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A COMPARISON OF COLOSTRUM AND MILK FEEDING SYSTEMS FROM BIRTH TO WEANING IN DAIRY CALVES

A Thesis in

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ABSTRACT

Experiments were conducted to determine the effect of different heating times with three levels of colostrum IgG content on passive transfer from a single feeding of colostrum and to compare the effects of feeding a combination of yeast-derived products to preweaned dairy calves. Calves were fed pasteurized milk, and we observed outcomes of intake, body weight gain, and structural growth under a once- or twice-a-day milk feeding system. In the first experiment, colostrum was collected from The Pennsylvania State University dairy herd and divided by quality (high, medium, or low) based on colostrometer measurements. Colostrum within each quality was pooled to create three unique batches. Each batch was further divided in thirds as follows: frozen to be fed without heat treatment, heated at 60˚C for 30 min, or heated at 60˚C for 60 min. Colostrum samples from each treatment were collected and analyzed for standard plate count, gram-negative non coliforms, coliforms, and total IgG concentration. Serum samples were collected from 108 Holstein calves before feeding colostrum and 24 h after birth to reflect colostrum feeding effects. These blood samples were analyzed for total protein, total IgG, and hematocrit. Colostrum quality (high, medium, or low), heat treatment (unheated, 60˚C for 30 min or, 60˚C for 60 min), and their interaction were analyzed as fixed effects, with calf sex included as a random block effect. Colostrum IgG was different between quality groups (92.5, 59.4, and 48.1 mg/mL of IgG; $P < 0.01$). Heating colostrum reduced IgG concentration compared to the control by 9% when heated for 30 min and by 12% when heated for 60 min. Colostrum heated for 60 min had a lower standard plate count than colostrum heated for 30 min or not heated (1.8, 2.0, and 3.6 log cfu/mL; $P < 0.01$). Serum

IgG concentration at 24 h increased as colostrum quality increased (18, 22.2, and 24.8 mg/mL; $P = 0.02$) and tended to increase as heat treatment time increased (19.7, 20.3, and 25 mg/mL of IgG; $P = 0.06$). Apparent efficiency of absorption was greater in calves that received medium quality colostrum compared to calves fed high quality colostrum (38.1 and 25%; $P < 0.01$). These results suggest there may be an upper limit to the amount of IgG absorption in a given time period and that medium or high quality colostrum yields similar blood IgG absorption given the same volume of intake.

In the second experiment, 48 Holstein heifer calves from The Pennsylvania State University dairy herd were fed 3.8 L of colostrum in one feeding and randomly assigned to 1 of 4 treatments (once-a-day milk feeding with or without yeast and twice-a-day milk feeding with or without yeast). Weekly growth measurements and blood samples were taken 3 h after the morning milk feeding for all animals. Growth measurement included body weight, hip width, and withers height. Calf starter refusal was recorded weekly, and a sample was taken to determine dry matter intake. Daily health scores were recorded for each calf using a standard scoring system. Intake, growth measurements, haptoglobin, and health scores data were analyzed using repeated measures analysis and the mixed procedure of SAS and calf was included as a random variable. Pre-weaning ADG was 553.4 and 512.1 g for calves fed milk once and twice a day, respectively, and there was no difference between treatments ($P = 0.11$). Pre-weaning calf starter intake was 242.3 and 198.7 g/d for calves fed milk once and twice a day, respectively, and there were no treatment differences ($P = 0.35$). Withers height and hip width were the same in calves fed milk either once or twice a day ($P = 0.32$ and 0.95, respectively). Haptoglobin concentration as a measure of stress had least square means of 4 and 9.5 ± 3.5μg/mL for

calves fed milk once or twice a day, respectively, and there was no difference among treatments ($P = 0.27$). Scours score and total daily score were the same for calves fed milk once or twice a day ($P = 0.36$ and 0.47, respectively.). These results suggest that feeding milk once a day can be successfully applied to a calf feeding system. Finally, yeast supplemented calves showed a tendency for increased withers height and hip width ($P = 0.06$ and 0.08, respectively.) and had lower health scores compared to calves without supplementation ($P < 0.05$).

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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Chapter 1

Introduction

Colostrum immunoglobulins provide passive immune protection to the immunodeficient newborn calf until their active immune system becomes functional (Weaver et al., 2000). Consequently, calves are required to ingest high immunoglobulin concentration colostrum to ensure adequate protection against diseases (Stott et al., 1979; Weaver et al., 2000). The absorption of immunoglobulins occurs in the small intestine, which is permeable to large molecules for the first 24 h of life (Stott et al., 1979; Larson et al., 1980). Successful absorption of immunoglobulin is determined by measuring serum IgG concentration, which should exceed 10 mg/mL at 24 h of life (Tyler et al., 1996).

One factor affecting successful passive transfer of immunoglobulin is the bacterial content in the colostrum (Gelsinger et al., 2015). Colostrum can be a source of infectious pathogens such as *Mycobacterium avium* subsp. *paratuberculosis, Mycoplasma* spp., *Escherichia coli,* and *Salmonella* spp. (Streeter et al., 1995; González and Wilson, 2003; Houser et al., 2008), which also can be absorbed in the small intestine during the first 24 h of life. Several studies have shown that heating colostrum at 60˚C for either 30 or 60 min, reduces or eliminates the colostrum bacterial population (Godden et al., 2006; Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009; Gelsinger et al., 2014). Feeding heat-treated colostrum to calves also increases serum IgG concentration by 15 to 30% (Elizondo-Salazar and Heinrichs, 2009b; Kryzer et al., 2015).

Colostrum can be heated 60˚C for either 30 or 60 min without affecting IgG concentration (Godden et al., 2006; Elizondo-Salazar et al., 2010). However, colostrum IgG concentration is extremely variable among cows and dairy farms (Kehoe et al., 2007b; Baumrucker et al., 2010), and there is a lack of information comparing both heating times at different colostrum IgG concentrations.

After colostrum feeding, calves are fed milk or milk replacer, calf starter, and water until weaning. Traditionally, calves are fed milk or milk replacer based on 10 to 12% of their body weight in 2 feedings a day. However, calves can be fed either milk or milk replacer once a day without affecting calf development (Wilson, 1968; Ackerman et al., 1969; Kehoe et al., 2007a). Another practice in a calf rearing system is the supplementation of additives such as yeast culture in the milk or starter. It has been shown that yeast culture increases growth measurements and induces minor improvements in rumen development of dairy calves (Lesmeister et al., 2004). However, there is a lack of information comparing once- or twice-a-day milk feeding, as well as the effects of yeast culture on calf development.

Therefore, the objectives of this research are:

a. Determine the effect of two different heating times with three levels of colostrum IgG content on passive transfer from feeding of colostrum.
b. Compare the effects of feeding a combination of Yea Sacc™ and Actigen™ to preweaned dairy calves as a supplement to pasteurized milk on intake, body weight gain, and structural growth under a once- or twice-a-day milk feeding system.

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Chapter 2

A review of bovine heat treated colostrum

The importance of bovine colostrum

Bovine colostrum, the first mammary gland secretion after parturition, is defined as a mixture of lacteal secretions and constituents of blood serum, mostly immunoglobulins and other serum proteins, that is accumulated in the mammary gland during the prepartum dry period (Foley and Otterby, 1978). The calf's immune system is immature at birth due to the structure of the bovine placenta that prevents transfer of maternal immunoglobulins before parturition (Davis and Drackley, 1998). Therefore, it is vital to feed colostrum to newborn calves because colostrum immunoglobulins provide immune protection until the calf's own immune system becomes entirely functional, which occurs around four weeks of age (Logan et al., 1974).

Besides immunoglobulins, colostrum contains bioactive molecules that promote the development of the calf in many ways including the small intestine. Growth factors, including IGF-1 and IGF-2, antimicrobial factors such as lactoferrin, lactoperoxidase, and lysozyme, hormones, and cytokines are some bioactive molecules found in bovine colostrum (Pakkanen and Aalto, 1997). It has been shown that IGF-1 increases intestinal DNA synthesis and growth (Baumrucker et al., 1994). Another study demonstrated that colostrum-deprived calves had smaller villus size and lower villus height to crypt depth ratios in the small intestine than calves fed colostrum (Blättler et al., 2001).

In addition to its roles in providing passive immunity and stimulating intestinal development, colostrum is an important source of essential nutrients for the calf after it is born. It contains fat, protein, vitamins and minerals (Foley and Otterby, 1978). Colostrum has approximately twice as much total solids as raw milk, however, lactose concentration is lower which matches up with newborn calves having low lactase activity (Kehoe et al., 2007). Total protein and fat are higher on colostrum because they are an important source of energy and antibodies (Foley and Otterby, 1978). Moreover, fat and lactose which are both energy sources, are critical to maintain body temperature in the newborn calf (Davis and Drackley, 1998).

Immunoglobulins and their importance

There are three classes of immunoglobulin present in bovine colostrum: IgG, IgA, and IgM (Butler, 1969). The predominant class, IgG, accounts for about 85 to 90% of the total immunoglobulins in colostrum, whereas IgA and IgM account for 5% and 7% respectively (Larson et al., 1980). Factors such as dry period length, energy intake, parity, and harvest time affect colostrum IgG concentration (Kehoe et al., 2011; Mayasari et al., 2015; Mann et al., 2016). Bovine IgG is divided into two subclasses, IgG1 and IgG2 (Butler, 1969). Although IgG1 and IgG2 are present in equal amounts in the ruminant blood stream (Larson et al., 1980), IgG1 is the main immunoglobulin in lacteal secretions (Butler, 1969) and represents 90% of the total IgG. IgG1 has a lower basic amino acid content and higher half-cystine than IgG2. Moreover, both differ antigenically in their Fc fragments (Butler, 1969).

The mobilization of immunoglobulins from serum to the mammary gland begins several weeks before parturition (Brandon et al., 1971), but maximum mobilization is reached 1 to 3 days prior parturition (Sasaki et al., 1976). IgG1 is transported mainly from blood across mammary alveolar cells by an active receptor known as FcRn (Baumrucker and Bruckmaier, 2014), meanwhile, IgG2 either comes from blood or is synthesized by the plasma or epithelial cells of the mammary gland and then transported to mammary secretory cells. The plasma or epithelial cells also synthesize IgA and IgM (Gapper et al., 2007).

Each immunoglobulin has a role in the calf's immunity. IgG is the main contributor of the immune system of the calf (Davis and Drackley, 1998). IgM is important in protection against septicemia. IgA is important for the mucosal immune system (Quezada-Mendoza et al., 2011). IgA is effective in secretions at mucosal surfaces to prevent the penetration and adherence of antigens (Butler, 1986). It has been demonstrated that feeding purified individual immunoglobulin classes to calves is less effective in preventing diseases compared to feeding all the immunoglobulin classes present in colostrum (Logan et al., 1974). Even though all immunoglobulin classes have an important physiological role, serum IgG concentration in neonates is an adequate indicator of passive transfer of immunity (Besser and Gay, 1985).

The amount of total serum IgG in calves has an impact on calf health, growth, and survival later in life. It is known that the lack of colostrum feeding is highly related to calf death in the United States, Wells et al. (1996) reported a 75% mortality rate when calves were colostrum deprived. In another study, necropsy determined that all calves that were deprived of colostrum died of septicemia (Smith and Little, 1922). Nocek et al. (1984)

demonstrated that colostrum-deprived calves had poor weight gain, longer scours episodes, and high mortality. Also, calves who received colostrum with high immunoglobulin concentration gained weight from birth to day 4, while calves who received colostrum with low immunoglobulin concentration lost weight. Additionally, severe scours lasting for a longer time were seen in calves who received colostrum with low immunoglobulin concentration compared to calves who received colostrum with high immunoglobulin concentration (Nocek et al., 1984). Besser and Gay (1985) showed that calves with IgG concentration more than 10 g/L had lower mortality rates from enteritis and respiratory disease than calves with lower IgG concentration. Robison et al. (1988) reported that serum immunoglobulin concentration at 24 to 48 hours was a significant factor affecting the growth rate of heifers from birth to 180 days. In the same study, they concluded that maternally derived antibodies play a significant role in calf immunity. Insufficient serum immunoglobulin concentration at 24 to 48 hours could require an immune response by the calf before its immune system is able to handle pathogenic invasions. Pathogenic invasions often promote illness in the calf that affects normal growth and development of the calf. Calves with adequate passively acquired serum immunoglobulin often are able to inactivate pathogens earlier than calves with low serum immunoglobulin that must mount an immune response for protection. For this reason, calves with adequate serum immunoglobulin will continue to grow normally and not be hampered as low immunoglobulin calves are (Robison et al., 1988).

