ABSTRACT

GLOSSON, KRISTEN. The Effects of Supplementing Pasteurized Milk Balancer Products to Pasteurized Whole Milk on the Health and Growth of Dairy Calves. (Under the direction of Dr. Brinton Hopkins.)

Two experiments were conducted to determine the health and growth effects of supplementing pasteurized whole milk with pasteurized milk balancer products in an enhanced calf feeding program. Two locations were used in the study, the North Carolina State University Lake Wheeler Dairy (Raleigh, NC) and the North Carolina Department of Agriculture Piedmont Research Station (Salisbury, NC), with calves fed according to the protocol at each site. All calves were removed from their dams after birth (d 0), fed 3.8 L pasteurized colostrum, and received their respective treatments from d 1 until weaning at d 56. All calves were fed pasteurized whole milk from d 1 through d 56. Calves were weighed and measured for wither height (**WH**), hip height (**HH**), and hip width (**HW**) every 7 d from birth until weaning at d 56. Average daily gain (**ADG**) and feed efficiency (**FE**) were calculated from d 0 through d 56. Feed efficiency was the ratio of total gain to total DMI, which included milk DM, milk balancer DM, and calf starter DM consumed.

In experiment 1, 80 Holstein heifer calves were used to investigate the effects of supplementing two levels of pasteurized whole milk with or without a pasteurized all-milk balancer product (Land O'Lakes All-Milk Pasteurized Milk Balancer Product). There were four dietary treatments (n=20 calves per treatment). Calves receiving treatment milk (**M**) and milk plus balancer (**M+B**) were fed 3.8 L milk divided into two equal feedings from d 1 through d 56. Calves on treatment increase milk (**IM**) and increase milk plus balancer (**IM+B**) were fed 3.8 L milk divided into two equal feedings from d 1 through d 14, 5.7 L milk divided into two equal feedings from d 15 through d 42, and 2.85 L milk fed once daily

from d 43 through d 56. Treatments M+B and IM+B included pasteurized all-milk balancer fed at a rate of 0.23 kg per 3.8 L of pasteurized whole milk. Calves fed treatment IM+B had greater body weight (BW) (P ≤ 0.05) and ADG ($P \leq 0.05$) through 56 d of age. Calves fed 5.7 L of whole milk had greater FE then those fed 3.8 L of whole milk regardless of balancer added ($P \leq 0.03$).

In experiment 2, 72 Holstein heifer calves were used to investigate the effects of supplementing two pasteurized milk balancer products (Land O'Lakes All-Milk Pasteurized Milk Balancer Product and Land O'Lakes Protein-Blend Pasteurized Milk Balancer Product). There were three dietary treatments (n=24 calves per treatment). Calves were fed 3.8 L milk divided into two equal feedings from d 1 through d 14, 5.7 L milk divided into two equal feedings from d 15 through d 56. Treatment increase milk (**IM**) did not include any supplements. Treatment increase milk plus balancer (**IM+B**) included a pasteurized all-milk balancer and treatment increase milk plus protein-blend balancer (**IM+PB**). Balancer was supplemented at a rate of 0.23 kg per 3.8 L of pasteurized whole milk. Calves fed treatment IM+B and IM+PB had greater BW when compared to calves given treatment M ($P \leq 0.01$). Calves fed M+PB had comparable BW and FE to calves receiving IM+B.

Calves that were fed 5.7 L of pasteurized whole milk supplemented with a milk balancer had greater BW when compared to treatments with lower total milk DM consumed. In experiment 1, FE was greater in calves receiving 5.7 L of whole milk when compared to calves receiving 3.8 L, regardless of the use of balancer. In experiment 2, the protein-blend balancer produced comparable results. The enhanced calf feeding programs evaluated in this study were successful in increasing growth in preweaning calves when supplementing a milk balancer product to whole milk.

Supplementing Pasteurized Milk Balancer Products to Pasteurized Whole Milk on the Health and Growth of Dairy Calves

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Animal Science and Nutrition

Raleigh, North Carolina

2014

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DEDICATION

To my family.

For all the walking phone calls.

BIOGRAPHY

Kristen Glosson was born in Greensboro, North Carolina on October 2nd, 1990, to Garry and Susan Glosson. She was raised in Gibsonville, North Carolina with her older brother, David Glosson, under the guidance of grandparents Samuel and Thelma Glosson and Harold and Evelyn Stanley. She graduated from Eastern Guilford High School in May of 2008 and began her Bachelor's of Science degree as a Pre-Vet Animal Science student at North Carolina State University. In May of 2012 she graduated with a B.S. in Animal Science with minors in Nutrition and Microbiology.

During her undergraduate career, she attended all regional and national meetings of the American Dairy Science Association as a student member and competed in regional and national Dairy Challenge conferences. The traveling involved in these activities provided her with a new understanding of the dairy industry. She began to focus on research opportunities through an internship at the Purina Longview Research Center in Grey Summit, Missouri, and continued with an undergraduate research project comparing cyclicity in purebred versus crossbred Holstein and Jersey dairy cattle. In October of 2011, she began her graduate thesis research project while finishing her B.S. at North Carolina State University. Continuing directly into a graduate degree under the guidance of Dr. Brinton Hopkins, she focused on how preweaning dairy calf health and growth could be impacted by enhanced calf feeding of a milk balancer product with pasteurized whole milk.

Graduate school allowed her to expand her knowledge in the dairy industry and gain more applied experience in managing a research study.

ACKNOWLEDGMENTS

I would like to first thank Dr. Brinton Hopkins for his support and guidance that allowed me the freedom to explore new ideas and the foundation for future success. I hope that his examples of personal and professional integrity will continue to influence me long after this project is completed.

I am especially grateful to Dr. Shannon Davidson who was an integral part of this study in both knowledge and experience. I truly appreciate her constant encouragement and understanding. I also want to thank the other members of my committee Dr. Steve Washburn and Dr. Geoffrey Smith. Their contributions to this project were both immeasurable and indispensable.

I would like to acknowledge the dedication and commitment shown by the faculty and staff at the North Carolina State University Dairy Farm and the North Carolina Department of Agriculture Piedmont Research Station that made this study possible.

I want to give a special thanks to the faculty and staff in the Animal Science Department at North Carolina State University that provided a community that fostered all graduate students regardless of species concentration or career focus.

I would also like to extend my appreciation to the North Carolina Dairy Foundation that funded my research assistantship position as well as the companies that contributed to this project, Land O'Lakes Animal Milk Products and Dairy Tech Inc.

Finally, I must recognize my family for their constant love and support. I am truly blessed. I want to especially extend my gratitude to my brother, my best friend.

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ABBREVIATIONS

FE: feed efficiency

FPT: failure of passive transfer

HH: hip height

HTST: pasteurization using high temperature short time

HW: hip width

IgG: immunoglobulin G

IM: treatment with increased quantity of milk

IM+B: treatment with increased milk and supplemented with an all-milk milk balancer product

LTLT: pasteurization using low temperature long time

LWD: North Carolina State University Lake Wheeler Dairy

M: treatment milk

M+B: treatment milk with supplemented all-milk balancer product

MAP: *Mycobacterium avium* subspecies *paratuberculosis*

NAHMS: National Animal Health Monitoring System

PRS: North Carolina Department of Agriculture Piedmont Research Station

VFA: volatile fatty acid

WH: wither height

LITERATURE REVIEW

INTRODUCTION

The success of a dairy cow is dependent on a strong foundation beginning at birth and continuing through maturity. In the pursuit of improving, enhancing, or accelerating calf growth in the preweaning period, many feeding and management strategies have been developed. Reasons for investing in such strategies include reducing health issues, improving nutrient efficiency, and lessening the economic cost during the non-milk stage of life. There has also been a link between enhanced calf feeding programs regarding increases in future productivity of the lactating cow. However, there are many components that must be addressed to create a successful calf feeding program. This review will include studies associated with management of colostrum feeding, on-farm pasteurization methods, and overall nutrition throughout the preweaning period for dairy calves.

The first topic discussed within this review focuses on colostrum, which is one of the essential on-farm resources required in the beginning development of a healthy calf. Within the colostrum topic, subsections about the importance of transferring passive immunity, considerations regarding the use of colostrum-replacers and supplements, and alternative strategies in the handling and storage of colostrum, are discussed.

The second topic area covers the effectiveness of on-farm pasteurization, as well as, key concerns when feeding raw milk to replacement heifers. Colostrum safety is also discussed within this topic as an effort to reduce bacterial contamination through pasteurization techniques.

The final main topic area focuses on overall nutrition provided to calves throughout the preweaning period. Sections within this topic concentrate on the general plane of nutrition, differences in liquid feed, calf starter composition, and a summary of studies regarding the impact that preweaning nutrition has on future milk production.

By investigating these three key topic areas, this review illustrates the many options producers have to create a successful dairy calf feeding program, as well as identify opportunities to further advance research within this field.

COLOSTRUM

It is essential to provide adequate amounts of clean, high quality colostrum to the neonatal dairy calf. Colostrum has been linked to the development of healthy calves with greater growth rates when compared to calves that did not receive adequate amounts of colostrum (Furman-Fratczak et al., 2011). The immunoglobulin G protein (**IgG**) has been linked to the health benefits of colostrum which play a pivotal role in activating the calf's own immune system at birth. The resecretion of IgG also provides localized protection in the small intestines after initial absorption (Jaster 2005). The quality of colostrum is determined by an IgG concentration, where >50 g of IgG/L is considered a high quality sample. A study by Faber et al. (2005) looked at health performance in calves fed different volumes of high quality colostrum which would differ in IgG mass, but not concentration. The results showed that the number of medical treatments needed during the preweaning period was doubled when reducing the colostrum offered from a standard four L dose to a two L dose (Faber et al., 2005). It is, therefore, recommended to provide at least 3.8 L of high quality colostrum administered directly after birth (Beam et al., 2008).

Passive immune transfer

The importance of feeding high quality colostrum with adequate IgG concentrations is directly related to a process called passive immune transfer that protects the calf by increasing immunoglobulin levels in the circulating blood serum (Trotz-Williams et al., 2008). Calves are only able to produce, on average, 1g of IgG per day starting at 36 hours after birth and continuing through 3 weeks of life (Devery et al., 1979). Based on this limitation it has been established that the maternal supply of IgG is essential for the initial activation of the calf's immune system as well as for localized intestinal protection. Failure of passive transfer (**FPT**) is defined as <10 mg/mL of IgG in blood serum sampled between 24 to 48 hours after birth (Weaver et al., 2000). It has been proven difficult to recover the initial immune development through enhanced nutrient programs later in the preweaning period shown in a study by Quigley et al. (2006). This is mostly due to the closure of the gap junctions in the small intestines beginning at birth. This causes a linear decrease in the calf's ability to absorb large protein molecules, such as IgG, until 24 hours after birth when the gap junctions are completely closed (Quigley et al., 2006). Therefore, the time limitation of colostrum feeding is based on the physiological change in the calf's gastrointestinal tract.

Some studies have focused on how to optimize IgG absorption through management techniques. The general method of feeding colostrum is to offer a one-time large dose directly after birth when the rate of absorption is the greatest, while others feed multiple doses of colostrum to provide a steadier rate of IgG mass through the intestines. Jaster (2005) split the colostrum feeding from a one-time four L colostrum dose at birth, to two L at birth and 2 L at 12 hours after birth which showed an increased rate of absorption of IgG from the intestines. However, in another study, a dose of 150 g of IgG from a milk replacer product directly after birth was more effective then feeding half at birth and the other half seven hours after birth (Hammer et al., 2004). Berge et al. (2008) extended the colostrum feeding program further through 28 days of life and showed a lower incidence of gastrointestinal disease and, therefore, required less antimicrobial treatments. After 24 hours the continued feeding of colostrum would no longer affect the immune system, but provided additional gut health through the feeding program (Berge et al., 2008). However, the availability of high quality colostrum throughout a calf's preweaning period is greatly limited by maternal production, which begins to transition to whole milk after the first collection. Because of this, a long-term colostrum feeding protocol is both difficult and expensive to maintain. The main goal of colostrum management is to insure passive immune transfer to newborn calves.

