Pasteurization and Freezing Effect on Colostrum Characteristics, Morbidity, IgG Absorption, and Growth Rates of Holstein Heifer Calves

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Abstract

The objective of this study was to determine if fresh frozen, pasteurized frozen, or fresh refrigerated colostrum impacted morbidity, growth rates, and the overall serum immunoglobulins (IgGs) in newborn calves. The second objective was to compare the effects that pasteurization and freezing had on colostrum IgG concentration and total bacteria counts compared to raw refrigerated colostrum. A controlled trial was completed on a 6,000-cow dairy in California where colostrum was harvested twice daily, pooled, and then divided into three different treatment groups and processed accordingly. One treatment was pasteurized at 60^{o}C for 60 min and then frozen, the other two were both fresh, one going into the freezer and the other into the refrigerator. Samples of all treatment groups were taken to determine total plate count, total E. Coli count, and total IgG immediately before the colostrum was fed. Newborn calves were randomly assigned to be fed 4 quarts of either pasteurized frozen (Past, n=60), fresh-frozen (FF, n=60), or fresh-refrigerated (FR, n=60) colostrum within 2 hours of birth. Calves were weighed daily and picked up after receiving 2 or 3 feedings of treatment colostrum (dependent on time of birth) and taken to a custom heifer raising facility where they were weighed and monitored for morbidity (number of treatments) until weaning (60d). Pasteurization improved the serum IgG count in the calves compared to fresh and fresh frozen treatments. Pasteurization decreased bacteria counts and appeared to decrease IgG concentration in the colostrum but had no effects on morbidity or weaning weight.

Key words: colostrum, pasteurized, frozen, immunoglobulin

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Introduction

Raising replacement dairy heifers has become a huge opportunity for producers to leverage their herd towards future success and higher margins of profitability. When dealing with replacements, you are dealing with the future of your business. The initial investment in following recommended standard operating procedures (SOPs) is not always seen right away, but it will positively impact the health of your calves and pay off later in their lives. Many studies show that following recommended SOPs as babies may result in less mortality, less morbidity, higher rates of gain, lower age at first calving, and the possibility of producing more milk throughout their lactations. In order to accomplish this, we have to keep our calves as healthy as possible, starting from the day they are born and their first feeding of colostrum. We need to minimize failure of passive transfer and maximize the amount of immunoglobulins fed and absorbed. One of the opportunities that a dairy manager can take advantage of is in his colostrum management program. Though there are some areas that are out of the managers control, amount of IgG in colostrum and quantity of colostrum harvested, there are still ways to make sure the calf gets what she needs to gain passive transfer. Two tools that are used compensate for the lack of control are pasteurizing and freezing of the colostrum. Pasteurization kills off pathogens, ensuring good quality, and increases the colostrums shelf life. Freezing of colostrum ensures minimal bacteria growth and preserves supply for future use. These two tools are ways that the manager could have more control over what the calves are consuming. Although many studies have shown that pasteurizing increases IgG absorption and decreases bacteria in the colostrum, This study wanted to see if it would really work on a more commercial setting that would apply to large California dairies. As I was organizing our protocols on our

colostrum management, I was left with two questions: should we be pasteurizing and/or should we be freezing our colostrum? Which then sparked the idea to research and find out for myself.

Literature Review

Many studies have been published over the years evaluating the effects of colostrum on calves and the difference that their first feeding can have on the rest of their life. These studies have shown how critical maternal colostrum is for the calf and how important it is for dairy producers to focus on the first colostrum feeding. One study in particular was a case study on the effects of colostrum on lactation performance (Faber et al., 2005). In this study, calves were provided 2 or 4 L of colostrum with some additional meals over a 4 d period. The calves were then monitored for 2 lactations, where three major observations were made: A 30% increase in pre-pubertal growth rates based on a higher colostrum feeding level, a 16% increase in survival to the end of the second lactation of calves fed the 4L of colostrum, and 2263 lbs more milk was produced by the calves that received 4L after the 2nd lactation. Another study concluded that for each unit of serum immunoglobulin (IgG) concentration, measured at 24 to 48 h after colostrum feeding, above 12 mg/mL, there was an 18.7 lb increase in mature equivalent milk (DeNise et al., 1989). These studies prove the importance of colostrum, not only in the early life of the calf, but throughout the rest of the calf's life as well. So IgGs proved to be important in gaining passive transfer, but they still wanted to know if there was more to colostrum than just IgG concentration. A study was then done in 2001 comparing raw colostrum with colostrum replacement. In this study calves fed colostrum replacer had nearly the same plasma IgG concentrations, but grew at a rate 30% less than the raw colostrum calves (Mowrey, 2001). According to these studies, there are still factors other than Ig's than are beneficial to the calf and the quantity of the colostrum fed also plays a major role in determining the health of the calf.