Immunoglobulin absorption by the calf

The absorption of immunoglobulins in the calf is a process that takes molecules from the lumen of the intestine to the blood (Bush and Staley, 1980) and cessation occurs 24 to 36 hours after birth (Brambell, 1958). Immunoglobulins are absorbed by the small intestinal epithelium, travel through the lymphatics, and enter the bloodstream via the thoracic duct (Comline et al., 1951; Besser and Gay, 1994). The intestine of the neonatal calf has no selectivity in the capacity of absorption (Pierce and Feinstein, 1965). It is generally accepted that non-selective pinocytosis is the mechanism of transport of immunoglobulins across the small intestinal epithelium (Besser and Gay, 1985). However, another study concluded that the intestinal epithelium can apply some degree of selectivity in the transfer of protein to the bloodstream (Staley et al., 1972). For selective absorption to occur, specific receptors to transport protein into bloodstream must be present (Brambell, 1958). The Fc receptor was first described as an IgG transporter in the neonatal rodent's gut (Rodewald, 1976). The neonatal bovine receptor (bFcRn) was later characterized and has been found in the mammary gland, small intestine, kidney, and liver (Kacskovics et al., 2000).

Passive transfer of immunity

Passive transfer refers to the transfer of immunoglobulins from the dam the neonate (Weaver et al., 2000). Passive transfer is determined by the amount of IgG in serum at 24 to 48 hours after birth. Successful passive transfer is defined as serum IgG concentration > 10 mg/mL; below this point is considered failure of passive transfer (Quigley, 2004). It has

been shown that achieving successful passive transfer confers many benefits, such as reduced age at first calving, reduced mortality rate, improved rate of gain and feed efficiency, improved milk production, and reduced culling during first lactation (Robison et al., 1988; DeNise et al., 1989; Wells et al., 1996; Faber et al., 2005). However, there are many factors affecting serum IgG concentration such as sex of the calf, age at first feeding, body weight, amount of IgG consumed, and colostrum quality (Quigley and Drewry, 1998). In 2014 the National Animal Monitoring System (NAHMS) reported 14% of calves had passive transfer failure in the United States. In order to understand the nature of immunoglobulin absorption and the management required to provide adequate passive immunity, it is important to calculate the apparent efficiency of absorption. The apparent efficiency of absorption measures the efficiency of immunoglobulins absorbed and not the total IgG absorption (Quigley and Drewry, 1998). The main factors contributing to failure in passive transfer are colostrum quality, time of first feeding, volume fed (Weaver et al., 2000), and colostrum bacterial count (Godden, 2008).

Colostrum quality

IgG concentration traditionally has been the primary indicator of colostrum quality because IgG represents 85% of the total immunoglobulins in colostrum (Godden, 2008). A colostrometer, which is a specialized hydrometer, can be used to determine IgG concentration in colostrum on farm (Fleenor and Stott, 1980). High quality colostrum contains more than 50 mg/mL of IgG (McGuirk and Collins, 2004). However, there are many factors affecting colostrum IgG content, including breed of the dam, dry period

length, parity, calving season, and prepartum ration (Foley and Otterby, 1978; Weaver et al., 2000). Also, it is important to consider the amount of bacteria in colostrum because bacteria reduce IgG absorption. Bacteria may bind to free immunoglobulins or block the absorption of immunoglobulins in the intestine (James and Polan, 1978). This was demonstrated in a study where calves fed heat-treated colostrum had higher IgG concentration (27.5 mg/mL) than calves fed unpasteurized colostrum (23.3 mg/mL) at 48 hours after birth (Gelsinger et al., 2014). Godden et al. (2012) demonstrated that calves fed heat-treated colostrum had a significantly lower risk for scours treatment compared to calves fed raw colostrum due to a lower total bacterial count and total coliform count in heat-treated colostrum. It is recommended that calves be fed colostrum that contains < 100,000 cfu/mL total bacterial count and < 10,000 cfu/mL total coliform count (McGuirk and Collins, 2004).

Time of first feeding

The ability of the gut to absorb immunoglobulins is optimal in the first 4 hours postpartum. After 12 hours postpartum, the ability of absorption begins to drop rapidly (Stott et al., 1979a; Bush and Staley, 1980). Thus, calves fed earlier will have a higher serum IgG concentration than calves fed the same colostrum quality later (Stott et al., 1979b).

Volume fed

Davis and Drackley (1998) calculated that a newborn calf should receive 100 g of IgG as soon as possible. Based on this, a newborn calf should be fed 2 liters of colostrum with more than 50 mg/mL of IgG within 4 hours after birth. However, Godden (2008) suggested that calves should be fed 4 liters of colostrum containing at least 50 mg/mL as soon as possible after birth.

Methods to determine IgG concentration

Radial immunodiffusion (RID) and the enzyme-linked immunosorbent assay (ELISA) are the only direct methods to measure IgG in colostrum and serum. RID is a more consistent method compared to ELISA. ELISA requires more extensive dilution to test colostrum and serum, whereas RID requires minimal sample dilution (Gelsinger et al., 2015). However, there are indirect methods to estimate IgG including total serum protein by refractometer, sodium sulfite turbidity test, zinc sulfate turbidity test, serum gamma glutamyltransferase activity, and whole blood glutaraldehyde coagulation test (Weaver et al., 2000). Many studies have compared the accuracy between indirect methods and direct methods to estimate serum IgG concentration on farm. A Brix refractometer can be used successfully to estimate colostrum and serum IgG concentration (Deelen et al., 2014). Also, a serum protein refractometer can be used to estimate serum IgG concentration (Calloway et al., 2002). All of these studies showed that at serum values more than 5.2 g/dL (Calloway et al., 2002) or 7.8% Brix (Morrill et al., 2013), passive transfer of immunoglobulins is successfully achieved. Colostrum IgG concentration can be estimated with a colostrometer,

which was developed from the linear relationship between colostrum specific gravity and immunoglobulin concentration (Fleenor and Stott, 1980). The colostrometer should be used with colostrum at 22˚C, because at lower temperatures the colostrometer overestimates colostrum IgG concentration. At colostrum temperatures above 22˚C, the colostrometer underestimates the colostrum IgG concentration (Mechor et al., 1991). Another way to estimate IgG concentration in colostrum is using a Brix refractometer (Quigley et al., 2013; Deelen et al., 2014). A meta-analysis showed that a Brix value ≥ 22% corresponds to colostrum with ≥ 50 mg/mL of IgG and a Brix value <18% means low quality colostrum (Buczinski and Vandeweerd, 2016).

Heat treated colostrum

Colostrum is a potential source of pathogens such as *Mycobacterium avium* subsp. *paratuberculosis, Mycoplasma* spp., *Escherichia coli,* and *Salmonella* spp. (Streeter et al., 1995; González and Wilson, 2003; Houser et al., 2008) to the newborn calf. These pathogens come from the mammary gland, contamination during the harvest process, or proliferation during storage (Fecteau et al., 2002; McGuirk and Collins, 2004). However, multiple studies have shown that pathogens are reduced when colostrum is heat treated. Heat treatment of colostrum was first done to avoid transfer of tuberculosis to newborn calves (Ragsdale and Brody, 1923). During their experiment, Ragsdale and Brody (1923) noticed that heating colostrum at 62.5˚C or 72.5˚C for 60 or 75 seconds respectively, resulted in thick colostrum pudding. However, when colostrum was heated at 60˚C for 20 minutes the tuberculosis organism was inactivated and colostrum consistency was

maintained (Ragsdale and Brody, 1923). Nevertheless, this experiment did not evaluate the effects of heating colostrum on immunoglobulins, especially IgG, and their findings are based on colostrum appearance only.

In order to see how IgG was affected by heat treatment, Meylan et al. (1996) conducted a laboratory experiment where they heated 5 mL from 18 colostrum samples at 63˚C for 30 minutes. The IgG mean was 44.4 ± 30.3 mg/mL for fresh colostrum and 37.2 ± 23.8 mg/mL for pasteurized colostrum. The authors reported that IgG was reduced by 12.3 ± 8.7%. Later, McMartin et al. (2006) conducted preliminary work to determine which heat treatment would produce no significant changes in colostrum viscosity, IgG concentration, and immunoglobulin activity. Heat treatment temperatures ranged from 59 to 63 ˚C for 120 minutes using a Rapid Visco Analyzer (RVA). After reviewing the results from their preliminary work, the authors determined that heating at 60 ˚C for up to 120 minutes did not affect viscosity and IgG concentration. To confirm these results, 50 mL from 30 unique batches of colostrum were heated at 60 and 63 ˚C. However, when colostrum was heated at 63 ˚C, IgG concentration decreased 34%. Finally, they concluded IgG concentration was not affected when colostrum was heated at 60 ˚C for up to 120 minutes (76.4 ± 26.5 to 74.5 ±24.3 mg/mL; McMartin et al., 2006).

Further studies focused on the effects of heat treatment temperature on colostrum viscosity, IgG concentration, and bacterial count. Elizondo-Salazar et al. (2010) conducted a study to identify the optimal temperature and time at which colostrum would have a significant bacterial reduction without affecting viscosity and IgG concentration. Colostrum samples with 74.8 mg/mL of IgG were heated at 57, 60 or 63˚C for 30, 60 or 90 minutes using a water bath. In contrast to McMartin et al. (2006), Elizondo-Salazar et al.

(2010) suggested that colostrum could be heated at 60˚C from 30 to 60 minutes without significant changes in viscosity, while achieving a minimal reduction in IgG concentration and a significant reduction in bacterial count. Godden et al. (2006) also found that heating colostrum at 60˚C for 30 minutes eliminated pathogens such as *Mycoplasma bovis*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella enteritidis*. However, *Mycobacterium avium* subsp. *paratuberculosis*, the causative agent of Johnes disease, was eliminated when colostrum was heated at 60˚C for 60 minutes (Godden et al., 2006). A following study used a commercial pasteurizer for 60˚C for 60 minutes and reported that total plate count and total coliform count were significantly decreased after heating (Donahue et al., 2012). However, Donahue et al. (2012) reported greater losses in IgG concentration when initial concentration was high (>70 mg/mL) compared to a lower quality colostrum. Later, Gelsinger et al. (2014) reported a loss of 6.5 mg/mL in IgG concentration with an initial IgG of 98.8 mg/mL of IgG, whereas colostrum with an initial IgG concentration of 56.4 mg/mL of IgG lost 3.8 mg/mL of IgG during heat treatment (60˚C for 30 minutes). Other studies (Godden et al., 2006; McMartin et al., 2006) have found similar results where the higher IgG concentration in colostrum, the greater the loss of IgG after heat treatment in comparison with colostrum of lower IgG concentration.