While IgG concentrations remain the standard to which colostrum is measured for quality, other studies have shown that a level of serum IgG of >10 mg/mL is not the only important aspect of colostrum. Key components such as growth factors, specifically insulin-like growth factor-I, have also been linked to growth within the gastrointestinal tract (Pakkanen et al., 1997). In one study focusing on other immunoglobulins absorbed from colostrum, a serum γ-globulin concentration of >15 g/L was shown to reduce respiratory tract infections and levels >10 g/L showed improvement of overall calf health (Furman-Fratczak et al., 2011). Maternal colostrum contains a variety of components, not limited to immunoglobulin proteins, which can greatly benefit a calf's performance. Recent research focusing on these different components may lead to a reevaluation of the standards used to determine the quality of harvested colostrum.

The national animal health monitoring system (**NAHMS**) survey of heifer raising facilities in 2010, reported that 4.2 +/- 1.2% of heifers died in the preweaning period, 1.6 +/- 0.3% died postweaning, and 0.2 +/- 0.01% died while pregnant. Pneumonia (2.3 +/- 0.9%), diarrhea (1.4 +/- 0.3%), navel infection (0.4 +/- 0.3%), and lameness (0.2%) account for the observed preweaning deaths reported in this survey (NAHMS, 2011). Adequate IgG absorption from colostrum helps protect the gastrointestinal tract through secretions into the intestinal lumen. Corely et al. (1977) looked more closely at the bacterial colonization in the gut of calves, specifically *E. coli*. The results showed exponentially more bacterial binding with apical plasma membranes in calves that received no colostrum, little to no binding when fed at the same time as colostrum, and no risk of invagination of *E.coli* colonies within the gut one hour after colostrum feeding (Corely et al., 1977). The resecretion of IgG prevents gastrointestinal distress such as diarrhea by transferring in antigen-binding antibodies into the intestinal tract lumen. Intestinal immunity is then continued after initial colostrum ingestion as IgG antibodies are then resecreted until the calves own immune system is fully established (Besser et al., 1988). Looking at long-term effects, a comprehensive study analyzing key factors affecting milk production in the first lactation identified days labeled as ‘ill’ before four months of age having a major negative effect on later production (Heinrichs et al., 2011).

Passive immune transfer is a direct result of the mass of IgG consumed and absorbed. In a colostrum management program, when feeding fixed levels of colostrum, the quality of the colostrum should be determined to insure proper IgG intake. Specific cut-offs have been established to recommend the quantity and quality of colostrum producers should feed newborn calves to insure successful passive transfer of immunity. Trotz-Williams et al. (2008) showed that the quantity of colostrum given to calves in the first 6 hours after birth was significantly and negatively correlated with failure of passive transfer, with calves that were left with their dam for the first 3 hours doubling the chances of FPT occurring. Cow movement away from the calf directly after parturition can be attributed to a significant delay in feeding and cause a reduction in the calf's ability to absorb adequate amounts of IgG. The two main methods to manually feed colostrum include nipple bottles and esophageal tubing. Both methods have been compared and proven to be equally effective in providing a way for producers to administer recommended volumes of colostrum and test for quality before feeding (Godden et al., 2009; Chigerwe et al., 2012).

Differences in colostrum quality can affect recommended colostrum volumes. One variation in colostrum can be related to cow breed, which determines the volume produced and immunoglobulins transferred from the blood stream to the lacteal secretion during colostrogenesis (Murphy et al., 2005). Other differences in management of dry cows, dry cow treatments, and the age of the cow can also affect colostrum by increasing or decreasing colostrum volume (Nowak et al., 2012). However, as a standard, it is generally recommended that calves should receive at least 3.8 L of high quality colostrum immediately after birth or within the first four hours after birth (Beam et al., 2009).

Using colostrum replacers and supplements

In situations where harvested maternal colostrum is not appropriate to use, alternatives are available to replace or supplement on-farm colostrum. One circumstance where colostrum replacers may be used is when there is a risk of disease transfer from the dam to the calf through colostrum. While using colostrum replacers can reduce the risk of introducing a disease to newborn calves, studies have shown that there are significantly lower serum IgG levels in those calves fed replacers versus maternal colostrum which can lead to FPT (Swan et al., 2007). In one study by Poulsen et al. (2010) a combination of colostrum replacers and supplements was used to provide calves with enough IgG in two two L doses. One dose was administered directly after birth or within two hours and the other dose between two and 12 hours after birth to provide a continuous flow of colostrum through the intestines. However, the trial did not follow calves through production age or assess other colostrum properties that may be needed for optimal growth in newborn calves (Poulsen et al., 2010). Other studies that have looked at long term-effects of feeding colostrum replacement products showed no differences in production performance or life expectancy. However, there was an emphasis placed on the use of products containing only plasma or serum extracts to formulate the IgG dosage (Pithua et al., 2010; Quigley et al., 2001).

When using serum derived colostrum replacers, many studies have shown that multiple doses of the recommended concentrations were required to insure successful passive immune transfer. To avoid high incidences of FPT, it is recommended producers only use tested colostrum replacers to determine if multiple doses are required to achieve adequate IgG absorption (Fidler et al., 2011; Godden et al., 2009; Smith et al., 2007). However, maternal colostrum remains the best choice when feeding newborn calves, even when feeding a colostrum replacer at a similar IgG concentration (Priestley et al., 2013). Priestly et al. (2013) attributed this difference to the absorption efficiency of maternal colostrum that allowed more IgG to be absorbed from the maternal source.

A possible problem regarding the use of replacers is the IgG molecules found in colostrum replacer are not from the local environment. Using maternal colostrum allows calves to develop immunity for pathogens present on a specific farm which they will need protection from as they join the milking herd (Godden, 2008). Priestly et al. (2013) compared maternal colostrum to colostrum replacers, both colostrum-derived and plasma-derived, and found calves fed maternal colostrum had higher serum total protein and serum IgG concentrations, which led to greater overall incidences of adequate passive immune transfer. Colostrum-derived replacers are superior to plasma-derived options, but calves fed a colostrum replacer instead of maternal colostrum had higher morbidity and mortality rates (Priestley et al., 2013). There is a large variation in processed replacer products, with options to increase the concentration of the IgG content to >200 g/dose. However, while the IgG content of colostrum replacers can be increased to greater than a calf's requirements, there may be an imbalance of other components that can cause a decrease in the effectiveness of the colostrum (Jones et al., 2004). In any management system, the costs and potential benefits of replacer products needs to be considered. The ability to increase the concentration of IgG dosage in colostrum replacers is a large advantage, but the increased dosage will increase the cost per calf with no evidence that future profit will outweigh the increase in cost (Pithua et al., 2010).

Colostrum supplements can also be used to fortify on-farm colostrum resources with IgG and nonimmunoglobulin proteins. Davenport et al. (2000) added 200 g of either whey protein or casein directly to maternal colostrum and reported no effect on the plasma urea N, total protein or plasma IgG levels. However, when adding over 500 g of colostrum supplement, containing nonimmunoglobulin proteins, there was a negative effect on IgG absorption linked to the competition of protein absorption in the calf's intestines (Davenport et al., 2000; Quigley et al., 2001 and 2002). Colostrum supplements may also include growth factors; insulin like growth factor-I and transforming growth factor-beta, but those additions can decrease IgG absorption by calves and lower the efficacy of using colostrum supplements (Hammer et al., 2004). Other supplementation focuses on adding or increasing different elements maternal colostrum may lack. One option is to include alpha-tocopherol to colostrum to off-set the limitation of vitamin E in newborn calves, but it is usually coupled with other recommended supplements (Quigley et al., 1995). However, supplementation of any preweaning feed is an added expense to producers and should be linked to future gains or improved health to justify the cost.

Handling and storing colostrum

The quality of the colostrum is only one consideration in reducing cases of FPT in newborn calves. Handling and storage protocols play a vital role in providing calves with clean, high quality colostrum. Colostrum quality can be determined quickly and efficiently through the use of on-farm testing instruments. For colostrum to be labeled as high quality IgG levels must be >50 g of IgG/L (Godden, 2008). Colostrometers use specific gravity to determine the IgG concentration, where >1.050 is equivalent to >50 g of IgG/L. However, this method is vulnerable to temperature changes that affect specific gravity and so temperature gauges are required in order to adjust the temperature of the colostrum to 21˚C. Specifications from the testing equipment manufacturer require the temperature of the colostrum to be controlled in order to avoid false positives (Chigerwe et al., 2008). Another method is using a Brix refractometer, which is not susceptible to changes in temperature, but instead measures the amount of sucrose through light refraction. Values range from 0 to 35% and a value of 22% corresponds to an IgG content of 50 g/L. Through this method, any samples scoring 22% or higher can be labeled as high quality colostrum (Quigley et al., 2013). It is, also, recommended to avoid pooling colostrum, because the higher quality colostrum tends to be smaller in volume and can be quickly overwhelmed by large quantities of low quality colostrum (Elizondo-Salazar et al., 2013).

The mean concentrations of nutrients in colostrum have increased as a result of improved general management and handling. Nationwide evaluation of the quality of colostrum on dairy farms in the US in 2012 showed differences between large and small farms and in different regions or climates (Morrill et al., 2012). Large farms reported longer times between calving and collecting colostrum, with a higher somatic cell count and lower nutrient content. Small farms had less consistency in freezing colostrum, using a colostrometer or supplementing a mineral-vitamin mix to calves (Kehoe et al., 2007). The Midwest reported greater overall IgG concentrations. On the other hand, the Southeast had a higher percentage of samples containing bacterial concentrations of >100,000 cfu/mL, which is the industry recommended cut off for microbes. It is the standard recommendation to feed colostrum fresh or freeze immediately without refrigeration in all regions and the colostrum is considered acceptable to use for up to 1 year (Morrill et al., 2012; Godden, 2008).

As alternatives to freezing collected colostrum, Stewart et al. (2007) showed that using potassium sorbate as a colostrum preservative alone is not enough to keep bacterial counts low. Colostrum must be stored at refrigerated temperatures if treated and frozen temperatures if not (Stewart et al., 2007). Another option is formaldehyde which can be used to treat colostrum at an inclusion rate of 0.05%, which can maintain colostrum pH for four weeks when stored at ambient temperatures (Mbuthia et al., 1997). In comparison, non-treated colostrum will become unstable after the first 3 days. However, in a study by Mbuthia et al. (1997), there were significant drops in IgG and IgM levels in formaldehyde treated colostrum, while the IgA level was constant between samples. The authors concluded that formaldehyde treated colostrum should be reserved for older calves and not newborn calves requiring passive immunity (Mbuthia et al., 1997). It has been shown that if the pH of the colostrum is kept between 7.5 and 5.0 there is no difference in IgG absorption or passive immune transfer (Quigley et al., 2000). This consideration allows for acidic preservation techniques to be used successfully in a calf feeding program.

While many options are available to clean, preserve, or use alternative colostrum products, the handling and care of colostrum and specific feeding protocols are still a necessary part of insuring passive immune transfer to newborn calves.

