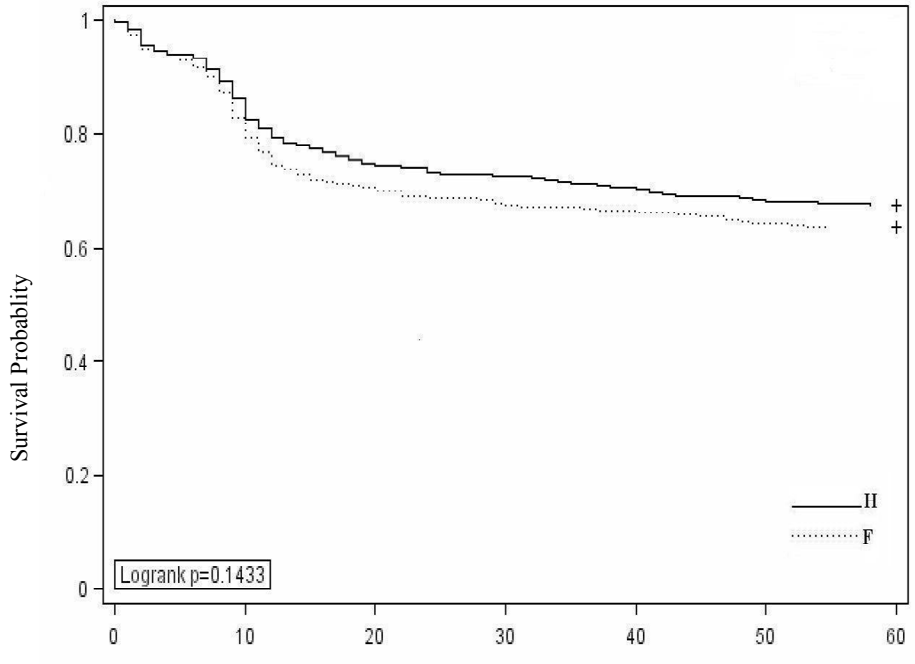
Death Event					
	n	%	O.R.	95% C.I.	p-value
Heat-Treated	14 / 554	2.5	1.51	0.56, 3.56	0.35
Fresh	9 / 519	1.7	referent	.	.

Table 6: Survival Analysis for the Effect of Feeding Heat-Treated Colostrum for the Hazard of a Treatment or Death Event in the Preweaning Period

Treatment Event				
	Estimate (se)	H.R.	95% C.I.	p-value
Heat-Treated	-0.13 (0.10)	0.88	0.72, 1.08	0.22
Fresh	referent	.	.	.

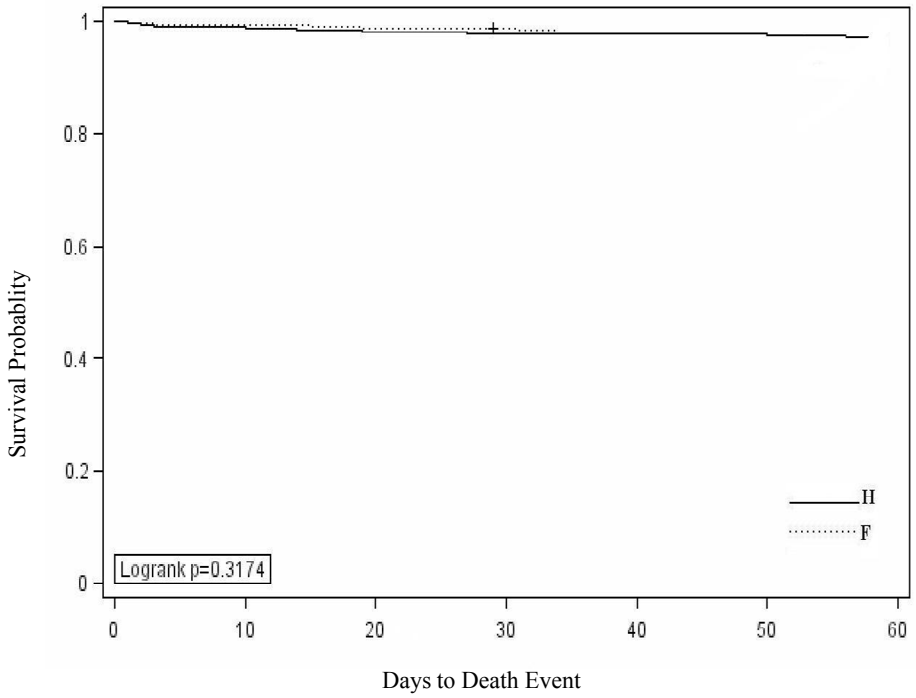
Death Event				
	Estimate (se)	O.R.	95% C.I.	p-value
Heat-Treated	0.40 (0.43)	1.49	0.64, 3.48	0.35
Fresh	referent	.	.	.