Knowing that colostrum could be heated at 60˚C for 30 or 60 minutes, researchers started to investigate the effects of feeding heat-treated colostrum to calves on passive immunity. Johnson et al. (2007) compared passive transfer in dairy calves fed heat-treated colostrum (60˚C for 60 minutes) vs. raw colostrum. Calves who received heat-treated colostrum had significantly greater serum total protein and IgG concentration at 24 hours than calves fed raw colostrum. Another study found that calves fed heat-treated colostrum

(60˚C for 30 minutes) had nearly 20% greater serum IgG concentration at 24 and 48 hours after birth than calves fed raw colostrum (23.4 vs. 19.6 mg/mL, and 23.9 vs. 20.2 mg/mL, respectively; Elizondo-Salazar and Heinrichs, 2009). Since then, many field studies have shown that IgG absorption is improved when calves are fed heat-treated colostrum (Godden et al., 2012; Kryzer et al., 2015). Also, heat-treated colostrum enhanced the colonization of *Bifidobacterium* and reduced the colonization of *Escherichia coli* in the calf small intestine soon after birth (Malmuthuge et al., 2015). This could be one reason for the observation of fewer enteric infections in calves fed heat-treated colostrum (Godden et al., 2012). However, growth measurements of calves fed heat-treated colostrum have been found to be similar to calves fed raw colostrum (Elizondo-Salazar and Heinrichs, 2009; Teixeira et al., 2013).

Since previous studies (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009; Godden et al., 2012) had compared IgG absorption between calves fed heat-treated colostrum and unheated colostrum containing 60 to 75 mg/mL of IgG, Gelsinger et al. (2014) conducted a study to investigate IgG absorption in dairy calves fed colostrum of different IgG quality. Colostrum used in this study was divided into three qualities based on IgG concentration (high, medium, and low) and they were significantly different. Gelsinger et al. (2014) reported that heat treatment of colostrum increased plasma total IgG concentration by 18.4% compared to unheated colostrum. The greatest benefit of heating colostrum appeared to be accomplished in medium quality colostrum (71 mg/mL of IgG) by increasing apparent efficiency of absorption and decreasing the possibility of failure of passive transfer when colostrum of lower quality is fed (Gelsinger et al., 2014).

Several studies have tried to explain the reason why calves fed heat-treated colostrum have a greater serum IgG concentration than calves fed unheated colostrum. One reason could be that heat treatment reduces bacterial load, so IgG absorption is improved as an indirect result of a reduction in bacterial load (Johnson et al., 2007). By reducing the amount of bacteria in colostrum, antibodies are going to be free for absorption, because it has been shown that bacteria can bind to colostrum antibodies before absorption occurs (Johnson et al., 2007). So, more IgG molecules are going to be free for absorption in the neonatal gut (Johnson et al., 2007). Also, bacteria can hamper the receptors that are responsible for IgG absorption, because bacteria can bind to nonspecific receptors on neonatal enterocytes (James and Polan, 1978; James et al., 1981; Staley and Bush, 1985). Another reason could be that heat treatment denatures some non-immunoglobulin proteins that would compete for receptors on neonatal enterocytes, so more receptors are available for IgG uptake (Elizondo-Salazar and Heinrichs, 2009). Also, results from Gelsinger et al. (2014) support this hypothesis that heating colostrum increases IgG absorption by decreasing the absorption of non IgG protein. Heating colostrum at 60˚C for 60 minutes has been shown to decrease IGF-1 and lactoferrin in colostrum (El-Fattah et al., 2014). Another study observed reduce cytokine absorption in calves fed heated-treated colostrum for 60˚C for 60 minutes compared to calves fed unheated colostrum (Gelsinger and Heinrichs, 2017). Recently, a functional analysis based on gene ontology showed that the majority of low abundance proteins whose ratios changed due to heat treatment were associated with immune functions, implying that calves fed heat-treated colostrum may experience a different degree of immune support compared to calves fed unheated colostrum (Tacoma et al., 2017). Finally, one last reason could be that the IgG molecule

gets fragmented during the heating process, and the method use to quantify IgG is not able to differentiate between whole molecules or IgG fragments (Bush et al., 1981). However, no one has validated this theory.

Feeding heat-treated colostrum increases serum IgG concentration and improves apparent efficiency of absorption, regardless of the initial concentration of IgG in colostrum. However, there is a need to determine the effect of different heating times using colostrum of a wide range of IgG concentration on passive transfer from a single feeding of colostrum.

Summary

Bovine colostrum is a rich source of both nutrients and immunoglobulins. The immunoglobulins are vital to provide immune protection until the calf's immature immune system becomes entirely funtional. The predomintant immunoglobulin in bovine colostrum is IgG, and it accounts for 85 to 90% of the total immunoglobulins in colostrum. For this reason, serum IgG concentration is an indicator of passive tranfer of immnuglobulins. There are two direct methods to measure serum IgG concentration (RID and ELISA) and multiple indirect methods to estimate serum IgG concentration, such as serum protein by refractometer. Serum IgG concentration can be influenced by many factors like colostrum IgG concentration and colostrum bacterial load. In order to reduce bacterial load, it has been established that heating colostum at 60˚C for 30 or 60 min is enough to reduce bacteria without decreasing IgG concentration and increasing viscosity. Calves fed heat-treated colostrum will have greater serum IgG concentration as well as greater apparent efficiency

of absorption than calves fed raw colostrum. The predominant theory about this greater serum IgG concentration suggest that the increase in serum IgG may be due a reduction in bacteria, leaving IgG molecules free for absorption. Regardless of the initial IgG colostrum, heat treatment of colostrum increases IgG absorption. However, there is a need to determine the effect of different heating times using colostrum of a wide range of IgG concentration on passive transfer from a single feeding of colostrum.

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Chapter 3

Once-a-day milk feeding system, pasteurized milk and yeast supplementation in dairy calves development: A review

Once-a-day milk feeding system

Feed and labor are the biggest expenses in a dairy calf rearing system. Feed accounts for 60.3% of the total cost to raise a heifer in a milking operation, while labor is the second largest expense accounting for 13.8% of the total cost to raise a heifer in a milking operation (Gabler et al., 2000). However, it is possible to reduce some of these expenses by adjusting the milk feeding system. Traditionally, calves are fed milk or milk replacer based on 10% of their body weight in 2 feedings a day. More recently milk intake has been increased to 12% of body weight (Jones and Heinrichs, 2006). Implementing once-a-day milk feeding is one way to reduce labor cost without affecting calf development. In a once-a-day milk feeding system, calves can start drinking milk once a day when they are 3 d old (Ackerman et al., 1969), or calves can be fed twice a day during the first week (Stanley et al., 2002) or 2 wk (Kehoe et al., 2007) before being switched to once-a-day milk feeding. Milk replacer has been recommended to be mixed at 10 to 13% solids and offered at 8% of body weight as well as free choice of calf starter in the past (Jenny et al., 1982). Current milk feeding recommendations have kept up with more modern understanding of calves nutrient requirements (Jones and Heinrichs, 2006).

After 3 yr of experience and over 500 calves involved in the research at The Unilever Research Laboratory, Wilson (1968) showed the convenience and advantages in labor savings when calves were fed milk once a day. Calves fed once a day required 39%

less labor for milk feeding compared to calves fed twice a day (Galton and Brakel, 1976). Time and labor spent on filling and cleaning buckets is reduced in half, and feeding can be done either in the morning or in the afternoon according to Wilson (1968). Also, he stated that feeding once a day left more time to pay attention to the details of calf rearing. The key to success in raising calves on once-a-day milk feeding is providing access to a high quality calf starter, fresh water, and good hay (Harshbarger and Fryman, 1969). Currently, companies in Europe and New Zealand are extensively marketing once-a-day feeding (Ngahiwi; Nutrition, 2017).

Several studies (Wilson, 1968; Ackerman et al., 1969; Willett et al., 1969; Stanley et al., 2002) have shown no difference in growth and health between calves fed milk replacer once versus twice a day. Wilson (1968) saw no difference in concentrate intake and live weight gain between calves fed milk replacer once a day and calves fed twice a day during the pre-weaning phase. Other research by Ackerman et al. (1969), using Holstein and Ayrshire calves, found no difference in growth rate or health problems between calves fed milk replacer once or twice a day. Willett et al. (1969) followed calves fed milk replacer once or twice a day up to 3 mo of age and observed no differences in weight gain, wither height gain, and heart girth gain between calves fed milk replacer once versus twice a day.

Stanley et al. (2002) concluded there was no difference in glucose metabolism, weight, or starter intake between calves fed milk replacer once versus twice a day. Galton and Brakel (1976) measured liver, heart, kidney, adrenal, rumen, reticulum, omasum, abomasum, small and large intestine, caecum, and rectum size as well as mineral content of each organ and found no difference between calves fed milk once versus twice a day.

Finally, Kehoe et al. (2007) reported no difference in body weight, hip and withers height, or heart girth post-weaning between calves fed once or twice a day.

Pasteurized milk

Newborn calves should ingest colostrum as the first meal because it provides essential nutrients that are important for their development. After colostrum feeding, calves must receive a liquid feed until rumen development has occurred. Common liquid feeds used to raise dairy calves are: saleable whole milk, non-saleable milk, which may include transitional milk, abnormal milk, and milk from treated cows, and milk replacer. Whole milk was the predominant liquid feed used for calves before 1956 (Otterby and Linn, 1981). Milk replacer was first developed in the late 1950s as a way to use by-products of the milk and cheese industry. The principal ingredients were dried skim milk, dried buttermilk, dried whey, and animal fat (Otterby and Linn, 1981). However, the conventional 20% protein and 20% fat milk replacer has changed. Newer milk replacers are higher in protein to be more similar to whole milk and fat content remains the same or lower (NAHMS 2014). Milk replacers were very popular in the US for many years, and in 2011 they were fed to 89.9% of the dairy calves and used on 85.9% of dairy farms in the US. In the latest National Animal Health Monitoring survey of 2014, milk replacer was fed to just 53% of calves and used on 63.9% of farms. This decrease is largely a result of changes in the price of ingredients and mailbox milk prices, all of which favor moving to feeding whole milk and waste milk.

In order to choose the right liquid feed for calves there are multiple factors to consider, including: nutritional targets, ease of managing the feeding program, risk of potential diseases, and economics. Calves are exposed to a large variety of bacteria and disease-causing organisms from the moment that they are born. Many of the things they are exposed to are quite harmless and eventually colonize the gut of the calf. Others are not beneficial and can be detrimental to the calf either immediately or later in life. Detrimental species of bacterial pathogens, such as *Mycobacterium avium* subsp. *paratuberculosis*, *Salmonella* spp., *Mycoplasma* spp., *Listeria monocytogenes*, *Campylobacter* spp., *Mycobacterium bovis*, and *Escherichia coli*, are often found on farms and can cause diseases that are economically important to the dairy industry (Lovett et al., 1983; Farber et al., 1988; Mcewen et al., 1988; Streeter et al., 1995; Selim and Cullor, 1997; Steele et al., 1997; Walz et al., 1997). Many of these pathogens can be found in raw, unpasteurized whole milk. Some also can be sourced from the mammary gland when it is infected with various mastitis-causing organisms. Some are found in the blood of infected animals. Other sources of these organisms are related to milking practices, such as pre- and post-harvest contamination, improper storage, or poorly cleaned feeding equipment. However, these pathogens can all be reduced or eliminated by pasteurization, which is the process of heating milk to a target temperature for a certain time, resulting in a reduction of pathogens. The definition of successful pasteurization is a 2-log reduction in bacteria counts, which in many cases will reduce the levels to zero or near zero.