The neonatal calf is born agammaglobulinemic, which is a condition in which the body forms no antibodies to sustain a healthy immune system, resulting in the lack of ability to fight infections. The placenta of the cow, separating the maternal and fetal blood supplies, causes Agammaglobunlinemia (Arthur, 1996), preventing the transmission of protective immunoglobunlins, immune cells, cytokines, nutritional elements, and other important growth factors (Barrington and Parish, 2001). This condition forces the calf to be fully dependent on the maternal colostrum to transfer over the necessary immunoglobulin to provide the calf with immunity during the first weeks of life (Tizard, 1996). This helps establish a good immune system, fight against invasive pathogens, and establish passive transfer, helping the calf to continue to mature and grow healthily.

Colostrum: Colostrogenesis and composition

Colostrum is created when the dam goes through the process of colostrogenesis. During this process many lactogenic hormones are released preparing to give birth, starting the formation of colostrum. While the dam is going through her dry period- lacteal secretions, serum proteins, immunoglobulins, maternal leukocytes, growth factors, cytokines, nonspecific antimicrobial factors, and nutrients accumulate in the mammary gland (Godden, 2008). All of these factors play an important role, impacting the future life and productivity of the calf.

Immunoglobulins

One of the most important factors in colostrum is the high level of immunoglobuns (Ig) it contains. These Ig's play an important role in establishing passive immunity in the young calf (Weaver et al., 2000; Jaster, 2005). The Ig intake of the calf depends on the amount of colostrum consumed and the concentration of Ig in that colostrum. Many factors such as lactation number, breed of cow, and the length of the dry period influence the volume and the total Ig concentration

in the colostrum (Muller and Ellinger, 1981; Pritchett et al., 1991; Tomkins and Jaster, 1991). In the maternal colostrum, there are a total of 2 isotypes of IgG: IgG_1 and IgG_2. These two IgG's work together to provide the calf with passive immunity until the calves own active immunity develops (Jaster, 2005). Although IgG is the dominant immunoglobulin transported into colostrum, there are still others such as IgM, IgA, and IgE that are present in the colostrum (Thatcher and Gershwin, 1989). In fact, IgG accounts for 85-90%, IgA accounts for 5%, and IgM accounts for a 7% of the total Ig in maternal colostrum, with IgG_1 accounting for 80 to 90% of the total IgG (Larson et al., 1980).

Other important factors

Colostrum also contains more than 1 million cells/mL of active maternal leukocytes, including macrophages, T and B-lymphocytes, and polymorphonuclear neutrophils (PMN) (Larson et al., 1980; Liebler-Tenorio et al., 2002). Early studies suggest that colostral leukocytes help increase the lymphocyte response to mitogens, which then increases phagocytosis and bacterial killing ability, and stimulates humoral immune responses in the calf (Reidel-Caspari, 1993; Le Jan, 1996; Donovan et al., 2007). These macrophages (leukocytes and PMN's) help defend against bacteria in the calf by a process called phagocytosis. In this process the immunoglobulins (antibodies produced by the T and B lymphocytes) enable the phagocytes to engulf and kill the bacteria that is present. So as a result, the more circulating immunoglobulins that the calf has against certain types of pathogens, the greater chance that the immune system will be able to dispose of the microorganisms (Akers, 2002).

Components of colostrum also include cytokines and growth factors. These other components deal with antimicrobial activity as well include lactoferrin, lysozyme, and lactoperoxidase (Pakkanen and Aalto, 1997; Shah, 2000; Elfstrand et al., 2002). Oligosaccharides in colostrum may also provide protection against pathogens by competing with

them in inhibiting the binding sites on the epithelial surfaces of the intestine (Przybylska et al., 2007). Growth factors also exist in colostrum such as transforming growth factor beta-2, growth hormone, and insulin, but the exact function of these growth facters are not yet clearly understood (Pakkanen and Aalto, 1997). Colostral insulinlike growth factor-1 may be a factor that helps with intestinal growth (Baumrucker et al., 1994) and also many other vitamins and minerals including calcium, magnesium, zinc, manganese, iron, cobalt, vitamin A, vitamin E, carotene, riboflavin, vitamin B12, folic acid, choline, and selenium are also found in colostrum (Foley and Otterby, 1978; Przybylska et al., 2007) The concentrations of most of these components are greatest in the first secretions harvested after calving and then decrease over the next six milkings to match the lower concentrations found in whole milk (Foley, 1978).