PASTEURIZATION

To safely utilize whole milk on a commercial dairy operation as a liquid feed source for calves, on-farm pasteurization is an option for producers. The two primary on-farm pasteurization techniques are high temperature, short time (**HTST**) and low temperature, long time (**LTLT**), also known as continuous and batch pasteurization, respectively. Continuous pasteurization heats milk to 71.6˚C for 15 seconds, whereas in batch pasteurization, milk is heated to 62.7˚C for 30 minutes. These methods have both been shown, in a field study of six Pennsylvania farms, to be equally effective in significantly reducing the bacterial load (total plate count) to <5,000 cfu/mL in whole milk (Elizondo-Salazar et al., 2010). In addition to reducing the bacterial contamination in whole milk, pasteurization is used to limit disease transmission that can occur from feeding raw milk to replacement heifer calves. Many pathogens have the potential to spread through colostrum and milk, including *Salmonella, Listeria monocytogenes,* and *Eschericha coli,* which can be successfully eliminated through pasteurization (Godden et al., 2003).

Mycobacterium avium **subspecies** ***paratuberculosi*****s risk**

A major concern in the dairy industry is *Mycobacterium avium* subspecies *paratuberculosi*s (**MAP**), the causative agent for Johne's disease, which continues to be prevalent in U.S. dairy herds (Stabel et al., 2001). The spread of this disease has been linked to the ingestion of contaminated feces, bedding, colostrum and raw milk (Ruzante et al., 2008). Johne's disease causes a serious reduction of milk production while also increasing susceptibility to other mammary infectious diseases, loss of body weight, and reduced fertility that leads to premature culling of infected animals (Johnson-Ifearulundu et al., 1997). Clinical signs can stay hidden for 2 to 5 years after the initial infection making infected animals difficult to isolate (Stabel, 1998). The ability of this bacterium to remain hidden for the majority of a cow's life and have negative impacts on long-term productivity illustrates the challenge Johne's disease presents to completely eliminate from a specific herd.

Options to limit calf exposure to this bacterium include colostrum and milk replacers and pasteurization of potentially contaminated maternal colostrum and milk (Stabel et al., 2004). Using these methods can limit exposure, but neither can eliminate the spread of MAP to the calves. This is due to the risk of *in utero* exposure from MAP positive dams (Dieguez et al., 2008, Pithua et al., 2009). In a study conducted by Aly et al. (2005), calves born to dams infected with MAP were 6.6 times more likely to contract the disease with 84.6% attributed to having infected dams. Prevention of exposure sources after birth is still an important part of calf management, but will not eliminate all risk of infection. Monitoring of infected cows and selective culling is a required tool to completely rid a herd of the disease (Aly et al., 2005).

Milk replacers are heat-treated to eliminate bacterial growth and are one option for producers who have a MAP contaminated herd. When using whole milk with on-farm pasteurization the major challenge when targeting MAP is the tendency for the bacteria to form clumps. This occurs through a hydrophobic interaction that provides the bacteria some protection against pasteurization (Lund et al., 2002). There are many variations of pasteurization techniques that can be used by producers in order to safely utilize whole milk in their calf management feeding programs. An example of a method developed by Ruzante et al. (2008), used a batch pasteurizer and set the temperature at 65.5˚C for 30 minutes, which is above the temperature set for LTLT pasteurization. The risk of spreading MAP through whole milk can be successfully reduced using this technique and thereby provides dairy farms with a safe feedstuff for their calves (Ruzante et al., 2008). A continuous method with temperatures ranging from 72 to 78˚C and times ranging from 15 to 25 seconds, was also shown as effectively killing the MAP contamination of whole milk (McDonald et al., 2004). There are many other pasteurization methods that have been tested and approved for on-farm use with both batch and continuous pasteurization techniques. However, with any technique, it should be stressed that standard operating procedures and employee training is required to properly use pasteurization with reliable results (Ruzante et al., 2008).

Colostrum has been shown to be a transmission portal for Johne's disease in herds infected with MAP (Pithua et al., 2011). In one study by Stabel et al. (2008), subclinical MAP infections were tracked in replacement heifers by measuring the production of IFN-y antibodies. The results showed calves fed colostrum harvested from infected animals had higher levels of IFN-y response within the first year of life then those fed pooled pasteurized colostrum. This indicates colostrum can be a strong exposure risk for calves and pasteurization is a viable option for reducing transfer of MAP (Stabel et al., 2008).

Colostrum Pasteurization and other heat treatments

High quality colostrum is determined by the concentration of IgG >50 g/L of colostrum. In a study conducted by Godden et al. (2003), there was a significant difference in IgG concentration after pasteurization, which was greatly affected by the batch size. Smaller amounts of colostrum exhibited less variation and damage than larger pooled batches. Both standard HTST and LTLT proved to eliminate the risk of pathogens, but reduced IgG concentrations by almost 30% (Stabel et al., 2004). The reduction of IgG is shown as a percentage, which would result in a greater IgG mass reduction in higher quality colostrum when compared to low quality colostrum (Donahue et al., 2012). As an alternative to the standard LTLT method, a temperature of 60˚C for 60 minutes was established by Godden et al. (2006) as a way to effectively reduce the bacterial load without damaging total IgG concentrations. The lower temperature, longer time pasteurization treatment prevented viscosity changes while also maintaining the IgG concentration in colostrum (Godden et al., 2006). It was determined that on a small scale, colostrum could withstand 60˚C for up to 120 minutes without affecting the IgG concentration or viscosity, but other factors could be altered if heated for extended periods of time (McMartin et al., 2006). Another study by Elfstrand et al. (2012) measured the effects both pasteurization and freeze-drying had on secondary components of colostrum, including transforming factor beta-II, insulin-like growth factor-I and growth hormone, as well as immunoglobulins. The study suggested that IgG and growth hormone are the most sensitive to any processing and can be used to determine the effectiveness of a pasteurization process (Elfstrand et al., 2002; Johnson et al., 2007).

There are many benefits of pasteurizing colostrum, one being that pasteurizing colostrum decreases bacterial load interference that can negatively impact IgG absorption. Colostrum with a high bacterial load was theorized to limit immunoglobulin absorption because the bacteria bind to the already large protein molecules of immunoglobulin and hinder intestinal absorption. A study tracking immunoglobulin uptake under the influence of bacterial exposure showed a significant reduction of globulin absorption when administered with added bacteria (James et al., 1981). Colostrum that is pasteurized at 60˚C for 60 minutes may result in a slightly lower total IgG concentration in the colostrum but will contribute to a final outcome of higher serum IgG circulation because of the reduced bacterial interference (Godden et al., 2008). In later studies, pasteurized colostrum resulted in significantly greater serum IgG circulation over fresh raw colostrum which, in turn, resulted in a lower risk of illness and reduced incidences of being treated for scours during the preweaning period (Elizondo-Dalazar et al., 2009; Godden et al., 2012).

Mastitis in the late non-lactating period of dry cows can have a major effect on reducing colostrum volume and IgG mass because of bacterial growth. Large numbers of bacteria in mastitic colostrum inhibits absorption and impairs calf health (Maunsell et al., 1998; Ferdowsi et al., 2010). In a study conducted by Ferdowsi et al. (2010), calf weight gain through the first 30 days of life decreased linearly as colostral somatic cell count increased. On-farm batch pasteurization can be an effective method of significantly decreasing the total bacterial load present in colostrum without decreasing the IgG concentration.

NUTRITION

Colostrum provides a foundation for a calf's future success. However, calf management must continue throughout the preweaning period. Understanding the nutritional requirements of a preweaning calf includes the evaluation of both the liquid feed source and the calf starter composition. Differences in feeding methods can also contribute to meeting specific goals of feed efficiency (**FE**) and calf weight gain. By promoting optimal growth and health throughout the preweaning period, reviews comparing multiple studies have illustrated the link between increased growth rates before weaning and increased milk production in the later lactation (Van Amburgh et al., 2009; Drackley, 2008).

Plane of nutrition

The digestive development of calves follows three main phases: a pre-ruminant phase with only liquid nutrients for the first 2 to 3 weeks of life; a transitional phase with initial fermentation of calf starter and liquid nutrients; and a ruminant phase depending primarily on dietary carbohydrate fermentation to form an energy source. The age range of calves in the transition and ruminant stage relies heavily on diet compositions that can promote or retard rumen development. During the pre-ruminant phase, the esophageal groove allows for a direct pathway to the calf's abomasum through the omasum. The abomasum can use enzymes to break down the milk solids such as protein, lactose and triacylglycerol. With no fermentation, plant products are not as easily degraded and are less bioavailable to the calf. As the calf begins to consume starter, the butyric acid and propionic acid from the fermentation of carbohydrates begins to form volatile fatty acids (**VFA**), which stimulate rumen epithelial papillae growth. The three main VFAs are acetate, propionate, and butyrate, but butyrate is the primary component required for rumen development. As the papillae become functional, the VFAs are absorbed and the pH of the rumen stabilizes in order to host microbial growth (Drackley, 2008).

Calf growth requires an increase of skeletal and muscle systems. Protein is essential to grow bone matrices that collect minerals in order to harden. Energy is essential for tissue growth with additional energy used to form adipose tissue to store reserves. Therefore, energy and protein are the focus of preweaning diets while providing essential vitamins and minerals. Calves fed *ad libitum* liquid feed consume approximately 16 to 20% of their body weight (**BW**) (2 to 2.5% BW as solids DM) with FE rate approaching what lambs and pigs achieve during the preweaning period (Diaz, 2001). However the common practice in the US is to limit feed calves to provide 8 to 10% of their BW as liquid feed in order to promote the consumption of calf starter (Jasper et al., 2002, Drackley, 2008).

A study by Brown et al. (2005b) looked at body growth and carcass composition of preweaned calves fed differing levels of protein and energy. There were no increases in fat deposition in calves, but increasing energy and protein did increase overall BW, wither height (**WH**) and FE (Brown et al., 2005b). However, postweaning, heifers began developing greater amounts of body fat deposition on a high energy and protein diet. This would suggest that greater protein and energy diets during the preweaning period encourage lean growth, whereas weaned heifers readily convert excess energy levels to fat stores (Brown et al., 2005b). To prevent this, in the preweaning diet, the balance of energy to protein must be maintained to prevent excess energy being stored as fat and not used in growth, or excess protein being degraded and excreted in the urine (Drackley, 2011; Hill et al., 2008). Hill et al. (2009) illustrated how using CP at different levels, 27, 25, and 23%, in a 17% fat milk replacer, produced a linear decrease in preweaning ADG and FE as CP concentrations decreased (Hill et al., 2009).

Increased nutrition can also impact a calf's ability to respond to bacterial challenge. Ballou et al. (2012) compared a higher (28% CP and 20% fat) plane of nutrition to a lower (20% CP and 20% fat) plane of nutrition in the preweaning period using milk replacer. When challenged with *E. coli*, calves fed the higher plane of nutrition showed a greater immune response measured by an increase in plasma haptoglobulin concentrations 24 hours after the challenge (Ballou et al., 2012).

A possible negative effect of feeding large amounts of liquid feed is a slump in growth directly after weaning resulting from an abrupt change in the diet. This causes an increase in stress and a decrease in growth (Passille et al., 2011, Sweeney et al., 2010). The weaning slump can be avoided through management programs of gradual weaning, using a step down technique that weans calves in stages. Khan et al. (2007a,b) showed that with feeding large volumes of milk, with gradual increases and decreases in the milk fed to calves, there was a higher intake of calf starter before weaning that caused greater rumen development. As the calf was weaned slowly, the more developed rumen allowed for greater absorption of more limited digestible nutrients compared to the diet of consistently high liquid volume from birth through weaning (Khan et al., 2007a,b). Feeding frequency does not appear to have an impact on calf weight gain shown in a study by Kehoe et al. (2007).

Several studies have shown that postweaning feed consumption and rumen development is influenced more by a gradual weaning method than the liquid feed volume fed during the preweaning period (Khan et al., 2011; Roth et al., 2009). This should be considered in enhanced or accelerated calf feeding programs that require a greater volume of liquid feed. In a study by Terre et al. (2007), calves fed an enhanced calf feeding program without a step down protocol were less able to digest calf starter at weaning when compared to a conventional, restrictive feeding program (Terre et al., 2007). Calves fed greater amounts of liquid feed during the preweaning period are able increase ADG, but in a study performed by Hill et al. (2010) all calves evened out in weight after weaning when fed the same heifer grower.