Implementation of on-farm pasteurization is a practical and economical way to reduce the bacterial load present in raw milk. There are 2 main types of pasteurizer that can used on farms: batch pasteurizers, which utilize a low temperature and long time (LTLT),

and commercial continuous flow pasteurizers, also known as high temperature, short time pasteurizers (HTST). The concept of pasteurizing is a result of heating a substance at a certain temperature for a period of time; the higher the temperature, the shorter the time required to reduce the bacteria counts. Commercial batch pasteurizers generally are composed of a milk container surrounded by a heated water jacket (some designs use a different heating device), and an agitator. An HTST pasteurizer is composed of a plate or tube exchanger that uses hot water to heat the milk on the opposite side of a metal plate or tube. Batch pasteurizers used for milk normally heat at 63˚C for 30 min and then cool the milk to 37˚C prior to feeding. Differently than a batch pasteurizer, an HTST pasteurizer heats the milk at 72˚C for 15 sec and then rapidly cools the milk to 37˚C prior to feeding. Several studies have shown that either a batch pasteurizer or an HTST pasteurizer are able to destroy pathogens including *Salmonella* spp., *Staphylococcus aureus*, and *Mycoplasma* spp. (Butler et al., 2000; Stabel et al., 2004), and pasteurization has become popular for dairy farms around the world. .

Feeding pasteurized milk is more common on large farms. The 2014 USDA National Animal Health Monitoring System survey reported that 43.8% of large operations were feeding pasteurized milk. Overall, feeding pasteurized milk is gaining popularity among dairy farms; its use increased from 4.2% of farms in 2007 to 7.4% in 2014. One observational study compared the effect of feeding pasteurized colostrum and waste milk versus unpasteurized colostrum and waste milk on a California dairy farm (Jamaluddin et al., 1996b). This study reported lower mortality rate and higher growth rate in calves fed pasteurized colostrum and waste milk compared to calves fed unpasteurized colostrum and waste milk. Also, calves fed pasteurized colostrum and waste milk had fewer sick days

during the pre-weaning phase, which led them to be 3.7 kg heavier than calves fed unpasteurized colostrum and waste milk. Similar to Jamaluddin et al. (1996b), Godden et al. (2005) reported higher average daily gain and lower mortality rate in calves fed pasteurized milk compared to calves fed conventional 20:20 milk replacer.

An economic analysis done by Jamaluddin et al. (1996a) estimated that calves fed pasteurized colostrum and waste milk were worth an extra $8.13 in gross margin/calf compared to calves fed unpasteurized colostrum and waste milk, whereas Godden et al. (2005) estimated a savings of $0.69/calf per day by feeding pasteurized milk compared to milk replacer. As it was mentioned, feeding pasteurized milk improves average daily gain and calf heath, decreases mortality rates, and offers economic efficiencies (Jamaluddin et al., 1996a; Jamaluddin et al., 1996b; Godden et al., 2005), but the adoption of this technology depends on the farm needs.

Yeast supplementation

Yeasts are specialized fungi that have lost the ability to form a mycelia. This unicellular organism has adapted to live and multiply in high sugar concentration niches (Wallace, 1996). The yeast culture used in dairy rations is derived from *Saccharomyces cerevisiae*, which grows in a medium containing ground corn, hominy, corn gluten feed, wheat and rye middlings, corn syrup, cane molasses, and other substrates that provide high energy (Harrison et al., 1988; Williams et al., 1991; Cole et al., 1992; Mutsvangwa et al., 1992). Then, the mixture of yeast and medium is dried at low temperatures to preserve the viability of yeast cells. The total count of live yeast cells varies between products and

growth batches, which can explain the variability of results in the yeast culture literature (Williams et al., 1991).

The action mode of *Saccharomyces cerevisiae* has been described by Wallace (1996), where 3 hypotheses postulate the effects of yeast on rumen microorganisms. The first hypothesis states that the provision of vitamins and minerals from yeast cultures creates a possible interaction of stimulatory factors that support cellulolytic and lactate-utilizing bacteria in the rumen. Also, it has been suggested that yeast cultures stimulate the growth of lactic acid utilizing microorganisms such as *Selenomonas ruminantium* and *Megaspera elsdenii* and the uptake by these microorganisms (Nisbet and Martin, 1991; Quigley et al., 1992; Koul et al., 1998). Another hypothesis is that the yeast has the ability remove oxygen in the rumen, leading to a beneficial effect on rumen microorganisms, the majority of which are anaerobes (Newbold et al., 1996).

Yeast has been primarily studied and used in dairy cattle rations (Erasmus et al., 1992; Yoon and Stern, 1996), but has also been studied in heifer rations (Lascano et al., 2009). The use of yeast culture in the diet of calves was first described by Eckles et al. (1924). They were interested in yeast because it is potent source of vitamin B. As is it known, vitamin B stimulates growth (Eckles et al., 1924). The rate of growth was measured in calves from 20 to 180 d old, however it did not increase in calves fed dried yeast as a source of vitamin B. Also, there was no observed effect on calf health after yeast supplementation (Eckles et al., 1924). Later, studies focused on the effects of yeast on the rumen environment in young and adult animals (Erasmus et al., 1992; Quigley et al., 1992; Yoon and Stern, 1996; Kumar et al., 1997). Quigley et al. (1992) and Kumar et al. (1997) supplemented yeast culture to calves, and it increased rumen pH, total cellulolytic bacteria,

and total ruminal VFA. It was suggested that supplementing calves with yeast culture may increase rumen pH regulation by reducing lactic acid production (Williams et al., 1991).

Other studies have shown the effect of supplementing yeast on intake. Quigley et al. (1992) reported that yeast culture increased starter intake prior to weaning; however, intake decreased after weaning during the 12-wk experiment. Average daily gains have been measured when yeast was supplemented in rations with no advantage noted (Williams et al., 1991; Mutsvangwa et al., 1992; Quigley et al., 1992; Seymour et al., 1995). However, Lesmeister et al. (2004) reported increased average daily gain when yeast was included in dairy calf starters. Additionally, Yoon and Stern (1996) saw an increased yeast culture response during stressful situations, indicating that there is applicability of yeast culture for animal health. In a calf study, Cole et al. (1992) reported that calves fed yeast in the diet had lower morbidity and mortality rates and fewer sick days. Finally, Lesmeister et al. (2004) concluded that the inclusion of 2% supplemental yeast culture in calf starter, on a dry matter basis, increased total dry matter intake, average daily gain, hip height, and hip width, stating that yeast culture positively influenced dairy calf growth.

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Chapter 4

Effects of different heating time of high, medium and low quality colostrum on immunoglobulin G absorption in dairy calves.

Introduction

Calves are required to ingest and absorb adequate amounts of immunoglobulins present in colostrum during the first 24 h of life due to the fact that calves are immunodeficient at birth (Bush and Staley, 1980). Passive transfer of immunity, which refers to the transfer of immunoglobulins from the dam to the neonate, has been defined as being successfully achieved when serum IgG concentration is 10 mg/mL or more (Weaver et al., 2000; Quigley, 2004). In addition, when measured on farm, serum total protein was used as an indicator of passive transfer of IgG (Tyler et al., 1996) with concentrations equal to or greater than 5.2 g/dL considered successful (Calloway et al., 2002). Only 6.2% of all operations in the United States routinely measured serum protein as an indicator of passive transfer (NAHMS, 2014); however, this was an increase compared to 2.1% of all operations in 2007.

Colostrum quality, feeding time, volume fed, (Weaver et al., 2000), and colostrum bacterial population (Gelsinger et al., 2015) can affect passive transfer. Colostrum provides immunity to newborn calves, however, it also can be one of the first opportunities for infectious pathogen exposure (Swan et al., 2007). Colostrum is a potential source of *Mycobacterium avium* subsp. *paratuberculosis, Mycoplasma* spp., *Escherichia coli,* and *Salmonella* spp. (Streeter et al., 1995; González and Wilson, 2003; Houser et al., 2008). These pathogens come from the mammary gland in some cases and from contamination

during colostrum milking, storage, and feeding (Fecteau et al., 2002; McGuirk and Collins, 2004). Colostrum can be heat treated to reduce or eliminate pathogens (Godden et al., 2006).

Previous research has found that heating colostrum at 60˚C for 30 or 60 min significantly reduces bacterial content in the colostrum (Johnson et al., 2007; Elizondo-Salazar et al., 2010; Donahue et al., 2012). Heat treatment of colostrum does not affect viscosity when it was heated at 60˚C for 30 or 60 min (Elizondo-Salazar et al., 2010). On the other side, heat treatment increases serum IgG concentration and improves AEA (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009; Godden et al., 2012; Gelsinger et al., 2014). It has been shown that heat treatment does not affect colostrum IgG concentration (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009). However, some studies have reported that high quality colostrum has greater IgG loss compared to low quality colostrum (Donahue et al., 2012; Gelsinger et al., 2014).

Colostrum IgG concentration is extremely variable among cows and between dairy farms (Kehoe et al., 2007; Baumrucker et al., 2010). Colostrum IgG levels in individual samples ranged from 11.8 to 74.2 mg/mL in the Kehoe et al. (2007) study. Previous studies comparing heat-treated colostrum and unheated colostrum have used colostrum containing 50 g/L of IgG (Elizondo-Salazar and Heinrichs, 2009), 76.6 g/L of IgG (McMartin et al., 2006), or 60 g/L of IgG (Godden et al., 2006), all quite average in IgG level. One study showed the effects of heating high, medium, and low quality colostrum on IgG absorption (Gelsinger et al., 2014) using pooled colostrum containing 98.8, 71.9, or 56.4 mg/mL of IgG. In that study Gelsinger et al. (2014) heated colostrum for 30 min only, whereas the majority of commercial pasteurizers are preset for a 60-min heat treatment based on the

early research by Godden et al. (2006) and Johnson et al. (2007). Studies by Donahue et al. (2012) and Gelsinger et al. (2014) showed that colostrum with a high IgG concentration was affected more than medium or low IgG concentration colostrum when it was heated. Data from Tacoma et al. (2017) suggest that heating colostrum for longer periods may have more detrimental effects on proteins that are responsible for neonatal calf growth and development. For that reason, the objective of this study was to determine the effect of different heating times with 3 levels of colostrum IgG (adequate but low, medium, and very high) content on passive transfer from a single feeding of colostrum. It was hypothesized that heating for a longer time would influence high quality colostrum in a more extreme manner than lower quality colostrum.

Materials and methods

Colostrum heat treatment

This research was approved by The Pennsylvania State University Institutional Animal Care and Use Committee (protocol # 47457). First milking colostrum was collected from primiparous and multiparous Holstein cows at The Pennsylvania State University dairy, stored in 1.89-L plastic containers, and frozen at -20˚C. At a later time, colostrum was allowed to thaw at 4˚C and divided into three quality categories (low, medium, high) based on colostrometer measurement 48 h before heat treatment. Colostrum within each category was pooled and mixed to create three unique batches. Each batch was further divided in three uniform batches. The first part was transferred into 1.89-L plastic

containers and immediately frozen at -20˚C until needed for feeding (unheated colostrum). The remaining two batches of each quality were heated to 60˚C and maintained either for 30 or 60 min. The temperature was continuously monitored inside stainless steel buckets in a steam vat pasteurizer (Girton Manufacturing Co., Millville, PA). After heat treatment, hot water was replaced with ice water to cool the colostrum. Then colostrum was transferred into 1.89-L plastic containers and immediately frozen at -20˚C until needed for feeding. Each 1.89-L plastic container contained 1.90 kg of colostrum. Samples from each treatment were collected before freezing for IgG concentration and bacterial analysis. This system is similar to that used previously by our lab group (Elizondo-Salazar and Heinrichs, 2009, 2009b; Gelsinger et al., 2014).

Colostrum sample analyses

Colostrum samples were thawed at 20˚C to measure bacterial content as described by Jayarao et al. (2004). Colostrum samples were analyzed for standard plate count (SPC), coliform count (CC), non-coliform gram negative (NC), environmental streptococci (ES), contagious streptococci (CS), and coagulase negative staphylococci (CNS). Colostrum samples were mixed and 50 µL were pipetted on to the plate and spread using a sterile, L-shaped polyproplylene cell spreader. All samples were cultured in duplicate for SPC using Plate Count agar, coliform and non-coliform gram negative bacteria using MacConkey's agar, CNS and coagulase positive staphylococci using Baird-Parker agar, and ES and CS using Edward's Modified Agar. Cultures were incubated at 37˚C for 48 h. Colonies were counted after 24 and 48 hours of incubation. Counts from duplicate plates were averaged

and converted to logarithmic counts. Colostrum IgG concentration was measured using radial immunodiffusion (RID). Colostrum samples were diluted 1:10 in 0.85% saline solution. Then, manufacturer's instructions were followed to determine IgG concentration (#728411; Triple J Farms, Bellingham, WA). Colostrum samples were tested in duplicate and sample values with a coefficient of variation ≤ 10.5% were accepted and averaged for analysis.