Colostrum management in the United States.

Failure of the newborn calf to absorb the adequate amount of immunoglobulins into circulation within the first 24 h of life results in failure of passive transfer (FPT; serum IgG <10.0 mg/mL), resulting in an increased risk for mortality and a negative effect on the future health and performance in the calves (Robinson et al., 1988; DeNise et al., 1989; Wells et al., 1996; Davis and Drackley, 1998; Faber et at., 2005). Unfortunately, many producers across the United States continue to struggle with maintaining good passive transfer rates and are suffering the financial losses associated with not reaching them. In fact, it was estimated that about 31% of preweaning mortality events that occurred in the first 3 weeks of life were attributed to FPT (Wells et al., 1996). In the last two studies done between 1997 and 2002, by the Nation Dairy Heifer Evaluation Project, 40-41% of the heifer calves in the U.S. suffered from FPT and the mortality rates were between 8-11% (National Animal Health Monitoring System, 1996; National Animal Health Monitoring System, 2002). In 2007 another study was done in which

19.2% of the calves experienced FPT and mortality rates were 7.8% (National Health Monitoring System, 2007; USDA, 2007). As you can see from these studies that a large number of the calves in the U.S. today are not getting the colostrum they need to gain passive transfer, and the mortality rates are high because of it. This is due to a lack of immunoglobulins absorbed, resulting from inadequate quantity or quality of the colostrum. One study tested the colostrum quality around the U.S. and found that almost 60% of colostrum on dairies is inadequate (Morrill et al., 2012). These studies point to the need of producers around the United States to adopt a better colostrum management program to make sure these calves are getting what they need.

Diseases and pathogens present in colostrum.

Although colostrum is an important source of nutrients and immune factors, it can also expose dairy calves to infectious microorganisms including Mycoplasma spp, Mycobacterium avium subsp paratuberculosis, fecal coliforms, and Salmonella spp., and bovine leukemia virus (Streeter et al., 1995; Steele et al., 1997; Walz et al., 1997; McGuirk and Collins, 2004). Microorganisms, contaminating colostrum, are also a concern because it is hypothesized that bacteria in colostrum may inhibit the absorption of IgGs across the intestine, not allowing them into the circulation, reducing passive transfer of immunity in the calf (James et al. 1981; Poulsen et al. 2002; Johnson et al. 2007). One study indicated that calves with higher concentration of Ig's were able to engulf pathogens before gaining full immunity, which allowed them to have energy for maintenance and utilize nutrients for growth. The calves with a low concentration of Ig's were forced to use them to defend against bacteria instead of gaining passive immunity (Robison et al., 1988). Experts recommend that fresh colostrum fed to calves contain fewer than 100,000 cfu/mL total bacteria count and fewer than 10,000 cfu/mL total coliform count (McGuirk and Collins, 2004). Unfortunately average bacteria counts on commercial dairies in

the U.S. far exceed this benchmark in our industry today. This was proven in two separate observational studies of commercial dairy herds in the Midwest that reported 82% and 92% of colostrum samples collected exceeded this industry standard (Poulsen et al. 2002; Swan et al., 2007).

IgG concentrations influencing morbidity and FPT.

When calves fail to absorb adequate amounts of immunoglobulins after birth, they are more likely to suffer from high morbidity and mortality rates (Boyd et al., 1972; Logan et al., 1975; Muggli et al., 1983). Newborn calves need the absorption of the Ig's, to gain initial immunity to control the presence of bacteria. This allows the immune system to produce enough antibodies rather than having to constantly fight off the bacteria (Williams et al., 1975). This was proved when a low amount of serum Ig concentrations correlated with a higher risk of death during the first 50 d of life in beef calves (Williams et al., 1975). Also, in a study of 1000 Holstein heifers calves, the calves with an Ig concentration less than 18 mg/ml had a higher risk of mortality than those with a concentration of greater than 18 mg/ml of Ig (Robison et al., 1988). Another study was done where morbidity, with the lowest serum Ig concentration exceeding 10 g/L at 30-60 h of life in heifer calves with serum levels greater than 15 g/L fully avoided respiratory tract infections (Furman-Fratzak et al., 2011). In 2012, a randomized conrolled trial was completed where pasteurized colostrum had significantly higher serum IgG concentrations than raw colostrum and morbidity was also much lower (Godden et al., 2012). These studies all point toward the premise that a higher concentration of immunoglobuns absorbed in the calf leads to lower rates of morbidity, mortality, and the chance of FPT.