Whole Milk vs Milk Replacer

The NAHMS 2011 dairy heifer raiser survey reported that 85.9 +/- 3.9% of farms were feeding milk replacer (34.6 +/- 5.4% non-medicated, 62.8 +/- 5.5% medicated), 32.1 +/- 5.3% offered whole milk (23.1 +/- 4.8% pasteurized, 10.3 +/- 3.4% unpasteurized) and 6.4 +/- 2.8% used other liquid feed sources in their preweaning calf feeding programs (NAHMS, 2011). The NAHMS survey agrees with an earlier USDA survey in 2007 which reported 69% of dairy calves were fed milk replacer during the preweaning period in the US (USDA, 2007). With the advancement of technology and the development of on-farm pasteurizers, the 10.3% of producers using unpasteurized milk have the option to process a safer liquid feed source for their preweaning calves. However, the majority of producers are still using milk replacers.

Of the producers feeding milk replacer, a high majority (84.7 +/- 4.7%) reported feeding protein in a range of 20 to 24% and (82.8 +/- 5.0%) feeding fat within the same range of 20 to 24% (NAHMS, 2011). This would indicate that most producers that are using milk replacers are not investing in enhanced or accelerated calf feeding programs that provide protein and fat at levels similar to whole milk. The standard nutrient content of whole milk averages 25.6% CP and 29.6% fat and is commonly accepted at 12.5% DM. Regardless of farm size, 70.4 +/- 5.4% of farms feed 3.8 to 4.75 L/calf/day, 21.1 +/- 4.9% receive 5.7 to 6.65 L/calf/day and 8.5 +/- 3.3% consuming >7.6 L/calf/day (NAHMS, 2011). This reveals that regardless of the size of the farm and region, limit feeding to promote calf starter consumption is still used by the majority of producers.

Shamay (2005) looked at long-term effects of feeding *ad libitum* whole milk or *ad libitum* milk replacer. When fed whole milk, calves exhibited greater body weights at weaning and greater first lactation milk production (Shamay, 2005). Gorka et al. (2011) looked at the development of the small intestine when comparing whole milk to milk replacer. Calves fed whole milk showed greater intestinal development measured through the increase in papillae length and muscle layer thickness (Gorka et al., 2011). Whole milk has also been linked to a higher functioning immune system based on the presence of nonspecific immune factors, such as leukocytes. These immune factors found in whole milk can positively affect overall health (Godden et al., 2005). The benefits of whole milk can also be seen in the improvements of the gastrointestinal health of a scouring calf switching from milk replacer to whole milk. This is due to 4 to 5% of the weight of milk fat coming from butyrate that has a bacteriostatic effect on the upper small intestines (Drackley, 2008). When comparing whole milk and milk replacer for calf mortality, under thermoneutral conditions, there is no difference. However, under cold stress, there is a significantly higher incidence of fatality in calves fed milk replacer when compared to calves fed whole milk at equivalent volumes. This can be most easily explained by the higher level of available energy found in whole milk (Godden et al., 2005).

High quality milk replacers consist mainly of whey protein (dried whey, whey protein concentrate, delactosed whey, sweet and acid whey products) due to market costs of dried skim milk products. Whey protein is less digestible than skim milk, because it does not clot like casein and is not retained in the abomasum the normal 6 hours seen by whole milk. Instead it enters the intestines approximately 3 hours after ingestion which limits the calf's ability to degrade and absorb the protein before excretion. Soy protein has also been substituted for up to 70% of the protein content due to the reduced market price. However, calves were even less able to digest plant based proteins when compared to milk based proteins (Drackley, 2008). Based on this, another alternative to milk proteins is the addition of animal plasma proteins that are highly digestible in young calves, but are less economically efficient as substituting plant based proteins (Drackley, 2008). Milk replacers can also vary in the source of energy, with the energy source coming from fat which can increase body fat deposition when compared to carbohydrate sourced energy (Tikofsky et al., 2001). In varying milk replacers of animal and plant sources of protein and energy, Daniels et al. (2008) reported no differences in blood metabolites or hormone concentrations in young heifers.

Mucin production is essential in protecting the intestinal wall from acidic stomach acids, mechanical damage, bacterial and viral pathogens, and to assist in general movement of particles. The amount of mucin protein produced is directly affected by intake and source of proteins, with plant proteins increasing secretion. Too much secretion can cause an overall loss of protein digestibility through exceedingly rapid movement of digesta through the intestines and can be another contributing factor that influences the limited digestibility seen by plant proteins in the preweaning calf (Montagne et al., 2000). Due to this physiological reaction, animal protein is recommended for optimal absorption to take place in the early preweaning period.

An alternative non-milk sourced animal protein is plasma protein. It has been used to reduce the cost of milk replacers at an inclusion rate of 25% of the CP provided by whey protein (Morrill et al., 1995; Quigley et al., 1996). In 1997 it became illegal to use bovine plasma to feed cattle in order to prevent the spread of bovine spongiform encephalopathy. Therefore, porcine plasma protein became the primary source of plasma protein available for ruminants. However, preservation techniques are important when including animal blood protein in milk replacer. Blood meal is heated to a high temperature, destroying the functioning parts of the protein, which makes it unacceptable to be used in milk replacers. Spray-dried is an acceptable method that can be used to preserve the protein functionality and, in a study developed by Quigley et al. (2003), spray-dried porcine plasma was added to whey protein concentrate milk replacers to reduce morbidity and mortality. This supplementary dose of active protein, which included approximately 20% immunoglobulins, helped protect the calf during times of stress in the preweaning period (Quigley et al., 2003). Spray-dried animal plasma has also been shown to work as an alternative to oral antibiotics in improving attitude, hydration, body weight gain, and reduction of mortality (Quigley et al., 2000b). Other alternatives, such as hydrolyzed spray dried red blood cells have also been shown to effectively replace 43% of the CP provided by whey proteins in calf milk replacers without adversely affecting BW, FE, the intake of calf starter and milk replacer, or fecal scoring (Quigley et al., 2000c).

Economic advantages of feeding pasteurized non-saleable milk versus commercial milk replacer, regarding growth rate, morbidity and mortality rates, showed a benefit of $0.69/calf/day or $34/calf from birth to weaning fed at a rate of 10% BW. It is estimated that as few as 23 calves can justify economic feasibility of the pasteurization equipment when fed pasteurized non-saleable milk. Affordable on-farm pasteurizers make this a viable option for dairy producers to use with proper management (Godden et al., 2005).

Calf Starter

There is debate within the industry about the best roughage source for calves to have during the preweaning period. The rumen pH is less than 6.0 for the first 10 weeks of life and the physical size of the rumen is greatly limited. Therefore, feeding indigestible forages can limit voluntary intake by storing fibrous material in the rumen. However, texture is also needed to prevent abnormal papillae growth (Drackley, 2008). Greater intakes and daily gains are associated with coarse starters versus pelleted mixed rations for calves. Particle shape and size were determined in a study by Porter et al. (2007) to be more important than fiber level to stimulate rumen development. A study by Naeem et al. (2012) stressed the use of high quality, fermentable calf starter as the primary source of rumen development to provide more nutrients to the rumen epithelium.   Greater fiber levels have been shown to decrease incidences of diarrhea and lower fiber levels increase nutrient digestibility. A low fiber level in calf starter with a coarse texture is therefore recommended to promote rumen development while encouraging calf growth (Porter et al., 2007). When feeding a milk replacer with greater amounts of CP, when compared to a conventional replacer containing 20% CP and 20% fat, the calf starter must also have a higher CP. Stamey et al. (2012) recommended a calf starter containing 25.5% CP instead of the conventional 19.6% CP, which would reduce the growth slump seen at weaning when using enhanced or accelerated milk replacers. Forages in a calf starter should consist of highly fermentable grasses or legumes that are high in sugar and low in fiber concentration (Drackely, 2008).

Long-term effects of using enhanced calf feeding

There are many conflicting studies looking at long-term milk production related to preweaning nutrition. In a review, Van Amburgh et al. (2009) reported a range of 453 to over 1,360 kg increase in milk production during the first lactation based on increasing the balanced nutritional profile during the preweaning period. A study conducted by Drackley et al. (2007) comparing an intensified program (28% CP and 20% fat) over conventional milk replacer (22% CP and 20% fat) showed significantly higher milk production during first lactation. A possible explanation of the preweaning nutritional impact on later milk production is a form of epigenetic programming, which is not fully understood. Epigenetic is defined as a long-term effect that is altered by outside forces, such as nutrition. It is thought that this programming can increase milk yield when nutrition is increased during the preweaning period (Soberon et al., 2012). Soberon et al. (2012) proposed an increase in preweaning ADG by 1 kg would result in an increase of 850 to 1,113 kg of milk in the first lactation. The requirements for these calculations included an increase in nutrition beginning immediately after birth, continued for at least 5 weeks and be provided in a liquid feed form. The results shown in this study linked approximately 22% of the variation in the first lactation milk yield to preweaning growth rates (Soberon et al., 2012).

However, other studies have reported no positive long-term results from enhanced calf feeding programs (Moallem et al., 2010; Morrison et al., 2011). Treatments in the study by Terre et al. (2009) compared 12% DM and 18% DM. Long-term effects were evaluated when of increasing milk replacer DM in heifer calves on subsequent milk production and showed no statistical significance when comparing treatments. However, the author did note results approached significance with a large numerical difference of a 625 kg increase in milk production during the adjusted 305 d lactation that could be significant with a larger calf trial of > 60 calves (Terre et al., 2009). In a study comparing conventional milk replacer to intensive milk replacer, the conventional milk replacer contained 20 to 22% CP, 15 to 20% fat at 12.5% DM, while the intensive milk replacer contained 25% CP, 20% fat at 12.5 to 17.5% DM. Results showed no difference in first lactation milk production or time before calving between the treatments (Raeth-Knight et al., 2009). However, it was reported by Davis Rincker et al. (2011), that the greater cost in feed during the preweaning period was offset with reduced medical expenses and resulted in no economical differences. The difficulty of comparing different trials is that there is no set definition of an enhanced calf feeding program. Due to the variation in protocols, as well as dietary composition, there are many conflicting results.

Focusing on the prebubertal, postweaning period, and feeding high energy diets after weaning can cause heifers to reach puberty faster due to fat deposition. This is thought to be caused by the secretion of leptin by adipose tissue that has a direct effect on luteinizing hormone which can impact the onset of puberty. Davis Rinker et al. (2008) showed that fat deposition in the udder can result in decreased mammary cell development leading to lower future milk production potential. It was, therefore, recommended to limit grain fed to prepubertal heifers to <1 kg/d (Davis Rinker et al., 2008). There have also been conflicting data presented concerning the correlation between postweaning, prepubertal weight to first lactation milk yield. However, reduced mammary development was reported in multiple postweaining high energy diets (Van Amburgh et al., 1998; MacDonald et al., 2005). An explanation proposed by Choi et al. (1998) theorized that the excess energy in the prepubertal diet decreased growth hormone concentration and resulted in a less developed udder. It was reported, in a study by Meyer et al. (2006a,b), that with the additional fat deposition in the mammary fat pad seen in postweaning, prepubertal heifers resulted in a decrease in mammary tissue development. However, total DNA in parenchymal tissue was found to be related mostly to age and not diet. This would indicate the different stages of a cow's life postweaning have more impact on the growth of the mammary system through proliferation, accretion, and total population then dietary influence (Meyer et al., 2006a,b).