Colostrum samples were sent to Dairy One Inc. Check Mark Dairy Laboratory (Ithaca, NY) to be analyzed for fat (ether extraction/Mojonnier method), total protein (Kjeldahl method), and total solids (direct forced air oven method). Samples were analyzed using AOAC (1990) wet chemistry methods. Also, colostrum samples were sent to the Agricultural Analytical Services Laboratory at The Pennsylvania State University to be analyzed for ash, P, K, Ca, Mg, Fe, Na, S, Cu, Zn, and Mn. Samples were prepared by Environmental Protection Agency (EPA) method 3050B. Then samples were analyzed by EPA method 6010 with inductively coupled plasma spectrometry (US EPA, 1986).

Calf treatment enrollment and sampling

Holstein calves (54 females and 54 males) from The Pennsylvania State University dairy were separated from their dams within 20 min of birth before suckling occurred, weighed, and placed in individual pens. Calves weighing ≥ 33 kg were randomly assigned to 1 of the 9 colostrum treatments (3 × 3 factorial arrangement of heat treatment for 0, 30, or 60 min and high, medium, or low quality colostrum). Colostrum was thawed in a warm

water bath (50˚C) and provided in a single feeding of 3.8 L using an esophageal feeder within 2 h of birth. Calf identification number, weight, treatment allocation, date and time born, and time at colostrum feeding were recorded for each calf.

Before feeding colostrum and 24 h after birth, a jugular blood sample was collected into 10-mL serum and 10-mL sodium heparin Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). The serum Vacutainer tube was refrigerated overnight, centrifuged, and the serum separated from the clot within 24 h of collection (Johnson et al., 2007). Serum protein was determined using an optical refractometer (SPR-T2, Atago Co. Ltd., Tokyo, Japan). Distilled water was used to calibrate the refractometer before each use. Then, serum samples were stored at -20˚C until analyzed for IgG concentration. The sodium heparin Vacutainer tube was used to collect blood to measure packed cell volume. Each blood sample was transferred to a capillary tube, placed on a centrifuge for 3 min, and read using a micro-hematocrit reader (Adams micro-hematocrit reader, Clay-Adams, Inc. New York).

Serum IgG analysis

Serum IgG concentration was determined by ELISA. A 1:200 dilution of affinity purified bovine IgG (Bethyl Laboratories Inc., Montgomery, TX) was created to coat each well. Bovine reference serum (Bethyl Laboratories Inc.) was diluted in Tris saline buffer containing 0.05% Tween 20 (pH = 8.0) to create a standard curve ranging from 0 to 500 ng/mL. All standards were run in duplicate with each assay. Serum samples were diluted

1:100,000 in saline buffer containing 0.05% Tween 20 (pH = 8.0). Serum samples were tested in triplicate and sample values with a coefficient of variation ≤ 10.5% were accepted and averaged for analysis. Between each step in the ELISA protocol, except for the final enzyme reaction, a 60-min incubation at 20˚C was allowed and then the wells were washed 5 times using Tris saline buffer. 3, 3', 5, 5'-tetramethylbenzidine ELISA peroxidase substrate (Rockland Immunochemicals Inc., Gilbertsville, PA) was added in the final enzyme reaction and incubated for 15 min. Then a 0.2 M sulfuric acid stopping solution was added. Absorbance was read at 450 nm using a plate reader (VICTOR ^{3} Multilabel Counter model 1420; PerkinElmer Life Science, Waltham, MA).

Statistical analysis

Calf characteristics were analyzed using the mixed procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC). Colostrum quality (high, medium, or low), heat treatment (60˚C for 0, 30, or 60 min), and their interaction were analyzed as fixed effects, with calf sex included as a random block effect to compare body weight, serum IgG concentration, serum total protein, and AEA. Hematocrit was included as a covariate in the model for serum IgG concentration, serum total protein, and AEA. The Tukey adjustment was used to compare least square means for individual treatments. Colostrum IgG concentrations and bacterial population were compared using Proc Mixed in SAS. Significance was declared at $P < 0.05$.

Results and discussion

The compositional analysis of colostrum treatments is presented in Table 4-1. The fat content was lower than values reported by Foley and Otterby (1978), Kehoe et al. (2007), and Elizondo-Salazar and Heinrichs (2009) (6.7, 6.7, and 6.09% respectively). However, total protein and total solids were similar to the values reported by Foley and Otterby (1978; 14 and 23.9% respectively). Ash and mineral contents were similar to the values presented by Elizondo-Salazar and Heinrichs (2009b). The difference in fat content may be related to fat adhered to the sides of the initial storage container used prior to making composite batches.

A description of colostrum treatments are shown in Table 4-2. Colostrum IgG concentration was similar to colostrum treatments used by Gelsinger et al. (2014). Colostrum IgG concentration was different between quality groups ($P < 0.01$), which is consistent with the objective of the study to have three different levels of colostrum IgG concentration. The initial IgG concentration was 98.1, 65.7, and 52.3 mg/mL for high, medium, and low quality colostrum. The low quality colostrum was just above the cut off value between acceptable and unacceptable colostrum (< 50 mg/mL; Fleenor and Stott, 1980), and the IgG concentration was lower than a previous study (56.4 mg/mL; Gelsinger et al. 2014) where a low quality colostrum was used. Colostrum IgG concentration was higher in unheated colostrum compared to heated colostrum, showing that heat treatment caused a decrease in IgG concentration as has been shown before (Johnson et al., 2007; Elizondo-Salazar et al., 2010; Godden et al., 2012; Gelsinger et al., 2014). However, IgG concentration was similar between colostrum heated for 30 or 60 min ($P = 0.23$). Elizondo-

Salazar et al. (2010) reported a significant decrease in IgG_1 concentration after heating colostrum at 60˚C for 30 and 60 min compared to raw colostrum but there was no difference between heating colostrum at 60˚C for 30 or 60 min. However, IgG_2 did not change after heating colostrum at 60˚C for either 30 or 60 min.

The decreases in IgG concentration from unheated colostrum were 6.1 (6.2%), 10.5 (10.7%), 8.1 (12.3%), 10.8 (16.4%), and 6.3 (12%) mg/mL for 30-min heat-treated high, 60-min heat-treated high, 30-min heat-treated medium, 60-min heat-treated medium, and 30- and 60-min heat-treated low quality colostrum, respectively. Many studies have reported changes in IgG concentration after heat treatment. Donahue et al. (2012) reported a decrease of 6.7 to 9.8 mg/mL of IgG in colostrum containing more than 70 mg/mL of IgG using a commercial batch pasteurizer at 60˚C for 60 min. Also, Gelsinger et al. (2014) observed a decrease in colostrum IgG concentration of 6.5, 0.9, and 3.8 mg/mL in high, medium, and low quality colostrum, respectively. However, the decrease in IgG concentration was not significant in either study.

Colostrum bacterial counts are presented in Table 4-2. Heating colostrum for either 30 or 60 min caused reductions of SPC, CC, NC, ES, CS, and CNS. Previous on-farm and laboratory studies have reported a reduction in bacterial count when colostrum is heated for 30 or 60 min (Godden et al., 2006; Johnson et al., 2007; Elizondo-Salazar et al., 2010; Donahue et al., 2012; Gelsinger et al., 2014). The unheated colostrum SPC values were 3.9, 3.4, and 3.4 log cfu/mL for low, medium, and high quality colostrum, respectively. Comparing these values with previous studies (Elizondo-Salazar and Heinrichs, 2009; Gelsinger et al., 2014), SPC values were lower. The unheated colostrum CC values were 3.3, 0.9, and 0.7 log cfu/mL for low, medium, and high quality colostrum, respectively. As

it is recommended, colostrum should contain < 100,000 cfu/mL total bacterial count and < 10,000 cfu/mL total coliform count (McGuirk and Collins, 2004), so the unheated colostrum was considered clean colostrum. After heating colostrum for 30 or 60 min, CC and NC were zero. Another study reported zero values for CC and NC after heating colostrum for 30 min (Gelsinger et al., 2014). Colostrum heated at 60˚C for 60 min had lower SPC as well as ES, CS, and CNS compared to other colostrum treatments. This agrees with Elizondo-Salazar et al. (2010), who reported lower values for SPC and ES when colostrum was heated at 60˚C for 60 min.

A total of 12 calves (6 heifer and 6 bull calves) were assigned to each colostrum treatment. Least squares means for birth body weight, age at colostrum feeding, serum total protein at birth, IgG concentration at birth, and hematocrit at birth are reported in Table 4-3 and were not different between colostrum treatments ($P > 0.05$). Serum total protein at birth was as anticipated and similar to values reported in previous studies (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009, 2009b). Serum IgG concentration at birth was similar to values reported by Johnson et al. (2007) and Gelsinger et al. (2015), and hematocrit was similar to values reported by Gelsinger et al (2015).

Serum total protein and IgG concentration at 24 h and AEA are shown in Table 4-4. The interaction between colostrum quality and heat treatment time was not significant for any of the parameters measured. Serum total protein increased after colostrum consumption and was highest in calves fed high quality colostrum (5.26, 5.63, 6.15 g/dL for low, medium, and high quality colostrum respectively) within each heating time. However, there was no difference in serum total protein between calves fed low or medium quality colostrum ($P > 0.05$). Johnson et al. (2007) and Elizondo-Salazar and Heinrichs

(2009b) reported similar 24-h serum total protein. Also, both studies reported higher 24-h serum total protein values in calves fed heat-treated colostrum compared to calves fed unheated colostrum, despite calves being fed colostrum with the same IgG concentration. No effect of heat treatment on serum total protein was observed in the present study.

As expected, 24-h serum IgG concentration increased as colostrum quality increased ($P = 0.02$). There was also a tendency for increased IgG concentration as heat treatment time increased ($P = 0.06$). Early work by Stott and Fellah (1983) fed calves the same colostrum volume at different IgG concentration and demonstrated a positive relationship between serum IgG concentration and colostrum IgG concentration. Therefore calves fed colostrum with a higher IgG concentration showed higher serum IgG concentration than calves fed colostrum with a lower IgG concentration. In the present study, calves fed heat-treated high quality colostrum had higher 24-h serum IgG concentrations than calves fed heat-treated low and medium quality colostrum. However, calves fed medium quality unheated colostrum had lower 24-h serum IgG concentration compared to calves fed low quality unheated colostrum. Calves fed high quality unheated colostrum had higher IgG concentration than both, as expected.

Passive transfer is considered successful when the serum IgG concentration is > 10 mg/mL of IgG at 24 h after birth, below this point is considered failure of passive transfer (Quigley, 2004). In the current study, 9 calves had failure of passive transfer base on that definition and ranged from 7.2 to 9.5 mg/mL of IgG. Of these, 7 calves received low quality colostrum heated for 30 min and 2 calves received low quality unheated colostrum. These low serum IgG values in several calves explain why calves fed low quality colostrum heated for 30 min had the lowest IgG concentration compared to the other treatments. In a

similar study, 5 calves fed low quality colostrum heated for 30 min and 6 calves fed low quality unheated colostrum had failure of passive transfer and also had the lowest IgG concentration compared to the other treatments (Gelsinger et al., 2014).