IgG concentrations influencing weight gain.

In addition to lower mortality and morbidity, higher concentrations of serum Ig in calves are positively correlated with increased weight gain through weaning (Robison et al., 1988). A study was done comparing different quantities (3.78 L vs 1.89 L) of colostrum were fed which the larger amount obtained significantly higher rates of average daily gain (Faber et al., 2005). Also, in 2011 a study concluded that heifers with serum Ig levels greater than 10 g/L showed better mortality and achieved higher body weight gains allowing for a lower Age at First Calving (Furman-Fratzak et al., 2011). But a study in 2009, comparing pasteurized vs raw colostrum, showed otherwise. The pasteurized colostrum had a higher IgG concentration but over all weight gain was not affected. Therefore, it is not known yet whether pasteurizing colostrum denatures hormones and growth factors that help with weight gain (Elizondo-Salazar and Heinrichs, 2009). Although with studies pointing to high amounts of pathogens as one of the causes of morbidity, and knowing morbidity highly affects growth rates, one can assume that pasteurization can be a tool to indirectly help the calves grow and stay healthy by taking out that bacteria.

The effects of Pasteurizing Colostrum

An additional concern to high bacteria counts found in maternal colostrum, was the effect the bacteria was having on the calf. Some studies have reported that high concentrations of bacteria in colostrum are leading to a decrease in the Ig absorption rate, and resulting in a higher chances of failing in passive transfer of immunity (James et al., 1981; Poulson et al., 2002). Once this was taken into account then the concept of pasteurization began to be tested on colostrum. Early studies tried to pasteurize colostrum using the same conventional methods and high temperatures that are typically used to

pasteurize milk (63° C for 30 min, or 72° C for 15 seconds). This process did not work as planned and yielded unacceptable results including IgG denaturation and increases in viscosity (Meylan et al., 1996; Godden et al., 2003; Stabel et al., 2004). However, more recent research has determined that problems with viscosity or IgG denaturation can be avoided by using a lower temperature, longer time approach to pasteurize colostrum. Heating up colostrum to 60° C for 60 min in a commercial batch pasteurizer should be sufficient to maintain IgG concentrations and colostrum characteristics while eliminating or significantly decreasing important pathogens such as Listeria monocytogenes, E. coli, Salmonella enteritidis, Mycoplasma bovis, and Mycobacterium paratuberculosis (McMartin et al, 2006; Godden et al., 2006; Elizondo-Salazar., 2010). Two on-farm controlled studies resulted in pasteurization (60° C for 60 minutes) having no effect on the IgG concentration in the colostrum but it significantly reduced the total bacteria load (Johnson et al., 2007; Godden et al., 2012). In Johnsons study, the calves fed pasteurized colostrum had significantly higher serum IgG levels at 24 hours of age, plus greater apparent efficiency of IgG absorption (35.6%) versus calves fed 3.8 L of raw colostrum (26.1%) (Johnson et al., 2007). Then in 2009, similar results took place where pasteurizing at 60°C for 30 minutes had no effect on IgG concentrations but increased the serum IgG and IgG absorption rates, although in this study the high bacteria load in the colostrum did not play a significant roll in the IgG absorption rate (Elizondo-Salazar and Heinrichs., 2009). This could be attributed to the initial high amounts of IgG within the treatment group's colostrum, allowing them to gain passive transfer along with fighting the bacteria.

Materials and Methods

Study Location

This study was conducted on a 6,000 Holstein-cow dairy in California during the summer of 2012. Calves were collected from August 13, 2012 to September 1, 2012. The total target sample size of the trial was 180 calves with 60 calves in each of three groups: Pasteurized (Past), Fresh-refrigerated (FR), and Fresh-frozen (FF) colostrum.

Colostrum Collection, treatment, and sampling

The colostrum was harvested from fresh cows twice daily at 5:00am and 5:00pm, and then it was put into containers where it was tested for quality and measured with a digital brix refractometer (ATAGO). If the reading was > 22% (>50g/L IgG) then the colostrum was pooled into a larger container where it was to be used for the study. Once all the colostrum was harvested and pooled into the large container, another brix reading was taken and recorded. Then, a 10mL sample was taken out of the pooled maternal colostrum (MC) and labeled with batch code (time, date, fresh sample, and batch number). The sample was plated for total plate count (TPC) and total E. coli count and then frozen for lab tests on total IgG concentration. Once the sample was taken, the pooled MC was evenly divided into three separate treatment groups:

1. Fresh-refrigerated (FR): One third of the colostrum was taken from the pool and transferred directly into 4-quart Perfect Udder bags (Dairy Tech Inc., Windsor, CO).