When long-term milk production is linked to preweaning nutrition it relies on the physiological response to produce allometric mammary growth. Allometric mammary growth naturally occurs at two to three months of age and during pregnancy. It is defined as the mammary system growing at a faster rate than the body (Daniels et al., 2006). In a study by Brown et al. (2005), increasing the energy and protein intake in the preweaning period increased the parenchymal mass and the presence of DNA and RNA without the increase in fat deposition. It was also noted that an increase in proliferation of ductal epithelial cells was linked to the histological development of mammary parenchyma through the lumen area. Preweaned mammary transcriptome, the RNA component of gene expression, is very responsive to enhanced nutrition during the preweaning period and can influence long-term productivity. In a study by Piatoni et al. (2012), possible alternation of mRNA expression in molecular functions and pathways can be accomplished by doubling the intake of milk replacer. However, the results of this study were not linked to positive increases in later milk production (Piatoni et al., 2012). In another study by Daniels et al. (2009a,b), there was no general difference in the degree or pattern of ductal development when comparing conventional and enhanced calf and heifer feeding programs from birth until puberty. On the other hand, while there was no difference in mammary stem cell population or hormones (growth hormone and insulin-like growth factor-I) there was a positive correlation in telomerase production and mammary parenchyma growth (Daniels et al., 2009a,b). In multiple studies, it was noted that only during the preweaning period would nutrient compositions affect mammary system development. After calves were weaned, the mammary cell sensitivity to adjustments based on the diet could not be recovered (Meyer et al., 2006a,b; Brown et al., 2005).

SUMMARY

Future research is needed to determine what is required during the preweaning period in order to optimize future milking herd success. This review covered the importance of colostrum through the discussion of passive immune transfer, colostrum replacers and supplements, as well as handling and storage techniques. Milk and colostrum pasteurization techniques were also evaluated through several research studies. However, the central topic of overall preweaning nutrition covering liquid feeds, calf starter, and long term effects, demonstrates that many research opportunities are still available. To develop a true understanding of how to improve calf nutrition, more studies are necessary to expand the research that has already taken place. The two dairy calf nutrition experiments of the study described in the next chapter were conducted to provide producers with an applied enhanced calf feeding program to optimize the growth and health of their replacement heifer calves.

The following study used milk balancer products, a relatively new development in the industry, as a part of an enhanced calf feeding program. Milk balancers could allow producers to capitalize on the benefits of whole milk as an inexpensive source of high quality nutrition, while also increasing milk DM in an enhanced calf feeding program where the calf gut size may limit milk volume.

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CHAPTER 2

Experiment 1:

Supplementing a Pasteurized Milk Balancer Product to Two Feeding Levels of Pasteurized Whole Milk Fed to Holstein Calves

Experiment 2:

The Effects of Supplementing Two Pasteurized Milk Balancer Products to Pasteurized Whole Milk on the Health and Growth of Dairy Calves

INTRODUCTION

Early management of calf health and growth has a great impact on the overall development of a dairy cow. Methods of improving nutrient absorption and FE, along with decreasing disease risks, allow producers to optimize their calf management system in order to construct their best possible milking herd. Establishing a high average daily gain in the first few weeks of life can institute an earlier breeding schedule based on weight and can allow for greater milk production in the first lactation (Drackley, 2008). Studies have supported this effect through the use of milk replacers in accelerated feeding programs (Soberon et al., 2012), whereas others have focused on differing feeding frequencies and weaning ages (Kehoe et al., 2007). Many techniques and procedures are available for producers to employ, but the goal is always to feed the neonatal calf so that it develops into a productive cow.

Producers have the option of feeding preweaning calves whole milk or milk replacer as a liquid feed source. When using whole milk, producers are limited on the volume a calf can physically consume in an enhanced calf feeding program, as well, an economical constraint based on the cost of salable milk. These restrictions mean producers cannot achieve the benefits available in an adjustable concentrated milk replacer accelerated feeding program. However, with the addition of a milk balancer product to whole milk, an increased amount of milk DM can be consumed by the calf without the limitations presented when feeding fluid milk alone. This supplementation is a technique that can allow producers to increase milk DM in calf management practices using whole milk available as an on farm resource with a product created to increase the plane of nutrition.

Milk replacers and milk balancers can also contain alternative protein products to further reduce costs, by substituting milk proteins for a mixture of soy proteins and other animal proteins. These more economically feasible alternatives can reduce the cost of the initial investment. However, caution has been advised in other studies due to results showing reduced growth rates based on a calf's inability to effectively break down plant protein sources early in life (Drackley, 2008). Alternative animal proteins, such as non-ruminant plasma protein, have been shown to be more effectively digested by the calf, but there are many products available that are less effective (Quigley et al., 2003). The goal of these alternatives is to match the growth rates and efficiency of all milk proteins as much as possible, while reducing the cost of raising replacement heifers in the preweaning period.

A large industry concern when dealing with whole milk revolves around the health of the calf and the possibility of disease transmission via contaminated raw milk. High morbidity and mortality rates in preweaning calves prompt most operational protocols, regarding calf management, to act either in the prevention or treatment of illnesses. The current study drew on the use of on-farm batch pasteurizers to decrease the bacterial load and destroy high risk diseases associated with feeding raw fluid milk, shown as effective by published studies (Godden et al., 2006; Elizondo-Salazer et al., 2010). Pasteurizing whole milk has been proven to be an essential step in protecting the future milking herd by not only giving the calf a higher quality feed, but, also, reducing the incidences of Johne's disease (Stabel, 2001). *Mycobacterium avium* subspecies *paratuberculosis* is most commonly spread through fecal consumption, but viable amounts have also been recorded in colostrum and whole milk from infected dams (Stabel, 2008). Pasteurization of both colostrum and whole milk has been shown to reduce the risk of passing Johne's disease to replacement heifers (Stabel, 2001, Godden et al., 2006).

Providing high quality colostrum is also a fundamental part of calf management that has been well documented based on the necessity of passive immune transfer from dam to calf through IgG proteins (Furman-Fratczak et al., 2011). Studies have ranged from creating management practices (Stewart et al., 2005; Johnson et al., 2007; Trotz-Williams et al., 2008; Godden, 2008; Beam et al., 2009), to instituting quality controls (Nia et al., 2010), and developing alternative cleaning techniques (Mbuthua et al., 1997; Campbell et al., 2007; Godden et al., 2012). The current study was able to provide exclusively pasteurized liquid feed sources to the calves from birth through weaning using a method of pasteurizing colostrum while still maintaining adequate IgG concentrations (Donahue et al., 2012).

The objective of experiment 1 was to determine the effects of supplementing two levels of pasteurized whole milk, divided into two equal feedings daily, with or without a balancer product (Land O'Lakes All-Milk Pasteurized Milk Balancer Product) on the growth and health of dairy calves.

The objective of experiment 2 was to determine the effect of feeding 5.7L of pasteurized whole milk, divided into two equal feedings daily, supplemented with or without an all-milk pasteurized milk balancer (Land O'Lakes All-Milk Pasteurized Milk Balancer Product) or a protein blend pasteurized milk balancer (Land O'Lakes Protein-Blend Pasteurized Milk Balancer Product) on the growth and health of dairy calves.

MAERIALS AND METHODS

Animals and Treatment Assignments (Experiment 1)

Eighty Holstein female dairy calves, 40 calves from the North Carolina Department of Agriculture Piedmont Research Station (**PRS**) and 40 calves from the North Carolina State University Lake Wheeler Road Dairy Educational Unit (**LWD**), were randomly assigned to four treatment groups. Heifer calves were added to the trial as they were born at each location and within every group of four calves the four treatments were represented in a continuous randomized bock design. Twenty heifer calves were assigned to each of the four treatments with 10 coming from PRS and 10 coming from LWD. Calves were raised according to the trial protocol and remained at their respective locations throughout the trial.

At LWD the first calf was born on October 8th, 2011 and the last calf left the trial on November 24th, 2012. At PRS the first calf was born September 29th, 2011 and the last calf left the trial June 12th, 2012.

Treatment one, milk only (**M**), calves were fed 3.8 L of pasteurized whole milk divided into two equal feedings daily until weaning at 56 d of age. This treatment supplied the calf with 0.45 kg of milk DM per day, consisting of only whole milk.

Treatment two, milk plus balancer (**M+B**), calves were fed 3.8 L of pasteurized whole milk with 0.23 kg of supplemented all-milk balancer per 3.8 L. This was divided into two equal feedings daily, 0.11 kg of milk balancer per feeding, until weaning at 56 d of age. This treatment supplied the calf with 0.68 kg of milk DM per day, consisting of whole milk and pasteurized milk balancer.

Treatment three, increased milk (**IM**), calves were fed 3.8 L of pasteurized whole milk divided into two equal feedings daily for the first 14 d after birth; then fed 5.7 L of pasteurized whole milk divided into two equal feedings daily for 35 d (until 49 d of age); and then fed 2.85 L once daily in the morning feedings for 7 d until weaning at 56 d of age. This treatment supplied the calf with 0.45 kg of milk DM per day, consisting of only whole milk, from 1 to 14 d of age, 0.68 kg of milk DM per day from 15 to 49 d of age, and 0.34 kg of milk DM per day from 50 to 56 d of age.

Treatment four, increased milk plus balancer (**IM+B**), calves were fed 3.8 L of pasteurized whole milk, with 0.23 kg of supplemented Land O'Lakes Pasteurized All-Milk Balancer per 3.8 L or 0.11kg per feeding, divided into two equal feedings for the first 14 d after birth; then fed 5.7 L of pasteurized whole milk divided into two equal feedings daily for 35 d (until 49 d of age), with 0.23 kg of supplemented all-milk balancer per 3.8 L or 0.17 kg per feeding; and then fed 2.85 L once daily in the morning feeding for 7 d until weaning at 56 d of age, with 0.23 kg of supplemented all-milk balancer per 3.8 L or 0.17 kg per day. This treatment supplied the calf with 0.68 kg of milk DM per day, consisting of whole milk and pasteurized milk balancer, from 1 to 14 d of age; 1.02 kg of milk DM per day, from 15 to 49 d of age; and 0.51 kg of milk DM per day, from 50 to 56 d of age.

Animals and Treatment Assignments (Experiment 2)

Seventy-two Holstein female dairy calves, 36 calves from PRS and 36 calves from LWD, were randomly assigned to three treatment groups. Heifer calves were added to the trial as they were born at each location and within every group of three calves, the three treatments were represented in a continuous randomized bock design. Twenty-four heifer calves were assigned to each of the three treatments with 12 coming from PRS and 12 coming from LWD. Calves were raised according to the trial protocol and remained at their respective locations throughout the trial.

At LWD the first calf was born on October 20th, 2012 and the last calf left the trial on June 22nd, 2013. At PRS the first calf was born October 1st, 2012 and the last calf left the trial May 15th, 2013.

Treatment one, increased milk (**IM**), calves were fed 3.8 L of pasteurized whole milk divided into two equal feedings daily for the first 14 d after birth; then fed 5.7 L of pasteurized whole milk divided into two equal feedings daily for 35 d (until 49 d of age); and then fed 2.85 L once daily in the morning feedings for 7 d until weaning at 56 d of age. This treatment supplied the calf with 0.45 kg of milk DM per day, consisting of only whole milk, from 1 to 14 d of age, 0.68 kg of milk DM per day from 15 to 49 d of age, and 0.34 kg of milk DM per day from 50 to 56 d of age.

Treatment two, increased milk plus balancer (**IM+B**), calves were fed 3.8 L of pasteurized whole milk, with 0.23 kg of supplemented all-milk balancer per 3.8 L or 0.11 kg per feeding, divided into two equal feedings for the first 14 d after birth and then fed 5.7 L of pasteurized whole milk divided into two equal feedings daily for 42 d (until weaning at 56 d of age), with 0.23 kg of supplemented all-milk balancer per 3.8 L or 0.17 kg per feeding. This treatment supplied the calf with 0.68 kg of milk DM per day, consisting of whole milk and pasteurized milk balancer, from 1 to 14 d of age and 1.02 kg of milk DM per day, from 15 until weaning at 56 d of age.