Figure 1: Survival Plot for Risk for Treatment for Calves fed Either Heat-Treated or Fresh Colostrum



H = Calves fed Heat-Treated Colostrum
F = Calves fed Fresh Colostrum

Figure 2: Survival Plot for Risk for a Death Event for Calves fed Either Heat-Treated or Fresh Colostrum



H = Calves fed Heat-Treated Colostrum
F = Calves fed Fresh Colostrum

Table 7: Effect of Feeding Heat-Treated Colostrum on Weight Gain in the Pre-Weaning Period on Three Farms

	Heat-Treated		Fresh		p-value
Number of Calves		n		n	
Birth Weight (kg)	40.1 (0.3)	278	41.4 (0.3)	272	0.18
Wean weight (kg)	76.8 (0.6)	186	76.5 (0.6)	184	0.70
Weight gained (kg)	35.5 (0.7)	183	34.8 (0.7)	182	0.47
Average Daily Gain (kg)	0.62 (0.01)	183	0.62 (0.01)	182	0.995

*Crude means and (standard error) reported

Table 8: REFLECT Guidelines for Calves fed either Heat-Treated or Fresh Colostrum

REFLECT Checklist			
Paper section and topic	Item	Descriptor	Reported on Page #:
Title and Abstract	1	How study units are allocated, clearly state if the intervention was natural exposure or deliberate challenge	41-42
Introduction, Background	2	Scientific background and rationale	42-44
Methods, Participants	3	Eligibility criteria and study units	45
Interventions	4	The level the intervention was allocated	46
	4b	Precise details of the agent and challenge model	45-47
Objectives	5	Clearly state primary and secondary objectives	44
Outcomes	6	Clearly defined primary and secondary outcome measures, level of measurement	44
Sample Size	7	How sample size was determined	47
Randomization – sequence generation	8	Method used to generate the random allocation sequence at the relevant level	46
Randomization – allocation concealment	9	Method used and concealment of sequence	46
Randomization – implementation	10	Who generated the sequence, enrolled study units, and assigned study units to group	46
Blinding (masking)	11	Were participants blinded to the intervention of justification if no blinding used	46
Statistical Methods	12	Methods used to compare outcomes and clearly state the level of analysis	47-48
Results, Study Flow	13	Study Units through each stage for each level of the organization	49-52
Recruitment	14	Dates defining the recruitment	44
Baseline Data	15	Baseline demographic and clinical data	Tables 1-2
Numbers analyzed	16	Number of study units, and state “intention to treat”	46
Outcomes and estimation	17	A summary of results for each group, accounting for relevant structure	Table 3–7 Figure 1-2
Ancillary analyses	18	Address multiplicity of analysis preformed	47-49
Adverse events	19	All important adverse events	47-48
Discussion, Interpretation	20	Interpretation of results, bias or imprecision, herd immunity if relevant and the relevance of the disease challenge	53-58
Generalizability	21	Generalizability (external validity) of the findings	58
Overall Evidence	22	General interpretation of the results in the context of the current evidence	58