Then the bags were labeled (time, date, Fresh-Refrigerated, batch number, and bag letter) and transported to the refrigerator. At feeding the bags were removed from refrigerator (held for no longer than 24-48h) and warmed in a bucket of water held at approximately 125^{o}F. Once the colostrum warmed to feeding temperature, then a duplicate 10mL sample was taken for TPC, E. Coli count, IgG testing, and labeled (time, date, Fresh-Refrigerated, batch number, and bag letter) before feeding.

2. Fresh-frozen (FF): One third of the colostrum was taken from the pool and transferred to 4-quart Perfect Udder bags. Then the bags were labeled (time, date, Fresh-Frozen, batch number, and bag letter) and transported to a freezer. Once bags were completely frozen they were then used in the trial. At feeding time, the bags were removed from the freezer and thawed/warmed in a bucket of water held at approximately 125^{o}F. Once the colostrum was warmed to feeding temperature, a duplicate 10mL sample was taken for TPC, E. Coli count, IgG testing, and labeled (time, date, Fresh-Frozen, batch number, and bag letter) before feeding.

3. Pasteurized: One third of the colostrum was taken from the pool and transported immediately to the 10 gallon Dairy-Tech batch pasteurizer system where it was then heat-treated at 60^{o}C for 60 minutes. Following the heat-treatment, the colostrum was then cooled to 40.5^{o}C (105^{o}F) and a duplicate 10mL sample of the colostrum was aseptically collected and labeled (time, date, Pasteurized, batch number) This sample was then tested for TPC and E. Coli count immediately and then frozen for later lab IgG testing. Then the pasteurized colostrum was transferred into the 4-quart Perfect Udder bags and then transported into the freezer (held at -3^{o}F). Once the bags were completely frozen they

were then used in the trial. At feeding time, the bags were removed from freezer and thawed/warmed in a bucket of water held at approximately 125^{o}F. Once the colostrum was warmed to feeding temperature, a duplicate 10mL sample was taken for TPC, E. Coli count, IgG testing, and labeled (time, date, Pasteurized, batch number, and bag letter) before feeding.

Calf Enrollment

All the calves (n=180) were collected within a two and a half week span and enrolled into their different treatment groups at birth. The maternity area on farm was monitored 24 h a day and calves were removed from their dam within 30 minutes post-calving. Calves were placed in a separate pen with rice hull bedding and a heat lamp for comfort. A pre-feeding (0-1h) 8mL blood sample was collected immediately prior to colostrum feeding using 1.5 inch 20 gauge needle and a red top serum vacuum tube, labeled with calf number, and placed in the refrigerator for further lab testing (pre-feeding IgG count in blood). Colostrum was then prepared, with a sample taken out for TPC and E. Coli testing and treatment group randomly selected. Calves were then fed 4 quarts of the selected colostrum with an esophageal tube feeder. The second feeding took place when the next batch of colostrum was harvested (5:00am and 5:00pm), tested for higher than 22% brix reading, then 2 quarts of colostrum were placed into Perfect Udder bags and labeled either fresh or pasteurized 2nd feeding. Calves were fed the 2nd feeding type of colostrum according to the treatment group they were enrolled into. Every morning the calves were then weighed on an individual electronic scale and then picked up and transported to the calf ranch, where they were fed, housed, and treated all the same. Due to some calves being born right after the calves were taken to calf ranch, some calves received a 3rd feeding (same type of colostrum as treatment group and >22% brix reading) before they were taken the next day. At the

calf ranch (48h old) a post-feeding blood sample was collected and sent to the lab for passive transfer detection and serum IgG concentration. The calves then followed the same feeding, and treating protocols used by the calf ranch and they were weighed a total of three times until weaning. The first weight recorded was at ages between 7 and 14 days, the second time recorded was between 28 and 35 days, and then the third time was just before weaning on day 60.

Prior to calf ranch transport, the maternity supervisor recorded date, time of calving, dam ID, calf ID, colostrum treatment group assigned, colostrum batch number, bag letter that was fed, sex, calving difficulty score, and total number of calves born. All heifer calves born alive and healthy were enrolled into the study except those born as twins, light birth weight (<50lbs), born with abnormalities, and calves with difficulty pulling. All calves were identified within the herd using an ear tag radio-frequency identification (RFID) system.