Several studies (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009; Gelsinger et al., 2014) have shown that feeding heat-treated colostrum increases serum IgG concentration. When calves were fed heat-treated colostrum with an initial concentration of 72.6 mg/mL of IgG, Johnson et al. (2007) reported an increase of 23.6% in serum IgG concentration compared to calves fed unheated colostrum. Following that study, Elizondo-Salazar and Heinrichs (2009) reported that calves fed heat-treated colostrum had nearly 20% greater serum IgG concentration compared to calves fed unheated colostrum. Recently, Gelsinger et al. (2014) reported an increase of 18.4% in plasma IgG concentration in calves fed heat-treated colostrum compared to unheated colostrum, despite a significant reduction in colostrum IgG concentration after heat treatment. In the present study, serum IgG concentration increased 3.4% and 27.2% in calves fed colostrum heated for 30 min and 60 min, respectively, compared to calves fed unheated colostrum. The low increase in IgG concentration seen in calves fed colostrum heated for 30 min could be due to a lower bacterial concentration in the unheated colostrum. The initial colostrum bacterial count in previous studies (3.95, 5.48, and 4.4 $\log_{10}$ cfu/mL from Johnson et al. (2007), Elizondo-Salazar and Heinrichs (2009), and Gelsinger et al. (2014), respectively) was higher than in the colostrum used in the present study. This bacteria was not likely a factor in IgG absorption as has been demonstrated (Elizondo-Salazar and Heinrichs, 2009; Gelsinger et al., 2014). This finding supports the suggestion by Johnson et al (2007) that bacteria can bind to colostrum antibodies before absorption occurs, so by heating

colostrum, more IgG molecules are going to be free for absorption in the calf gut. As it is shown, calves fed 60-min heat-treated colostrum had the greatest IgG concentration and the lowest bacterial count.

Mean AEA values are shown in Table 4-4. AEA was greater in calves that received medium quality colostrum compared to calves fed high quality colostrum (38.1 and 25%; $P < 0.01$) and in calves that received colostrum heated for 60 min compared to calves fed colostrum heated for 0 or 30 min (41.5, 32.5 and 28%; $P = 0.01$). A previous study indicated that there was likely a certain amount of receptors to carry IgG from the intestinal wall to the blood stream (Jaster, 2005). However, when the receptors became saturated, there is no way to transport more IgG molecules (Jaster, 2005). It is likely that this is happening when a large volume of colostrum with high IgG concentration is fed. These results suggest there may be an upper limit to the amount of IgG absorption in a given time period and that medium or high quality colostrum yields similar blood IgG absorption given the same volume of intake, especially when it is high IgG, low bacteria colostrum.

Conclusions

Serum IgG concentration increased as colostrum quality increased in the calves on this study, and there was a tendency to increase serum IgG levels as heat treatment time increased. Heat treatment reduced SPC, CC, NC, ES, CS, and CNS levels in the colostrum. The AEA was greater in calves that received medium quality colostrum compared to calves fed high quality colostrum, as well as in calves that received colostrum heated for 60 min compared to calves fed colostrum heated for 0 or 30 min. These results suggest there may

be an upper limit to the amount of IgG absorption in a given time period and that medium or high quality colostrum yields similar blood IgG absorption given the same volume of intake, especially when it is composed of low bacteria colostrum. These findings also support the hypothesis that IgG absorption is improved as a result of a reduction in colostrum bacterial load.

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Table **4- 1**. Compositional analysis of colostrum treatments

	Unheated			Heated 30 min			Heated 60 min		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
Fat, %	4.64	5.25	3.83	3.48	5.29	3.98	4.32	5.44	4.01
Total protein, %	11.59	13.68	15.91	11.78	13.6	15.92	11.79	13.75	15.97
Total solids, %	21.27	23.68	24.45	21.2	23.83	24.52	21.4	23.83	25.58
Ash, %	5.29	4.95	4.73	5.3	4.48	4.59	5.38	4.81	4.69
Ca, %	0.19	0.21	0.21	0.18	0.21	0.21	0.19	0.21	0.21
P, %	0.19	0.19	0.20	0.19	0.20	0.20	0.19	0.20	0.20
Mg, %	0.03	0.03	0.04	0.03	0.03	0.04	0.03	0.03	0.04
Na, %	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
k, %	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
S, %	0.11	0.13	0.16	0.11	0.13	0.16	0.11	0.13	0.16
Zn, mg/kg	59	82.3	79.6	59.2	81.1	77.4	60.7	82.3	79.2
Fe, mg/kg	< 3.7	< 4.4	< 4.1	< 4.0	< 4.1	< 3.9	< 3.8	< 4.2	< 4.1
Cu, mg/kg	< 0.75	< 0.61	0.65	< 0.72	< 0.60	0.65	<0.73	< 0.65	0.69
Mn, mg/kg	<0.5	< 0.4	< 0.4	< 0.5	< 0.4	< 0.4	< 0.5	< 0.4	< 0.4

Table **4- 2**. Colostrum IgG concentration and bacterial population[1] in each treatment

	Unheated			Heated 30 min			Heated 60 min			*P*-value[2]		
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Q	M	SEM
IgG (mg/mL)	52.3	65.7	98.1	46	57.6	92	46	54.9	87.6	<.01	<.01	0.84
SPC (log cfu/mL)	3.9	3.4	3.5	2.5	1.6	2.1	2	1.6	2	0.03	<.01	0.09
CC (log cfu/mL)	3.3	0.9	0.7	0	0	0	0	0	0	0.4	0.12	0.47
NC (log cfu/mL)	1.7	2.1	2.4	0	0	0	0	0	0	0.4	<.01	0.11
ES (log cfu/mL)	6	2.3	3.3	0	0	1.5	0	0	0.9	0.5	0.04	0.74
CS (log cfu/mL)	2.4	0	0	0	0	0	0	0	0	0.4	0.4	0.46
CNS (log cfu/mL)	3.6	2.8	3.4	0	0.7	0	0	0	0	1.0	<.01	0.22

[1]SPC = standard plate count; CC = coliform count; NC = noncoliform, gram negative count; ES = environmental streptococci; CS = contagious streptococci; CNS = coagulase negative staphylococci.

[2]Q = colostrum quality (high, medium, or low); M = heat treatment minutes (0, 30, or 60).

Table **4- 3**. Birth weight, age at colostrum feeding, and blood parameters at birth for calves in each treatment

	Unheated			Heated 30 min			Heated 60 min			*P*-value[1]			
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Q	M	Q × M	SEM
Body weight, kg	40.5	40.6	41	42.2	42	39.9	41.4	42.6	39.6	0.3	0.8	0.7	2.8
Age at colostrum feeding, min	90	65	91	69	84	77	74	79	72	0.8	0.6	0.2	10.3
Total protein, g/dL	4.2	4.1	4.2	4.0	4.1	4.1	4.0	4.0	4.1	0.9	0.5	0.7	0.1
IgG, mg/mL	0.4	0.3	0.7	0.4	0.3	0.5	0.3	0.6	0.5	0.6	0.9	0.9	0.2
Hematocrit, %	32.3	34.4	34.0	35.5	37.0	31.7	36.3	36.5	34.1	0.1	0.3	0.4	1.6

[1]Q = colostrum quality (high, medium, or low); M = heat treatment minutes (0, 30, or 60).

Table **4- 4**. Blood parameters at 24 h for calves in each treatment

	Unheated			Heated 30 min			Heated 60 min			*P*-value[1]			
	Low	Medium	High	Low	Medium	High	Low	Medium	High	Q	M	Q × M	SEM
Total protein, g/dL	5.3[b]	5.6[b]	6.1[a]	5.3[b]	5.6[b]	6.3[a]	5.2[b]	5.7[b]	6.1[a]	<.01	0.7	0.8	0.1
IgG, mg/mL	19.4	18.2	21.4	13.3	22.1	25.7	21.6	26.3	27.2	0.02	0.06	0.3	3.1
AEA, %[2]	37.3	26.2	20.8	32.6	39.6	25.4	47.3	48.5	28.9	<.01	0.01	0.2	5.1
Hematocrit, %	28.5	30.3	32.2	31.5	31.8	27.0	31.4	31.2	30.0	0.52	0.79	0.06	1.4

[a-b] Values within a heat time with different superscripts are different ($P < 0.05$) after accounting for multiple comparison by Tukey's adjustment factor.

[1]Q = colostrum quality (high, medium, or low); M = heat treatment minutes (0, 30, or 60).

[2]Apparent efficiency of absorption, calculated as: AEA = [(birth weight x 0.091 x serum IgG) / total IgG fed] x 100

Chapter 5

Effects of once- versus twice-a-day milk feeding supplemented with Yea Sacc™ and Actigen™ on growth and health in dairy calves.

Introduction

Feeding milk twice a day is the most common system to feed calves and was utilized by 94.6% of farms in the United States. In contrast, once-a-day feeding accounted for 0.8% of all operations (NAHMS, 2014). Therefore, 88.9% of US calves were fed milk twice a day (NAHMS, 2014). Early studies on once-a-day milk feeding showed the convenience and labor advantages of this system (Wilson, 1968; Galton and Brakel, 1976). As it is known, labor is the second largest expense accounting for 13.8% of the total cost to raise a heifer in a milking operation (Gabler et al., 2000). Feeding calves once a day could be an alternative to reduce labor expenses because it has been shown that feeding milk once a day requires 39% less labor than feeding twice a day (Galton and Brakel, 1976).

Several studies have compared once- versus twice-a-day milk feeding systems and have not seen differences in growth measurements, rumen development, blood constituents, or health scores between systems (Wilson, 1968; Ackerman et al., 1969; Willett et al., 1969; Stanley et al., 2002; Kehoe et al., 2007). Interestingly, no one has ever published data showing any significant difference to feeding once versus twice daily. The key to success in raising calves in a once-a-day milk feeding system is providing access to

a high quality calf starter, fresh water, and good hay (Harshbarger and Fryman, 1969). When casein and milk fat form a curd using whole milk or a casein-containing milk replacer, calves have the ability to slowly digest nutrients over a long period post feeding, which may be one reason for the success of such feeding systems (Davis and Drackley, 1998). European and New Zealand companies are aggressively marketing once-a-day feeding (Ngahiwi; Nutrition, 2017).

Casein represents approximately 76 to 86% of total milk protein (DePeters and Cant, 1992). The latest National Animal Health Monitoring System survey (NAHMS, 2014) reported a decrease in the use of milk replacer in dairy farms from 85.9% in 2011 to 63.9% in 2014. This decrease is largely a result of changes in the price of ingredients and mailbox milk prices, all of which favor moving to feeding whole milk and waste milk. This may represent a great opportunity to implement a once-a-day milk feeding system on more farms where management can make the changes and take advantage of lower labor costs.

When feeding whole milk or waste milk, calves may be exposed to pathogens such as *Mycobacterium avium* subsp. *paratuberculosis*, *Salmonella* spp., *Mycoplasma* spp., *Listeria monocytogenes*, *Campylobacter* spp., *Mycobacterium bovis*, and *Escherichia coli* (Lovett et al., 1983; Farber et al., 1988; Mcewen et al., 1988; Streeter et al., 1995; Selim and Cullor, 1997; Steele et al., 1997; Walz et al., 1997). However, these pathogens can all be reduced or eliminated by pasteurization, which is the process of heating milk to a target temperature for a certain time, resulting in a reduction of pathogens. The use of pasteurized milk to feed calves increased 7.4% in from 2007 to 2014 in the United States (NAHMS, 2014), demonstrating that this practice is becoming more popular. However, it is not the major way whole milk is currently being fed. The costs of pasteurizing whole milk are

often quite small. Jones and Heinrichs estimated the cost of feeding 60 calves pasteurized whole milk is $109.93/d and the cost of feeding the same animals with a 20:20 milk replacer is $114.10/d.