CHAPTER 4

SUMMARY AND FUTURE DIRECTIONS

INTRODUCTION

The neonatal calf is born agammaglobulinemic since the syndesmochorial placenta of the ruminant does not allow the passive transfer of maternal immunity in utero.[7, 12] Thus, the calf must consume colostrum of both sufficient volume and immunoglobulin concentration within the first few hours of life for adequate passive transfer of maternal immunoglobulins (Ig) to occur. Research in dairy production medicine has evaluated critical factors affecting both colostrum quality and its feeding and management to improve the passive transfer of immunity.[9-11, 50, 61, 63] One area of focus in contemporary colostrum research has centered on the hygienic quality of colostrum to identify critical control points for microbial contamination, including the heat-treatment of colostrum to reduce microbial counts and inactivate pathogens while preserving IgG.[74, 79, 80] Recent research in heat-treating colostrum aimed at reducing microbial counts, inactivating pathogens, while minimizing changes in viscosity and preserving IgG concentration has determined that colostrum can be heat-treated at 60^{o}C for 60 minutes.[79, 80, 85] Pilot studies found feeding heat-treated colostrum to dairy calves resulted in improved efficacy of Ig absorption and improved serum immunoglobulin G (IgG) concentrations as compared to calves fed untreated colostrum.[14, 67] However short and long-term health, performance, and economic benefits of feeding heat-treated colostrum under field conditions have not been described.

The objectives of this project were first to evaluate the effect of heat-treating colostrum at 60^{o}C for 60 minutes on microbial counts and colostral IgG concentration and second to evaluate the effect of feeding heat-treated colostrum on passive transfer of IgG, pre-weaning weight gain, morbidity and mortality events in calves raised under the working conditions of commercial dairy farms. Reduced pathogen exposure and improved passive transfer of Ig should theoretically result in a healthier calf.

The major objective of the first study was to describe the effect of heat-treating colostrum at 60^{o}C for 60 minutes on the microbial counts, Total Plate Count (TPC) and Total Coliform Count (TCC), cfu/ml and on IgG concentration (mg/ml) under the working conditions of six commercial dairy farms. Of the total 276 possible unique batches of colostrum, there were 266 matched pairs of colostrum, unheated and heat-treated, used in the analysis. The mean $\log_{10}$ TPC and TCC measurements for fresh colostrum were 5.4 and 4.4 (cfu/ml), respectively while the mean $\log_{10}$ TPC and TCC measurements for heat-treated colostrum were 3.6 and 2.3 (cfu/ml) respectively (p-value < 0.0001). The difference for the effect of heat-treatment was an estimated 1.79 and 2.06 decrease in the mean for TPC and for TCC respectively. All farms saw reductions in both measures of microbial counts, although the magnitude of the reduction varied by the farm. The mean colostral IgG concentration for fresh and heat-treated colostrum was 60.7 and 59.2 mg/ml respectively (p-value = 0.38). While there was no significant loss of colostral IgG concentration overall, there was evidence in a secondary analysis that individual batches of colostrum with higher IgG concentrations, greater than 70 mg/ml,

experienced significantly greater losses during the heat-treatment process as compared to batches of colostrum with IgG concentrations less than 50 mg/ml.

Limitations to this study include the fact, that aside from TPC and TCC, colostrum was not tested to describe other species of bacteria or other colostrum components for either treatment group. As an example, colostral leukocytes are another aspect of maternal immunity demonstrated to be absorbed by the neonatal intestine. In the current study, because colostral samples were frozen on the farm, there was no longer the option to evaluate the effect of heat-treatment on the viability or the functionality of these cells.