Sample Handing and Analysis

A 10mL colostrum sample was taken out of every pooled batch collected, another 10mL sample was taken after the colostrum underwent the heat-treatment, and then a final 10mL sample was taken from the Perfect Udder bag immediately prior to feeding. These samples' were all frozen immediately and transported to the freezer in the lab on dairy. They were then thawed and serially diluted 1:5 and 1:500 dilutions. Each dilution was plated on a 5% blood agar plate for total plate count (TPC) and E. Coli count. To determine E-coli on blood agar, a trained lab technician deciphered between colonies and tested them using a KOH test. Plates were then incubated for 24 h at 37^{o}C and the number of colonies recorded as cfu/mL. Then samples were refrozen and sent to the Westside Veterinary Services lab for determining the IgG concentration using a radial immunodiffusion (RID) analysis.

An 8mL blood sample was taken immediately prior to feeding (0-1h), refrigerated, and then sent to the Westside Veterinary Services lab along with a 48 hour post-feeding blood sample taken at the calf ranch. These samples were used to determine the serum immunoglobulin (IgG, mg/mL) levels by radial immunodiffusion (RID) analysis.

Results

Objective 1: Determine effects of freezing, pasteurization, and refrigeration on morbidity, growth rates, and serum IgG concentration post feeding.

A GLM procedure from the SAS system was used to create 3 separate models using serum IgG concentration, 60 day weight, and morbidity as the dependent variables. In the first analysis, to see the effect of treatment on serum IgG concentration, 149 observations were used out of the 154 read. This model contained serum IgG concentration as the dependent variable with 3 class effects: Treatment (P = .0191), number of feedings (P = .8190), and birth weight (P = .0119). The Least Squares means serum IgG concentration for calves fed FF, FR, and PAST colostrum was 3774, 3548, and 4438 mg/dL (P= .0191).

In the second analysis, to see the effect of treatment on 60 d weight, 151 observations were used out of the 154 read. This model contained 60 day growth as the dependent variable with 3 class effects: Treatment (P = .9583), number of feedings (P = .0509), and birth weight (P = <.0001). The Least squares means 60 d weight for calves fed FF, FR, and PAST colostrum was 171, 170, and 171 (P = .9583).

In the third analysis, to see the effect of treatment on morbidity, 152 observations were used out of the 154 read. This model contained morbidity as the dependent variable with 3 class

effects: Treatment (P = .7099), number of feedings (P = .96), and birth weight (P = .3833). The least squares means morbidity for calves fed FF, FR, and PAST colostrum was 2.2, 2.4, and 2.5 (number of times treated).

Table 1. Effects of treatment groups on morbidity, weight gain, and serum IgG

Variable	Past	FF	FR	P-value
Morbidity (x treated)	2.5	2.2	2.4	.7099
60d weight	171.4lbs	171.6lbs	170.8lbs	.9583
Serum IgG (mg/dL)	4438	3774	3548	.0191

Objective 2: Compare the effect of pasteurization on colostrum IgG concentration and bacteria counts compared to raw fresh colostrum.

A GLM procedure from SAS system was used to create a model using IgG concentration as a dependent variable. Within the IgG concentration there were two dilutions used in the study to determine the total IgG count, a 1:4 dilution and a 1:5 dilution. Within these two dilutions, treatment was used as a class effect. In the IgG dilution 1:5, treatment showed an effect (P= .0943). Raw colostrum having a higher amount of IgG's (9103 mg/dL) when compared to pasteurized colostrum (8361 mg/dL). In the IgG dilution 1:4, treatment showed little effect (P= .5041). Raw colostrum had a little higher IgG count (9601 mg/dL) when compared to pasteurized colostrum (9133 mg/dL).

Another model was made using E. coli and TPC (as fed) as independent variables with treatment group as a class effect. Treatment showed to decrease both E. coli (P= .13) and TPC (P=.0530).