Another practice in a calf rearing system is the supplementation of additives such as yeast culture in the milk. First, yeast culture was used as source of vitamin B in dairy calves' diets (Eckles et al., 1924). Then, studies focused on the effects of yeast on the rumen environment in young and adult animals (Erasmus et al., 1992; Quigley et al., 1992; Yoon and Stern, 1996; Kumar et al., 1997). When calves are supplemented with yeast culture intake increases, which could be attributed to stabilization of rumen pH due to lower lactic acid concentration (Williams et al., 1991). Similarly, Quigley et al. (1992) reported an increase in starter intake prior to weaning for calves supplemented with yeast; however, intakes decreased after weaning. Improved gain and efficiency are the reasons yeast is often added to the diet of older growing animals (Lascano and Heinrichs, 2007). However, there is an inconsistency in the results among studies when yeast is added to the diet; this may be partly due to different yeast inclusion rates in the diet (Lascano and Heinrichs, 2007). Adding yeast into a calf's diet can reduce morbidity and mortality (Cole et al., 1992), as well as scours and electrolyte and antibiotic treatments (Seymour et al., 1995). Lesmeister et al. (2004) reported an increase in growth measurements and slight improvement in rumen development parameters in yeast-supplemented calves. For this reason, the objective of this study was to compare the effects of feeding a combination of Yea Sacc™ and Actigen™ to preweaned dairy calves as a supplement to pasteurized milk on intake, body weight gain, and structural growth under a once- or twice-a-day milk feeding system.

Materials and methods

This research was approved by The Pennsylvania State University institutional animal care and use committee (IACUC # 47457). Forty eight Holstein heifer calves from The Pennsylvania State University dairy herd were separated from their dams soon after birth and placed in a 1.0- × 1.0-m holding pen until colostrum feeding. All calves were fed 3.8 L of colostrum in 1 feeding followed by whole milk feedings. After colostrum feeding, calves were placed in 1.2- × 2.4-m individual pens bedded with straw and sawdust located inside a naturally and mechanically ventilated barn until 7 wk of age. Calves were randomly assigned to one of the 4 treatments (once-a-day milk feeding with or without yeast and twice-a-day milk feeding with or without yeast). All calves were fed 6 L of pasteurized whole milk in 2 equal feedings from d 1 to 7. A batch pasteurizer (PLV model, 2c Duecinox, Guastalla, Italy) was used to pasteurize the milk at 65˚C for 30 min. Milk samples were collected periodically and frozen at -20˚C for bacterial and component analysis. Calves fed milk once a day were fed 6 L in the morning from d 7 until weaning. Milk feeding amount was reduced in half in both treatments by 35 d of age, and calves were weaned at 42 d. Calves assigned to yeast treatment were fed 1.5 g/d of mannan-rich fraction (Actigen®; Alltech, Nicholasville, KY) in the milk and offered texturized calf starter (Ridley Feeds, Lancaster, PA) containing live yeast culture (Yea-Sacc®; Alltech, Nicholasville, KY) ad libitum from 3 d of age. Control calves were offered texturized calf starter without yeast culture ad libitum from 3 d. Water was provided at libitum and replaced 2 times a day.

Milk samples were thawed at 20˚C to measure bacterial content as described by Jayarao et al. (2004) . Milk samples were analyzed for standard plate count (SPC) and coliform count (CC). Milk samples were mixed and 50 µL were pipetted on to the plate and spread using a sterile, L-shaped polyproplylene cell spreader. All samples were cultured in duplicate for SPC using Plate Count agar and CC using MacConkey's agar. Cultures were incubated at 37˚C for 48 h and then colonies were counted. Counts from duplicate plates were averaged and converted to logarithmic counts. Milk samples (Table 5-1) were sent to Dairy One Inc. Laboratory (Ithaca, NY) to be analyzed for fat, total protein, and total solids using the following methods 989.05, 991.20, and 990.20, respectively (AOAC, 1996). Calf starter samples (Table 5-2) were sent to Cumberland Valley Analytical Services, Inc. (Waynesboro, PA) to be analyzed for DM, CP, ADF, ash, and minerals (AOAC, 2000), and NDF (Van Soest et al., 1991).

Weekly growth measurements were taken 3 h after the milk morning feeding for all animals. Growth measurement included body weight, hip width, and withers height. A jugular blood sample from each calf was collected into 10-mL serum Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) at 24 h after birth to determine serum protein. Serum protein was determined using an optical refractometer (SPR-T2, Atago Co. Ltd., Tokyo, Japan) calibrated with distilled water before each use. Also, weekly jugular blood samples were taken 3 h after milk morning feeding and collected into 10-mL sodium heparin Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) for haptoglobin determination by ELISA. Samples were diluted 1:100 in the solution provided in the ELISA kit (HAPT-11; Life Diagnostics Inc., West Chester, PA). Then, manufacturer's instructions were followed to determined haptoglobin concentration. Calf starter refusal

was recorded weekly, and a sample was taken to determine dry matter intake. Daily health scores, which focused on scours, respiration, general appearance and total daily score, were recorded for each calf using a standard scoring system (CALF Track), (Lesmeister and Heinrichs, 2004).

Intake, growth measurements, haptoglobin, and health scores data were analyzed using repeated measures analysis and the Mixed procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). Calf was included as a random variable. Birth body weight and wk 1 hip width and withers height were considered as potential covariates, but they were removed from the final models because no significant differences due to these factors were found. The AR (1) covariance structure was used in the model for repeated measures of intake, growth measurements, and health scores. The ARH (1) covariance structure was used in the model for haptoglobin with Tukey's adjustment used to compare least square means. Significance was declared at $P < 0.05$ and trends at $P < 0.10$.

Results and discussion

All calves completed the study as there was no mortality or culling. Serum total protein at 24 h averaged 6.0 for all treatments ($P = 0.60$). However, 2 calves had serum total protein values < 5.2 g/dL, which is considered failure of passive transfer; 1 calf was from the once-a-day milk feeding without yeast treatment and the other was from the twice-a-day milk feeding without yeast treatment. Body weight least square means are shown in Table 5-3 and Figure 5-1. Calves fed milk once a day were slightly heavier than calves fed

milk twice a day. The reason during wk 2 may be related to a higher milk consumption in calves fed once a day before weighing; however, subsequent weeks would not have any additional impact of the feeding amounts prior to weighing. No differences between once or twice-a-day feeding were detected (P = 0.17), and the inclusion of a mannan-rich fraction in the milk and live yeast culture in the starter did not affect body weight (P = 0.33).

Average daily gain (ADG) least square means are reported in Table 5-3 and Figure 5-3. Pre-weaning ADG was 553.4 and 512.1 g for calves fed milk once and twice a day, respectively, and there was no difference between treatments (P = 0.11). As seen for body weight, ADG was higher in calves fed milk once a day compared to calves fed milk twice a day during wk 2 (P = 0.004) as previously explained. In both treatments, ADG decreased during weaning in wk 6. Post weaning ADG was not different among treatments (P = 0.14); however, overall ADG showed a tendency to be greater in calves fed milk once a day compared to calves fed milk twice a day (P = 0.07). Overall ADG was not affected by the inclusion of a mannan-rich fraction in the milk and live yeast culture in the starter (P = 0.39).

These results are in agreement with Wilson (1968), Willett et al. (1969), and Kehoe et al. (2007) who also found no differences in pre weaning ADG between calves fed milk once or twice a day. It should be noted that there are no studies reported that show any difference between once and twice daily feeding of milk to calves. Kehoe et al. (2007) reported greater pre-weaning ADG in calves fed milk once a day versus twice a day; however, no significant differences were reported between systems for overall ADG. The same occurred in the present study. A study supplementing yeast culture at 1% and 2% of

DM in the calf starter reported no difference between control and yeast culture-fed calves in pre-weaning ADG (Lesmeister et al., 2004). A similar study feeding 0.2% yeast culture in the calf starter found no difference between yeast culture-fed and control calves in pre and post weaning ADG (Quigley et al., 1992). Both studies agree with the results reported in the present study. However, Lesmeister et al. (2004) showed a difference between calves supplemented with yeast culture at 2% of DM in the calf starter and control calves for overall ADG.

Calf starter intake least square means are shown in Figure 5-2. After 2 wk of age, calves fed milk once a day had a numerically higher calf starter intake compared to calves fed twice a day; however, there was no difference between treatments (P = 0.47). Several studies have shown that feeding milk either once or twice day does not affect starter consumption (Willett et al., 1969; Stanley et al., 2002; Kehoe et al., 2007). Kehoe et al. (2007) reported higher values for calf starter intake compared to the present study. This difference could be attributed to a higher milk protein and fat content in the milk fed in the present experiment. Pre-weaning calf starter intake was 242.3 and 198.7 g/d for calves fed milk once and twice a day, respectively, but there were no treatment differences (P = 0.35). Also, Willett et al. (1969) reported greater starter consumption in calves fed milk once a day compared to twice a day, but the difference was not significant. As expected, calf starter intake increased after weaning in both treatments (P = <0.01). Similar to Quigley et al. (1992) and Lesmeister et al. (2004), the inclusion of a mannan-rich fraction in the milk and live yeast culture in the starter did not affect calf starter intake (P = 0.92).

Structural growth measurements such as withers height and hip width least square means are reported in Table 5-3. Withers height and hip width were the same in calves fed

milk either once or twice a day (P = 0.32 and 0.95, respectively). This result agrees with Kehoe et al. (2007), who also did not report differences in withers height between calves fed milk once or twice a day. The most interesting result was observed in calves fed mannan-rich fraction in the milk and live yeast culture in the starter, where withers height and hip width in these calves tended to be higher than control calves (P= 0.06 and 0.08, respectively). This result supports Lesmeister et al. (2004) findings, where for calves supplemented with yeast culture at a higher level in the starter, withers height tended to be higher than calves supplemented with yeast culture at a lower level and was greater than control calves. Also, Mir and Mir (1996) reported a numerical increase in carcass weight in steers fed yeast, suggesting that yeast has an effect on bone growth.

Haptoglobin concentration least square means were 4 and 9.5 ± 3.5µg/mL for calves fed milk once or twice a day, respectively, and there was no difference among treatments (P = 0.27). Haptoglobin concentration least square means were 10.8 and 2.8 ± 3.5µg/mL for calves without yeast and calves with yeast, respectively, and there was no difference among treatments (P = 0.11). This was attributed to the high variability in the data. There were 22 calf-weeks where haptoglobin concentration was higher than 10 µg/mL of which 14 wk were from control calves and 8 for yeast calves. Haptoglobin concentration increases after inflammatory stimuli or acute phase reactions, so for this reason haptoglobin concentration is used as indicator of inflammation (Morimatsu et al., 1992; Nakajima et al., 1993). Also, haptoglobin concentration can be used as an indicator of stressful management practices such as transportation or feedlot entry (Araujo et al., 2010; Cooke et al., 2012). In healthy animals, haptoglobin concentration is < 350 µg/mL (Horadagoda et al., 1994), so the calves were not sick or stressed in the present study.

Least square means for scours score and total daily score were the same for calves fed milk once or twice a day (P = 0.36 and 0.47, respectively.). This finding is also supported by previous research that has not observed differences in scours, digestive disorders, or health scores of calves fed milk once or twice a day (Willett et al., 1969; Owen and Larson, 1982; Kehoe et al., 2007). Least square means for scours score were the same for calves fed a mannan-rich fraction in the milk and live yeast culture in the starter and the control group (P = 0.21). However, total daily score, which included scours, respiratory, and general appearance score, tended to be lower in calves fed a mannan-rich fraction in the milk and live yeast culture in the starter than the control group (P = 0.06). This finding may be related to another experiment feeding yeast culture at 2% of the starter DM, where an improvement in calf heath and reduced treated days were reported (Magalhães et al., 2008). The soluble products present in yeast culture have been shown to modulate the immune system (Jensen et al., 2007), which can help explain the tendency observed.