The objective of the second part of this study was to describe the effect of feeding heat-treated colostrum on passive transfer of IgG and on growth, treatment and mortality risk in preweaned calves. It was hypothesized that feeding heat-treated colostrum would cause reduced microbial exposure from colostrum and improved serum IgG levels in the calf, ultimately resulting in healthier calves with improved growth and less morbidity and mortality. A total of 1099 calves were assigned to be fed either heat-treated (n = 576) or fresh (n = 523) colostrum. These calves were enrolled in the study from June to August of 2007 from six commercial dairy farms in Wisconsin and Minnesota. The mean (se) serum IgG and standard error was 18.11 (0.24) mg/ml for calves fed heat-treated colostrum as compared to 15.48 (0.25) mg/ml for the calves fed fresh colostrum. The final overall estimate of the effect of feeding heat-treated colostrum on serum IgG was a significant increase of 2.63 mg/ml (p-value < 0.0001). However, there was no effect of colostrum treatment on growth, risk for treatment, or risk for mortality in the preweaning

period. Mean average daily gain was 0.67 kg/day for calves in both colostrum treatment groups (p-value = 0.995). A total of 32.5% or 36.3% of calves were treated for illness in the heat-treated versus fresh group, respectively. A total of 2.5% or 1.7% of calves died in the heat-treated versus fresh group, respectively. Logistic analysis determined that feeding heat-treated colostrum had no effect on risk for treatment since the (O.R. = 0.84 (0.65, 1.10), p-value = 0.20) or risk for death (O.R. = 1.51 (0.65, 3.49), p-value = 0.35). Similarly, survival analysis showed there were no differences in the hazard for a treatment event ($HR_{heat\text{-}treated}$ = 0.88 (0.72, 1.08), p-value = 0.22) or a death event ($HR_{heat\text{-}treated}$ = 1.49 (0.64, 3.48), p-value = 0.35).

There are several hypotheses as to why the authors did not see improved growth or health in calves fed heat-treated colostrum, the first being high levels of passive transfer in the control calves. The management on these farms was very good as evidenced by the high mean serum IgG levels of 15.48 and 18.04 mg/ml for calves fed fresh and heat-treated colostrum, respectively. These levels of passive transfer of IgG are well above the adequate mark of 10 mg/ml. A second possible explanation may rest in low-to-moderate exposure to bacteria in the control group fed fresh colostrum, resulting in a decreased disease challenge in control calves. The association between microbial levels in colostrum and the passive transfer of immunity and calf health remains to be determined. More studies are needed to elucidate the mechanisms involved with the improved efficacy of absorption of IgG in the neonatal intestine and to better understand the factors involved in gut closure. Another possibility considers that, while feeding heat-treated colostrum resulted in improved serum IgG levels and decreased microbial

exposure, these effects might potentially be partially negated by inactivating colostral leukocytes. Further research is needed to describe the function and significance of colostral leukocytes to the neonatal calf. In order to detect a difference, if one truly exists, this study should be repeated with either a larger number of calves or in herds where there is more variation in management and the levels of disease. Feeding heat-treated colostrum may have some long term benefits in animal health and performance. This study is ongoing to determine the effect of feeding heat-treated colostrum on the risk of testing positive for *Mycobacterium avium* subsp. *paratuberculosis* in the first three lactations.

CONCLUSION

This thesis represents the first large scale clinical trial under the working conditions of commercial dairy farms. This study found that heat-treatment of colostrum at 60 ºC for 60 minutes resulted in significant reductions in colostrum bacteria counts but had no negative effect on colostrum IgG. Serum IgG and TP was improved in calves fed heat-treated colostrum. However, treatment had no effect on growth, treatment risk, or mortality risk in the preweaning period. Future research needs to evaluate the effect of feeding heat-treated colostrum on the passive transfer of maternal leukocytes and its effect on the maturation of the immune system in the neonatal calf. Future research needs to evaluate whether feeding heat-treated colostrum results in any meaningful differences in production measures for lactation performance or the risk for diseases with strong risk factors for colostral transmission such as Johne's Disease.

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