Table 2. Effects of pasteurization on IgG concentration, TPC, and E. Coli counts

Variable	Fresh	Pasteurized	% decrease
In colostrum fed:			
IgG concentration (mg/dL)			
1:4 dilution	9601	9133	4.8%
1:5 dilution	9103	8361	8%
TPC (cfu/mL)	117,065	3,676	97%
TCC (cfu/mL)	1,292	1.2	99%

Discussion:

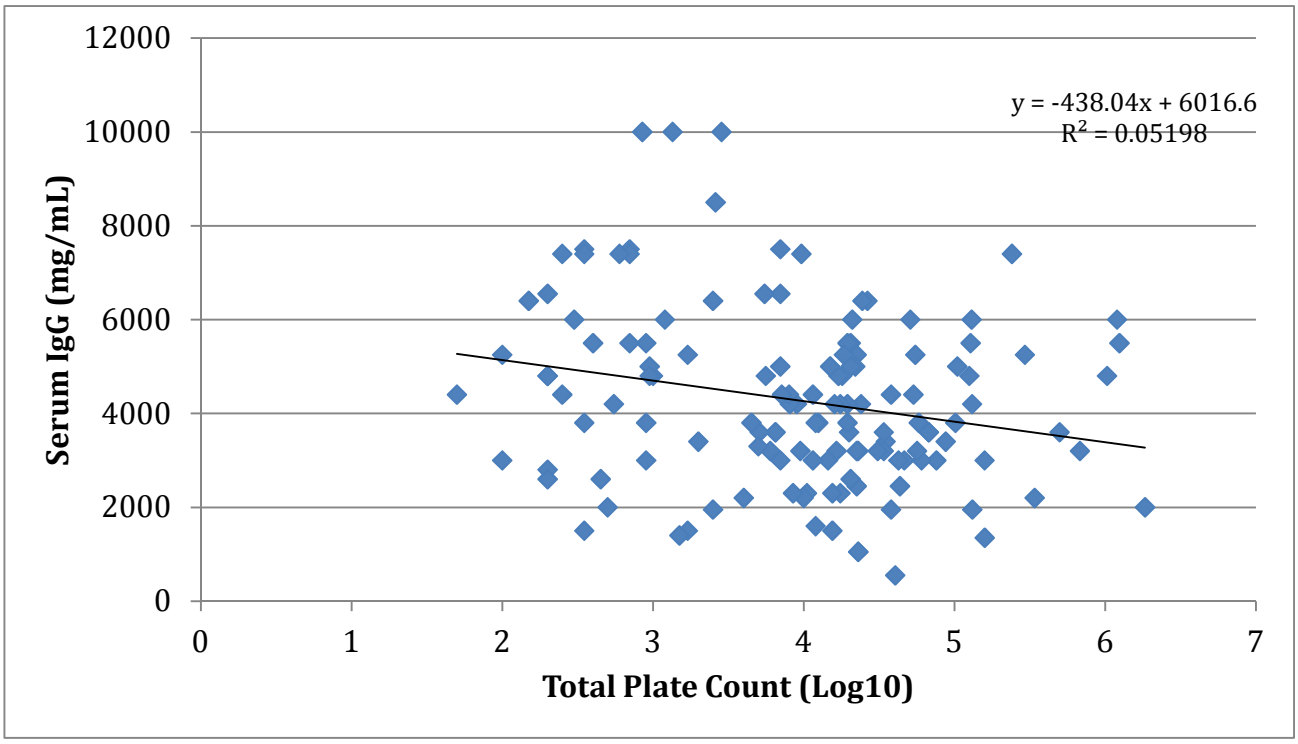
Objective 1: Determine effects of freezing, pasteurization, and refrigeration on morbidity, growth rates, and serum IgG concentration post feeding.

Serum IgG concentration (IgG absorption)

In this study pasteurized colostrum had a positive effect on serum IgG concentration (P = .0191). Pasteurized colostrum had the most serum IgG concentration (4438 mg/dL), frozen colostrum had less (3774 mg/mL), and refrigerated colostrum had the least amount (3548 mg/mL). These results conveyed that pasteurization had a positive effect on IgG absorption over both frozen and refrigerated. Freezing colostrum increased serum IgG concentration by 6% but the results were not significant in that we could not attribute the act of freezing to have a positive effect. This indicates that the lower bacteria count, mainly in pasteurized, resulted in an increase in serum IgG concentration. Thus, agreeing with the theory stating that bacteria inhibits the absorption of IgGs. As you can see in the figure below (Figure 1) there is a relationship between lower bacteria counts and higher serum IgG counts. These results were consistent with 3 other

similar studies in that the serum IgG was higher in pasteurized colostrum (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009; Godden et al., 2012).