Treatment three, increased milk plus protein blend balancer (**IM+PB**), calves were fed 3.8 L of pasteurized whole milk, with 0.23 kg of supplemented protein-lend milk balancer per 3.8 L or 0.11 kg per feeding, divided into two equal feedings for the first 14 d after birth and then fed 5.7 L of pasteurized whole milk divided into two equal feedings daily for 42 d (until weaning at 56 d of age), with 0.23 kg of supplemented protein-lend milk balancer per 3.8 L or 0.17 kg per feeding. This treatment supplied the calf with 0.68 kg of milk DM per day, consisting of whole milk and pasteurized milk balancer, from 1 to 14 d of age and 1.02 kg of milk DM per day, from 15 until weaning at 56 d of age.

Nutrition of Neonatal Calves

All calves were removed from their dams immediately after birth to prevent nursing of raw colostrum and moved to individual calf hutches. Calves were then fed 3.8 L of high-quality pasteurized colostrum, tested in the green zone (>50 g of IgG/L of milk at 22˚C) using a colostrometer[8], within 1 to 2 hours after birth with no subsequent colostrum feedings. Colostrum was pasteurized and stored in Dairy Tech (Windsor, CO) "Perfect Udder" bags during the trial. Using a model DT30G DairyTech, Inc.[1] commercial batch pasteurizer, colostrum and whole milk fed to the trial calves was pasteurized by pasteurizing to 60˚ C for 60 minutes. From d 1, all calves were offered a high quality calf starter and free-choice water. The calf starter contained 15% cottonseed hulls, ground corn grain, soybean meal – 48% protein, soy hulls, dried molasses, salt, Rumensin ®, vitamins and minerals. The N.C. State VTM pack included calcium carbonate, manganese, sulfate, zinc sulfate, copper sulfate, ferrous sulfate, sodium selenite, zinc oxide, cobalt sulfate, calcium iodate, vitamin E supplement, vitamin A supplement, vitamin D_3 supplement. Calves were weaned at 56 d of age, but remained in individual calf hutches until 63 d of age after they were given 7 d to adjust to the increase of cottonseed hulls to 30% in their weaned heifer diet. Calves were fed a total mixed ration (TMR) through 180 days of age. The TMR consisted of 30% cottonseed hulls, ground corn grain, soybean meal – 48% protein, salt, Rumensin ®, and minerals. Water was available to calves and heifers at all times.

In experiment 1, on d 63, calves were moved from the hutches and put into group housing within a calf barn for at least one week. The groups of calves were then moved to a pasture that was fenced into sections to separate the different age groups. Groups were not separated based on treatment. At LWD the groups averaged five calves, while at PRS the groups ranged from 11 to 14 calves. The differences in group sizes were due to the limited space and design of the pastures being used at each location.

In both experiments, the pasteurized whole milk fed to the calves consisted of available non-saleable milk with the addition of saleable whole milk collected from the bulk tank in order to match the requirements of the calves. Milk was pasteurized using a Dairy Tech[1] 30G batch pasteurizer. After pasteurization, the milk was cooled to under 24˚C and then stored in industrial 19 L milk bags that were used by the Dairy Processing Plant located in Schaub Hall on North Carolina State University's campus. The bags of milk were then stored under refrigerated conditions of approximately 4˚C between feeding times. At LWD, milk was pasteurized once a day after the scheduled morning milking and the milk was stored for the following evening and morning feedings; at PRS, the staff ran the pasteurizer twice daily and no milk was stored between feedings. The 19 L milk bags were kept at PRS in case of an equipment malfunction, but the bags were not used in the regular feeding procedure.

Pasteurization Equipment Maintenance

The Dairy Tech[1] 30G batch pasteurizer was cleaned after every use using the milk residue cleaner, Diton B[12]. Diton B is used by the Dairy Processing Plant at Schaub Hall to break down milk fat residue and clean surfaces that come in contact with milk products. The chlorinated manual cleaner was mixed, according to instructions labeled on the product, at between 4.4 to 59 mL of cleaner per 3.8 L of hot water between the temperatures of 38˚C to 49˚C. Using cleaning instruments designated for pasteurizer cleaning only, the powder-water mixture was used to scrub the inside metal bowl of the pasteurizer along with the agitator and then the outside surfaces. After thoroughly scrubbing the pasteurizer, in compliance with the stated instructions for the Diton B, hot water was used to rinse the cleaner off the pasteurizer, which was emptied into the floor drainage system.

The temperature of the milk was tested twice monthly during the pasteurization process to ensure consistency and the spigot was disassembled once weekly for additional cleaning. The temperature was taken using a Traceable waterproof thermometer[9] and verified monthly using an Exact temp oven thermometer[10].

These cleaning procedures and products were used at both research facilities.

Calf Feeding Procedures

In experiment 1, at LWD calves were fed at 6:00 h and at 17:00 h; while at PRS the feeding times were at 2:00 h and 13:00 h to coincide with each operations milk schedule. In experiment 2, at LWD calves were fed at 7:00 h and at 18:00 h; while at PRS the feeding times were at 2:00 h and 13:00 h in order to coincide with each operations milking schedule. At LWD, the amount of milk needed was transferred from the stored 19 L milk bags from the industrial refrigerator into the empty pasteurizer. The on-board computer was then programmed to use a profile loaded at the beginning of the trial to gradually reheat the milk to 41˚C for the feedings. The amount of time needed to heat the milk differed by the amount of milk required to feed the calves and was adjusted accordingly throughout the trial so that the milk was not held at a 41°C for longer than fifteen minutes before the assigned feeding time. At PRS, the milk that was in the pasteurizer after pasteurization was fed once it cooled to 41˚C. The milk balancer products recommended a temperature above 38˚C to dissolve the balancer with the milk.

While the milk reheated or pasteurized, according to the location's feeding procedure, feed buckets were sanitized using a zinicin-water mixture as an added cleaning step. The selected buckets where then set out onto a drying rack before the feeding. A measuring pitcher was also sanitized using this method to be used in the feeding process. After the feeding, everything was cleaned with hot water mixed with Dawn dish soap and then rinsed with the zinicin-water mixture.

At the assigned feeding time, once the milk had reached a temperature of 38˚C, milk was poured into the pitcher through a spigot located at the front of the pasteurizer. The measured amount, according to the protocol of the different treatments, was then poured into the bucket and the pasteurized milk balancer was added to each bucket. The all-milk balancer includes ingredients of dried whey, dried whey protein concentrate, dried whey product, dried skimmed milk, dried milk protein, animal fat (preserved with Ethoxyquin), lecithin, polysorbate 80, dicalcium phosphate, calcium carbonate, yeast extract, methionine supplement, l-lysine, calcium pantothenate, biotin, ascorbic acid, pyridoxine hydrochloride, folic acid, choline chloride, roughage products, calcium silicate, zinc methionine complex, manganese methionine complex, copper lysine complex, iron amino acid complex, cobalt sulfate, ethylenediamine dihydroiodide, vitamin A acetate, vitamin D_3 supplement, vitamin E supplement, thiamine mononitrate, riboflavin, niacin supplement, vitamin B_{12} supplement, selenium yeast, and natural and artificial flavor.

The alternative protein-blend milk balancer substituted 30% of the milk protein with a combination of soy protein and non-ruminant plasma protein. It included dried whey, dried whey protein concentrate, dried whey product, dried skimmed milk, dried milk protein, soy isolate, protein modified soy flour, animal fat (preserved with Ethoxyquin), lecithin, polysorbate 80, dicalcium phosphate, calcium carbonate, animal plasma, dried saccharomyces cerevisia fermentation extract, methioinine supplement, l-lysine, vitamin A acetate, vitamin D_3 supplement, vitamin E supplement, thiamine mononitrate, riboflavin, niacin supplement, calcium pantothenate, biotin, ascorbic acid, pyridoxine hydrochloride, folic acid, vitamin B12 supplement, choline chloride, calcium silicate, zinc methionine, manganese methionine, copper lysine, iron amino acid complex, ethylenediamine dihydroiodide, selenium yeast, and natural and artificial flavor.

Sampling and Observation Recording Protocol

Samples of colostrum were taken before and after pasteurization in the Dairy Tech "Perfect Udder" colostrum pasteurization bags. Pre and post samples were evaluated by the University of Saskatchewan, Canada[3], for IgG concentrations by single radial immunodiffusion assay[7]. This was necessary to verify pasteurization did not cause excessive denaturation and destruction of IgG's in amounts that would cause FPT. A standard plate count (**SPC**)[11] measurement was performed on the pre and post-pasteurization milk samples, as well as the colostrum samples by the PRS[4] as a verification of pasteurization. A weekly sample of the whole milk was taken before pasteurization, after pasteurization, and after the last calf was fed, then frozen until later analysis for SPC[11].

To determine the successful transfer of passive immunity in the calf, a jugular blood sample was taken from each calf between 24 and 48 hours after the colostrum was fed. Samples were immediately centrifuged[6] and the blood serum supernatant was extracted and frozen. The University of Saskatchewan, Canada[3], analyzed the serum samples for the IgG content through a single radial immunodiffusion assay[7].

Any milk refusals were weighed and measured daily and calf starter samples were taken weekly, composited by month, and analyzed for nutrient contents. The calf starter offered was recorded daily and feed refusals were recorded weekly. Samples of both were also taken weekly. Dry matter intake, ADG and FE were then calculated based on the collected data. Body weight, HH, HW, and WH of each calf was measured at birth and then every 7 d through 63 d of age using a sliding scale height stick with bubble leveling. After leaving the individual hutches, calves were weighed once a month at approximately 90, 120, 150, and 180 days of age.

While in the individual hutches, calves were monitored daily and scored based on: fecal appearance (1 to 4), respiratory health (1 to 5), and calf attitude (1 to 4). Any medication required or other health issues were recorded. Fecal scores were assigned as follows: 1 was normal, 2 was soft, 3 was runny, and 4 was watery. Respiratory scores were as follows: 1 was normal, 2 was a runny nose, 3 was heavy breathing, 4 was a moist cough, and 5 was a dry cough. Calf attitude scores were given as follows: 1 was normal and alert, 2 was slow to drink her milk or appears mildly depressed, 3 was moderately depressed, requiring encouragement to get up and slow to drink, 4 was severely depressed and an unwillingness to get up or drink milk. Calves were treated with scour boluses and electrolytes when scours was suspected at a fecal score of 4 or multiple scores of 3 according to the visual guidelines listed in the protocol regardless of illness verification. In cases of illness, PRS and LWD used a combination of oral oxytetracycline tablets (scour boluses) and Hydralyte[12], composed of dextrose, sodium acetate, potassium chloride, glycine, sodium citrate, and sodium chloride.

During the summer months at LWD the water buckets in the hutches were removed for 30 minutes directly after the milk feedings to prevent nutrient loss associated with greater water intake. This did not happen at the PRS location because the trial was completed before the summer months.

Milk samples from LWD were frozen, and then transported to PRS labs for analysis and PRS milk samples were frozen before testing to ensure similar treatment was given to all milk samples regardless of location. Calf starter samples of feed offered and feed refused were composited by month and analyzed by Cumberland Valley Analytical Services (Maugansville, MD)[5] for nutrient content at the end of the trial cycle.

In experiment 1, calves were followed through 6 months of age. The replacement heifers were weighed once a month and frame measurements were taken. The grower was also sampled once a week and composited by month for nutrient analysis.