Conclusions

Calves fed milk once a day were not different than calves fed milk twice a day in BW, ADG, calf starter intake, growth measurements, or health scores; indicating that feeding milk once a day can be successfully used. However, there was a tendency for increased withers height and hip width and lower health scores in calves supplemented with a mannan-rich fraction in the milk and live yeast culture in the starter. The inclusion of a mannan-rich fraction in the milk and live yeast culture in the starter did not affect ADG or calf starter intake.

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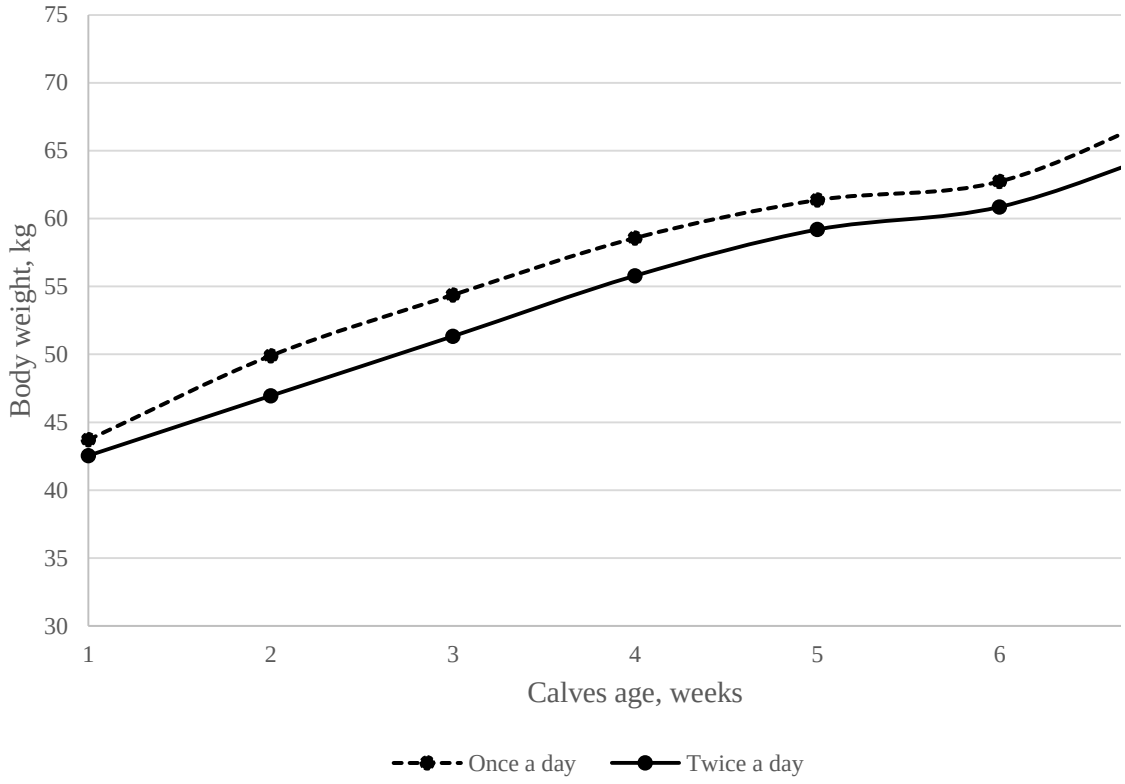

Figure **5- 1**: BW least squares means for calves fed milk once or twice daily.

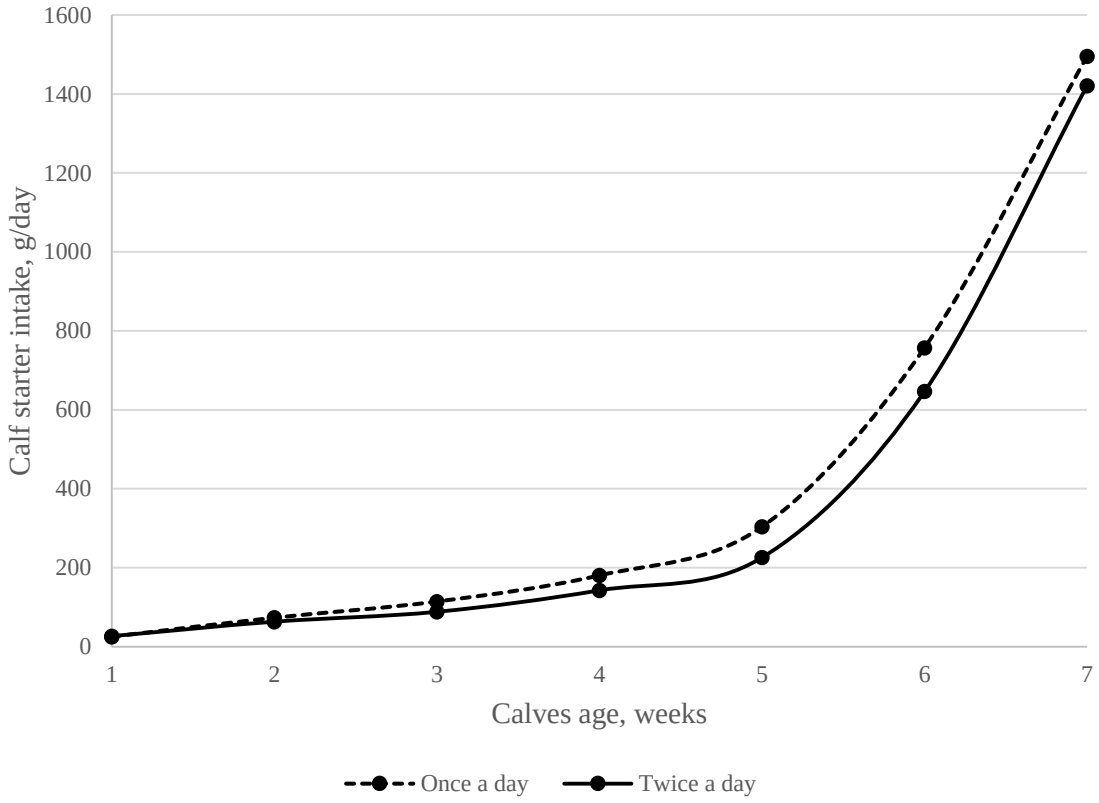

Figure **5- 2**: Calf starter intake for calves fed milk once or twice daily.

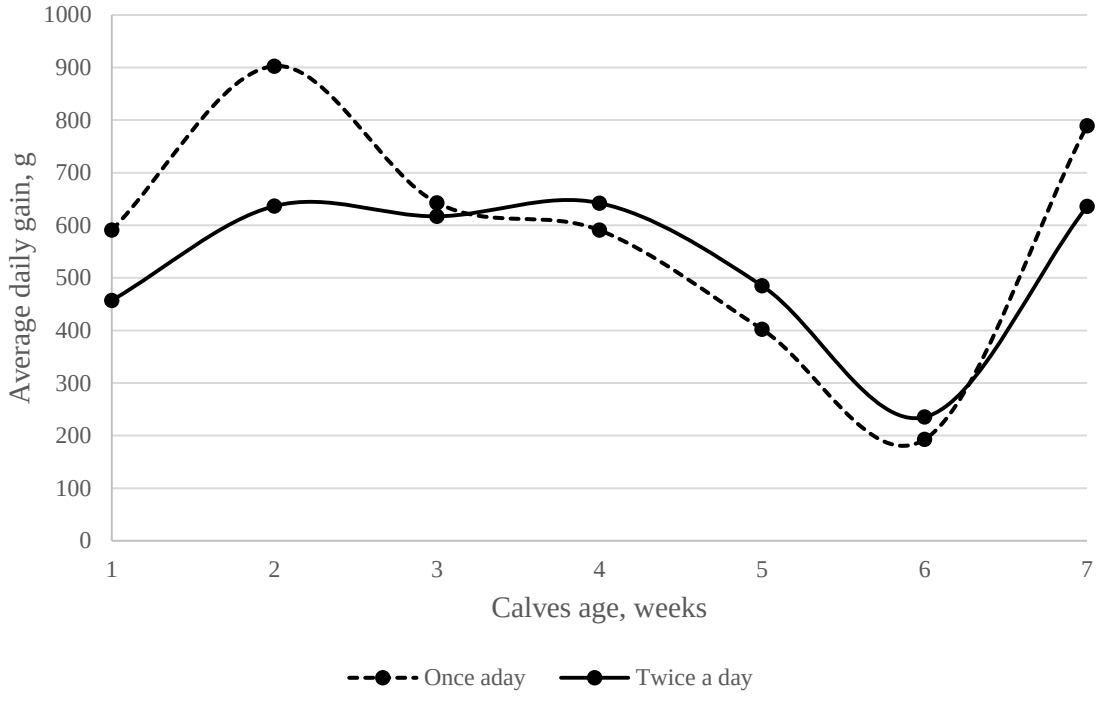

Figure **5- 3**: Average daily gain for calves fed milk once or twice daily.

Table **5- 1**. Milk compositional analysis and bacteria counts before and after pasteurization

Total solids, %	12.8
Protein, %	3.8
Fat, %	3.5
SPC[1], cfu/mL	
Before	3.3
After	1.5
Coliform, cfu/mL	
Before	1.5
After	0.4

[1]SPC = standard plate count.

Table **5- 2**. Compositional analysis of calf starters

	Calf starters	
	No yeast	Yeast
DM, %	96.3	96.3
CP, %	20.4	21.4
ADF, %	8.55	9.3
NDF, %	15.2	16.4
Ash, %	9.0	8.6
Ca, %	1.5	1.4
P, %	0.7	0.7
Mg, %	0.5	0.4
K, %	1.4	1.5
Na, %	0.8	0.6
Fe, mg/kg	452	437
Mn, mg/kg	260	258
Zn, mg/kg	378	867.5
Cu, mg/kg	91	99

Table **5- 3**. Least squares means of growth parameters for calves fed milk once or twice daily and yeast or no yeast supplementation

	Once a day	Twice a day	No yeast	Yeast	SEM
Weight, kg					
Initial	43.7	42.5	42.1	44.1	1.3
Weaning	62.7	60.9	61.1	62.5	1.3
Final	68.2	65.4	66.3	67.3	1.3
ADG, g/day					
wk 1-6	553.7	512.1	543.6	522.2	18.2
wk 7	789.3	635.8	742.2	683.0	73.3
wk 1-7	587.3	529.8	572.0	545.2	22
Hip width, cm					
Initial	17.1	17.1	16.9	17.3	0.2
Final	20.5	20.5	20.3	20.7	0.2
Withers height, cm					
Initial	76.5	75.5	75.3	76.7	0.5
Final	86.5	85.7	85.3	86.9	0.5

Chapter 6

Conclusions

Heating colostrum at 60˚C for either 30 or 60 min reduced bacterial content. Colostrum IgG concentration was similar between colostrum heated for 30 or 60 min. Serum IgG concentration at 24 h increased as colostrum quality, and there was a tendency to increase serum IgG concentration at 24 h as heat treatment time increased. Results from this experiment supports that IgG absorption is improved as a result of a reduction in colostrum bacterial load. In addition, feeding average quality colostrum with low bacterial content, yields similar blood IgG absorption to high quality colostrum. However, more research in high and medium quality colostrum with different bacterial content is necessary to determine the effect of heating time on IgG absorption.

Feeding milk once a day to dairy calves could be successfully use in dairy farms operations, especially in today's labor market with no impact on the calf. Calves fed milk once a day were not different than calves fed twice a day milk in BW, ADG, calf starter intake, growth measurements, or health scores. In addition, calves supplemented with Actigen™ and Yea Sacc™ showed a tendency for increased withers height and hip width and lower health scores. The use of these two additives did not affect ADG or calf starter intake. More research is necessary to determine long term effects of once versus twice a day milk feeding for dairy calves.