Figure 1. Relationship between bacteria and serum IgG



Morbidiy

In this study, the three treatment groups showed no effect on morbidity. This result did not agree with prior studies showed pasteurization resulting in lower rates of morbidity (Boyd et al., 1972; Logan et al., 1975; Muggli et al., 1983; Godden et al., 2012). But due to the lack of information regarding each treatment, morbidity data was a limiting factor in this project. This

could be the reason why treatment had no effect on morbidity. Another theory regarding this result is that each treatment group received high amounts of IgGs in the colostrum they consumed. This allowed each group to gain passive transfer, resulting in a high IgG absorption rate across the treatment groups.

Growth rates

In this study, the three treatment groups showed no effect on the overall 60-day weight gain (P = .9583). This outcome did not match our hypothesis and the results of studies in the past that suggested higher concentrations of serum Ig in calves are positively correlated with increased weight gain through weaning (Robison et al., 1988). This could be attributed to all treatment groups gaining high amounts of IgGs, allowing them all to gain passive transfer and grow well to be healthy. If the total IgG concentration in the colostrum was lower, then pasteurization may have had a bigger impact on weight gains by increasing absorption. Though our results did not match the results in past studies due to the high amounts of IgG they all received, a new result was discovered in the process. In the current study, each calf received either 2 or 3 feeding prior to leaving the dairy and taken to the calf ranch. The number of feedings the calf received was dependent on her time of birth, due to feedings given every 12 hours. The calves that received 3 feedings averaged 4.3 lbs more at 60 d of age (P = .0509). In creating a statistical model with number of feedings as the independent variable, there was no effect on serum IgG concentration (P = .8190) between the two groups. This finding has led me to believe that stress in the first 24 hours of the calf's life, taken to the calf ranch and placed in a hutch, may be more important than we think in the calf getting off to the right start. Another theory is that there could be other factors in colostrum, other that IgGs, which may have benefited the calves who received 3 feedings vs. the calves who received 2. Further research is

needed to look more closely at these two groups of calves to find what is causing this big difference in weight gain.

Objective 2: Compare the effect of pasteurization on colostrum IgG concentration and bacteria counts compared to raw fresh colostrum.

Colostrum IgG concentration

The colostrum concentration in this study was measured twice with a 1:4 dilution and a 1:5 dilution using a Radial Immunodiffusion Test. In the 1:4 dilution, pasteurization did not show to have a significant effect on the IgG's in the colostrum compared to raw colostrum (P= .5041), although pasteurization decreased IgG's by 4.8%. In the 1:5 dilution, pasteurization was closer to having a significant effect (P=.0943), with a 8% decrease in IgG concentration. These results agreed with other studies that showed pasteurization had little effect in decreasing IgG concentrations (McMartin et al., 2006; Godden et al., 2006) Although some studies found that pasteurizing colostrum, with higher concentrations of IgG's, showed to have a larger decrease in total IgG in the colostrum (Godden et al., 2012).

Bacteria counts

Pasteurization showed to decrease total bacteria counts by 97% and E. Coli by 99%, agreeing with prior indicating proving that pasteurization has an effect on bacteria counts (Johnson et al., 2007; Godden et al., 2012). Freezing appeared to have no effect on bacteria counts when compared to fresh colostrum. This could be attributed to the colostrum being fed within 2 days of being in the refrigerator, minimizing the time for bacteria to grow.

Conclusion

Calves fed pasteurized colostrum had significantly higher serum IgG levels (4438 mg/mL) when compared to fresh-frozen (3774 mg/mL) and fresh-refrigerated (3548 mg/mL) This showed that pasteurization increased serum IgG by 15% vs fresh-frozen and 20% vs fresh-refrigerated. This could be attributed to the lower levels of bacteria allowing more IgGs to be absorbed into the calves. There was no difference in 60-day weight gain or morbidity, but this result may have been due to the high amounts of IgGs that each treatment group received allowing them to all gain passive transfer. Another reason treatment may have had no effect on morbidy or weight gain may be due to the calves being raised at the calf ranch. While at the calf ranch, morbidity data is lacking which was a limitation in finding the true morbidity, and unknown stresses may have impacted overall weight gain. An interesting finding that we observed throughout this project was that the calves fed three feedings of colostrum gained about 4.3 lbs more at 60 days than calves fed twice. Looking further into this, there was no difference in serum IgG concentration between each group (2 vs 3 feedings). So we've come to the conclusion that less stress within 24 hours of the calves life, being at the dairy longer, may be more important than we think, or that there are other factors, other than IgG's, that may be benefiting the calves fed three times more than those who were fed two times. Further research is needed to test these theories.

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