Statistical Design (Experiment 1)

A randomized complete block design was used, where calves were assigned to a treatment at birth. The recorded data was analyzed in SAS ® as follows: the ADG values were calculated using PROC REG; ADG, FE, starter DMI, total DMI, and health scores used PROC GLM; BW, WH, HH, and HW used PROC MIXED for repeated measures.

Average daily gain was calculated by determining the growth regression curve for each calf and using the ADG slopes to compare treatments. Feed efficiency was calculated using total preweaning gain per unit of total DMI including milk DM standard (12.5% DM) and starter DM calculated weekly. Starter DMI and total DMI were calculated by subtracting feed refusal weight from feed offered weight with the milk DM added to total DMI. Health scores were divided between scores of 0 to 2 and 3 to 5. The number of days an animal scored greater than or equal to 3 for fecal, respiratory of attitude scores was averaged and compared between treatments.

Growth data was analyzed in PROC MIXED for the repeated measures over time. Treatment and time were fixed variables and calf was considered a random variable to allow for inference to the population. Separate analyses were conducted for BW, WH, HH, and HW. The fixed effects included the mean which represents the treatment at week and interactions between the treatments and time. The random effects are the calves of each treatment and the location. Statistical significance was determined at $P \leq 0.05$.

Other data were calculated using whole values with no comparisons: milk quality samples, SPC and IgG concentration, were averaged logarithmically with a standard deviation and general range; serum protein and IgG levels were recorded as mean values with a standard deviation and general range.

Statistical Design (Experiment 2)

A randomized complete block design was used where calves were assigned to treatment at birth. The recorded data was analyzed in SAS ® as follows: the ADG were calculated using PROC REG; ADG and health scores used PROC GLM; weekly FE, starter DMI and total DMI used PROC MIXED; BW, WH, HH, and HW used PROC MIXED for repeated measures. A contrast statement was used to compare unsupplemented treatment, M, versus the average of the supplemented treatments, M+B and M+PB, and between the supplemented treatments, M+B and M+PB.

Average daily gain was calculated by determining the growth regression curve for each calf and using the ADG slopes to compare treatments. Feed efficiency was calculated using total preweaning gain per unit of total DMI including milk DM standard for the treatment and starter DM calculated weekly. Starter DMI and total DMI were calculated by subtracting feed refusal weight from feed offered weight with the milk DM added to total DMI. Health scores were divided between scores of 0 to 2 and 3 to 5. The number of days an animal scored greater than or equal to 3 for fecal, respiratory of attitude scores was averaged and compared between treatments.

Growth data used PROC MIXED, for the repeated measures over time. Treatment and time were fixed variables and calf was considered a random variable to allow for inference to the population. Separate analysis were conducted for BW, WH, HH, and HW. The fixed effects included the mean which represents the treatment at week and interactions between the treatments and time. The random effects are the calves of each treatment and the location. Statistical significance was determined at $P \leq 0.05$.

Other data was calculated using whole values with no comparisons: milk quality, SPC and IgG concentration, samples were averaged logarithmically with a standard deviation and general range; serum protein and IgG levels were recorded as mean values with a standard deviation and general range.

RESULTS AND DISCUSSION

Verification of Methods

Transfer of passive immunity

Failure of passive transfer of immunity was determined by a serum IgG level of <10 mg/mL or serum protein level of <5 g/dL. In experiment 1, shown in Table 1, the mean values found in the serum protein were 6.0 ± 0.6 g/dL (ranging 4.8 to 8.6) and serum IgG of 25.3 ± 9.1 g/L (ranging 2.5 to 63.0), which are well above the minimum passive transfer requirements. In experiment 1, 98% of calves received successful immune transfer In experiment 2, shown in Table 1, the mean values found in the serum protein were 6.1 ± 0.7 g/dL (ranging 4.4 to 7.9) and serum IgG of 26.0 ± 11.8 g/L (ranging 6.2 to 59.4), which are well above the passive transfer requirements. In experiment 2, 95% of calves received successful immune transfer. All calves remained in the trial until completion. These results indicate that the pasteurization technique developed by Godden et al., 2008, pasteurizing colostrum at 60°C for 60 minutes did not prevent the calves from receiving successful passive immune transfer.

Table 1. Experiment 1 and 2 Passive Immune Transfer

	Mean	SD	Min	Max
Experiment 1 (n=80)	98% of calves received successful passive immune transfer			
Serum Protein[1] (g/dL)	6.1	0.6	4.8	8.6
Serum IgG[2] (g/L)	25.2	9.1	2.5	63.0
Experiment 2 (n=72)	95% of calves received successful passive immune transfer			
Serum Protein[1] (g/dL)	6.1	0.7	4.4	7.9
Serum IgG[2] (g/L)	26.0	11.8	6.2	59.4

[1]FPT <5 g/dL

[2]FPT <10 mg/mL

Verification of pasteurization

In experiment 1, the bacterial load of the whole milk and colostrum was analyzed as SPCount, $\log_{10}$ (cfu/mL) shown in Table 2. There was a multiple log reduction, from 4.9 ± 0.7 (ranging 3.3 to 6.6) to 1.7 ± 0.7 (ranging 0 to 3.3), between the mean values of pre and post pasteurization milk samples respectively. In experiment 2, shown in Table 2, there was a multiple log reduction, from 5.0 ± 1.2 (ranging 3 to 7.2) to 1.9 ± 0.6 (ranging 1 to 3.3), between the mean values of pre and post pasteurization milk samples respectively. Less than 3.5, or 5,000 colony forming units, is considered successfully pasteurized (Elizondo-Salazar et al., 2010). In both experiments the 'after last calf fed' samples were variable due to the amount of time it took to feed the different number of calves at any given period throughout the trial. Other studies have shown significant regrowth for after last calf fed samples, because pasteurization techniques can only be effective when coupled with good management techniques (Elizondo-Salazar et al., 2010). Minimizing the time the pasteurized milk stands at feeding temperatures of 40˚C will limit the opportunity for bacterial regrowth.

Table 2. Experiment 1 Whole milk and colostrum pasteurization verification

	Standard Plate Count, $\log_{10}$ (cfu/mL)[1]			
	Mean	SD	Min	Max
Experiment 1 Milk				
Pre-Pasteurization	4.9	0.7	3.3	6.6
Post-Pasteurization	1.7	0.7	0	3.3
After Last Calf Fed	2.0	0.6	1	3.5
Experiment 1 Colostrum				
Pre-Pasteurization	5.6	1.2	3	7.8
Post-Pasteurization	2.1	0.6	1.	4.4
Experiment 2 Milk				
Pre-Pasteurization	5.0	1.2	3	7.2
Post-Pasteurization	1.9	0.6	1	3.3
After Last Calf Fed	2.4	0.6	1	4
Experiment 2 Colostrum				
Pre-Pasteurization	6.1	0.8	4.5	7.8
Post-Pasteurization	2.3	0.7	1.3	4

[1]Log 10^X = X

Growth data (Experiment 1)

Average BW, HH, HW, and WH, ADG, starter DMI and FE are shown in Table 3. Calves fed M, M+B, and IM were not significantly different from one another in regards to body weight. Calves fed IM+B were significantly (P < 0.05) greater in average BW (58.8 kg) when compared to calves receiving M, M+B, and IM. It was interesting to note that when comparing treatment M versus M+B and IM with approximately 30% liquid feed DM increase there was no change in BW or ADG. However, when comparing the approximately 45% increase in milk DM from treatments M+B and IM versus IM+B there is a significant difference in both BW and ADG. This may lead to a proposed threshold in liquid DM that must be reached to encourage greater growth rates. However, there was not enough increase in milk DM to determine this effect. There were no significant differences in the overall HH (P < 0.6), HW (P < 0.3) or WH (P < 0.4) between treatments. This indicates no change in skeletal structure between treatments and so the greater average body weights in calves fed IM+B can indicate addition muscle and fat production. Postweaning, the addition of fat through high energy diets has been linked to earlier puberty. However, during the preweaning period, mammary system development can be impacted by increasing the energy in the diet and can cause an increase in mammary cell proliferation (Davis Rincker et al., 2008; Meyer et al., 2006). To link this to future milk production, further observations must be collected once the calves have reached the milking herd. As expected, calves receiving IM+B with the greater average BW also showed significantly greater ADG (P < 0.01). Calves receiving IM+B showed an ADG of 0.84 kg/d and calves receiving M averaged only 0.64 kg/d. It was also interesting to note that while calves fed M consumed significantly more calf starter, 31.6 kg ($P < 0.02$), than the other treatments, the calves receiving 0.68 kg and 1.02 kg of milk DM for the majority of the preweaning period consumed similar amounts of calf starter regardless of the 45% increase of milk DM from M+B and IM to IM+B. It could be concluded that treatment M (0.45 kg milk DM), similar to limit-feeding techniques, encouraged greater calf starter intake in response to receiving less milk DM. Another notable result was that calves fed M and M+B, were significantly ($P < 0.01$) less FE then calves fed IM and IM+B. This would lead to the conclusion that FE is more closely linked to liquid feed volume than milk DM. In other studies it has been shown that greater volumes of liquid feed offered to calves during the preweaning period can allow calves to reach FE levels of lambs and piglets. (Diaz 2001)

Table 3. Experiment 1 Growth data for preweaning period

	Treatment					
	M	M+B	IM	IM+B	SEM	*P* ≤
BW[1], kg	51.5[a]	54.0[ab]	52.9[ab]	58.8[c]	1.1	0.045
HH[1], cm	84.1	84.2	84.1	85.1	1.3	0.6
HW[1], cm	18.4	18.4	18.4	19.0	0.6	0.3
WH[1], cm	79.8	80.0	80.0	81.0	1.2	0.4
ADG, kg	0.64[a]	0.69[ab]	0.72[b]	0.84[c]	0.02	0.01
Starter DMI, kg	31.6[a]	24.4[b]	23.1[b]	19.1[b]	2.2	0.01
Total DMI, kg	56.9[ab]	62.4[bc]	55.6[a]	67.9[c]	4.9	0.01
FE, G:F	0.62[a]	0.63[a]	0.70[b]	0.68[b]	0.02	0.01

[a-c]Means with differing superscripts ($P < 0.05$)

[1]The average weight and frame measurements were calculated from birth until weaning

[2]Daily M: 0.45kg milk DM (d 1-56)

[3]Daily M+B: [0.45kg milk DM] + 0.23kg balancer per 0.45kg milk DM (d 1-56)

[4]Daily IM: 0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)

[5]Daily IM+B: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg balancer per 0.45kg milk DM (d 1-56)

Growth data (Experiment 2)

Average BW, HH, HW, and WH, average starter DMI, average total DMI, ADG, and FE are shown in Table 4. Calves fed IM+B and IM+PB were significantly ($P \leq 0.05$) greater in average BW (56.7 kg and 58.19 kg) when compared to calves receiving IM (53.8 kg). Calves receiving a supplemental balancer product also had greater average HW ($P \leq 0.04$) and WH ($P \leq 0.04$). The greater average BW of calves receiving IM+B when compared to calves receiving IM was consistent with the results from experiment 1. It was interesting to note that calves receiving IM+B were not significant in the overall average BW or frame measurements when compared to calves receiving equal amounts of the alternative balancer in treatment IM+PB. According to these results, it would seem that calves were able to utilize the alternative proteins in the protein blend milk balancer as well as the all-milk balancer. Calves receiving IM did consume more calf starter DM ($P \leq 0.01$); however, when calculating total DMI, calves receiving a supplemental balancer, IM+B or IM+PB, consumed more total DM ($P \leq 0.01$). Feed efficiency was not different between treatments which was expected from the results of FE in experiment 1 were FE was linked to milk volume rather than milk DM. Treatments IM, IM+B, and IM+PB, included the same volume of whole milk and only differed by supplemental milk DM. As shown in experiment 1, ADG was increased by calves receiving a balancer ($P \leq 0.01$) with no difference found between balancers ($P \leq 0.3$). If growth rates can be matched with a less expensive protein alternative product added to a low cost, on-farm, whole milk product this may give producers another option to enhance their calf feeding programs. To link this to future milk production further observations must be collected once the calves have reached the milking herd.

Table 4. Experiment 2 Growth data for preweaning period

	Treatment				IM vs ½(IM+B+IM+PB)[5]	IM+B vs IM+PB[5]
	IM[2]	IM+B[3]	IM+PB[4]	SEM	$P \leq$	$P \leq$
BW[1], kg	53.8[a]	56.7[b]	58.19[b]	1.0	0.01	0.3
HH[1], cm	85.0	85.5	86.3	0.7	0.2	0.3
HW[1], cm	18.8[a]	19.0[ab]	19.4[b]	0.2	0.04	0.1
WH[1], cm	80.4[a]	81.1[ab]	82.1[b]	0.7	0.04	0.1
Avg Starter DMI/wk, kg	2.40[b]	1.46[a]	1.51[a]	0.2	0.01	0.8
Avg Total DMI/wk, kg	6.77[a]	8.01[b]	8.06[b]	0.2	0.01	0.8
ADG, kg	0.70[a]	0.80[b]	0.77[b]	0.03	0.01	0.3
FE, total gain/total DMI	0.75	0.69	0.64	0.04	0.09	0.4

[1]The average weight and frame measurements were calculated from birth until weaning

[2]Daily IM: 0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)

[3]Daily IM+B: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg all milk balancer per 0.45kg milk DM (d 1-56)

[4]Daily IM+PB: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg protein blend balancer per 0.45kg milk DM (d 1-56)

[5]Contract between M and the average of MB and MPB; between MB and MPB

Health visual scoring (Experiment 1)

Evaluation of health scores, Table 5, showed fecal scores were significantly higher for calves fed IM+B, on average 3.2 days scoring greater than or equal to 3, during the 56 day preweaning period. The other scores for respiratory and attitude showed no significant differences between treatments with an average of less than 0.5 days scoring greater than or equal to 3. Even though calves fed IM+B had a greater number of days scoring greater than or equal to 3 for fecal, other studies have shown that increasing milk DM can result in looser stool. In a study conducted by Jenny et al. (1978), the incidences of scouring in liquid fed calves was directly related to DM concentration, with an increase in liquid feed or a decrease in calf starter resulting in an increase of calf scouring. With the other scores for respiratory and attitude showing no significant difference, it was not believed the higher value resulted exclusively from illness.

Table 5. Experiment 1 Health score analyses

Average number of days scoring ≥ 3	Treatment					
	M[4]	M+B[5]	IM[6]	IM+B[7]	SEM	$P \leq$
Fecal[1], d	0.9^{a}	2.0^{b}	1.4^{ab}	3.2^{c}	0.38	0.01
Respiratory[2], d	0	0.3	0.1	0	0.1	0.3
Attitude[3], d	0	0.3	0.1	0.2	0.1	0.2

[a-c] Means with differing superscripts ($P \leq 0.05$)

[1] 1=normal, 2=soft, 3=runny, 4=watery

[2] 1=normal, 2=runny nose, 3=heavy breathing, 4=moist cough, 5=dry cough

[3] 1=normal, alert; 2=slow to drink or appear mildly depressed; 3=mildly depressed, slow to get up and drink; 4=severely depressed, does not get up or drink

[4] Daily M: 0.45kg milk DM (d 1-56)

[5] Daily M+B: [0.45kg milk DM] + 0.23kg balancer per 0.45kg milk DM (d 1-56)

[6] Daily IM: 0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)

[7] Daily IM+B: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg balancer per 0.45kg milk DM (d 1-56)

Health visual scoring (Experiment 2)

Evaluation of health scores, Table 6, showed fecal scores were significantly higher for calves fed a supplemental milk balancer ($P \leq 0.05$). The other scores for respiratory and attitude showed no significant differences between treatments. From the results of experiment 1, looser stool was thought to be the result of greater amounts of milk DM fed in the supplemented calves fed 5.7 L of whole milk (Jenny et al., 1978). With the other scores for respiratory and attitude showing no significant difference, it was not believed the higher value resulted exclusively from illness. The health scores did not differ between the calves receiving IM+B versus IM+PB. It can be concluded that substituting the protein-blend milk balancer for the all-milk balancer will not negatively impact calf health.

Table 6. Experiment 2 Health score analyses

Average number of days scoring ≥ 3	Treatments				IM vs ½(IM+B + IM+PB)[7]	IM+B vs IM+PB[7]
	IM[4]	IM+B[5]	IM+PB[6]	SEM	*P* ≤	*P* ≤
Fecal[1], d	1.9	4.9	5.5	1.2	0.02	0.7
Respiratory[2], d	0	0	0	0	0.2	1.0
Attitude[3], d	0.2	0	0.1	0.1	0.3	0.2

[1]1=normal, 2=soft, 3=runny, 4=watery

[2]1=normal, 2=runny nose, 3=heavy breathing, 4=moist cough, 5=dry cough

[3]1=normal, alert; 2=slow to drink or appear mildly depressed; 3=mildly depressed, slow to get up and drink; 4=severely depressed, does not get up or drink

[4]Daily IM: 0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)

[5]Daily IM+B: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg all milk balancer per 0.45kg milk DM (d 1-56)

[6]Daily IM+PB: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg protein blend balancer per 0.45kg milk DM (d 1-56)

[7]Contrast between IM and the average of IM+B and IM+PB; between IM+B and IM+PB

Conclusion

In experiment 1, the findings demonstrate an option for producers to increase ADG, BW and FE in their preweaned calves through the use of whole milk and milk balancer products. In experiment 2, the findings demonstrate the new opportunity producers have to increase ADG, BW and FE in their preweaned calves through the use of whole milk and milk balancer products with alternative protein sources to decrease initial investment costs. The effectiveness of pasteurizing both colostrum and whole milk supported past studies and as an option to decrease disease transfer and bacterial contamination. However, the importance of investing in the preweaning period must be illustrated in future production and so further data is needed once the calves have entered the milking herd to determine the effects of neonatal nutrition on milk yield and composition.

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APPENDIX

APPENDIX A

Table 1. Experiment 1 and 2 All-Milk Balancer[1] nutrient analysis (AF)

Crude Protein, not less than	25.0%
Crude Fat, not less than	10.0%
Crude Fiber, not more than	0.15%
Calcium (Ca), not less than	0.70%
Calcium (Ca), not more than	1.25%
Sodium (Na), not more than	1.50%
Phosphorus (P), not less than	0.70%
Vitamin A, not less than	66,371 IU/kg
Vitamin D3, not less than	16,592 IU/kg
Vitamin E, not less than	331 IU/kg

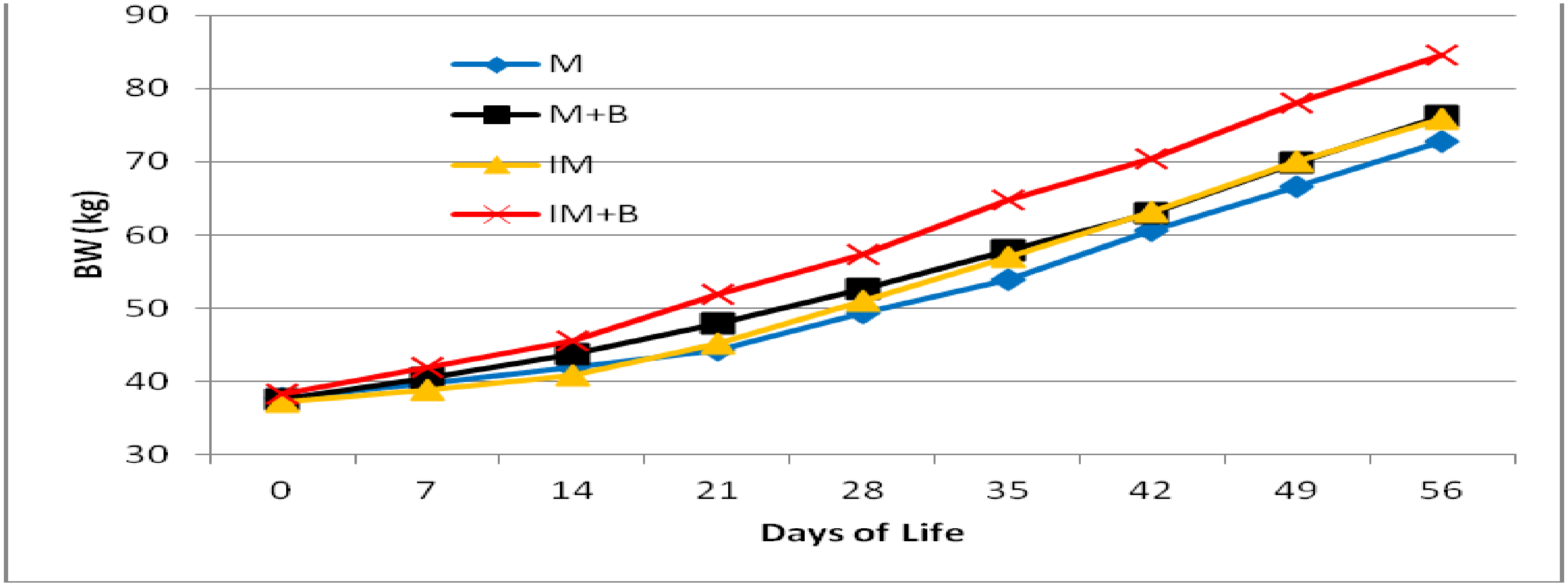
[1]dried whey, dried whey protein concentrate, dried whey product, dried skimmed milk, dried milk protein, animal fat (preserved with Ethoxyquin), lecithin, polysorbate 80, dicalcium phosphate, calcium carbonate, yeast extract, methionine supplement, l-lysine, vitamin A acetate, vitamin D3 supplement, vitamin E supplement, thiamine mononitrate, riboflavin, niacin supplement, calcium pantothenate, biotin, ascorbic acid, pyridoxine hydrochloride, folic acid, vitamin B12 supplement, choline chloride, roughage products, calcium silicate, zinc methionine complex, manganese methionine complex, copper lysine complex, iron amino acid complex, cobalt sulfate, ethylenediamine dihydroiodide, selenium yeast, and natural and artificial flavor.

Table 2. Experiment 2 Protein-Blend Milk Balancer[1] nutrient analysis (AF)

Crude Protein, not less than	25.0%
Crude Fat, not less than	10.0%
Crude Fiber, not more than	0.25%
Calcium (Ca), not less than	0.70%
Calcium (Ca), not more than	1.25%
Sodium (Na), not more than	1.50%
Phosphorus (P), not less than	0.70%
Vitamin A, not less than	66,371.68 IU/kg
Vitamin D3, not less than	16,592.92 IU/kg
Vitamin E, not less than	331.86 IU/kg

[1] dried whey, dried whey protein concentrate, dried whey product, dried skimmed milk, dried milk protein, soy isolate, protein modified soy flour, animal fat (preserved with Ethoxyquin), lecithin, polysorbate 80, dicalcium phosphate, calcium carbonate, animal plasma, dried saccharomyces cerevisia fermentation extract, methioinine supplement, l-lysine, vitamin A acetate, vitamin D3 supplement, vitamin E supplement, thiamine mononitrate, riboflavin, niacin supplement, calcium pantothenate, biotin, ascorbic acid, pyridoxine hydrochloride, folic acid, vitamin B12 supplement, choline chloride, calcium silicate, zinc methionine, manganese methionine, copper lysine, iron amino acid complex, ethylenediamine dihydroiodide, selenium yeast, and natural and artificial flavor.

Figure 1. Experiment 1 BW through the preweaning period in days



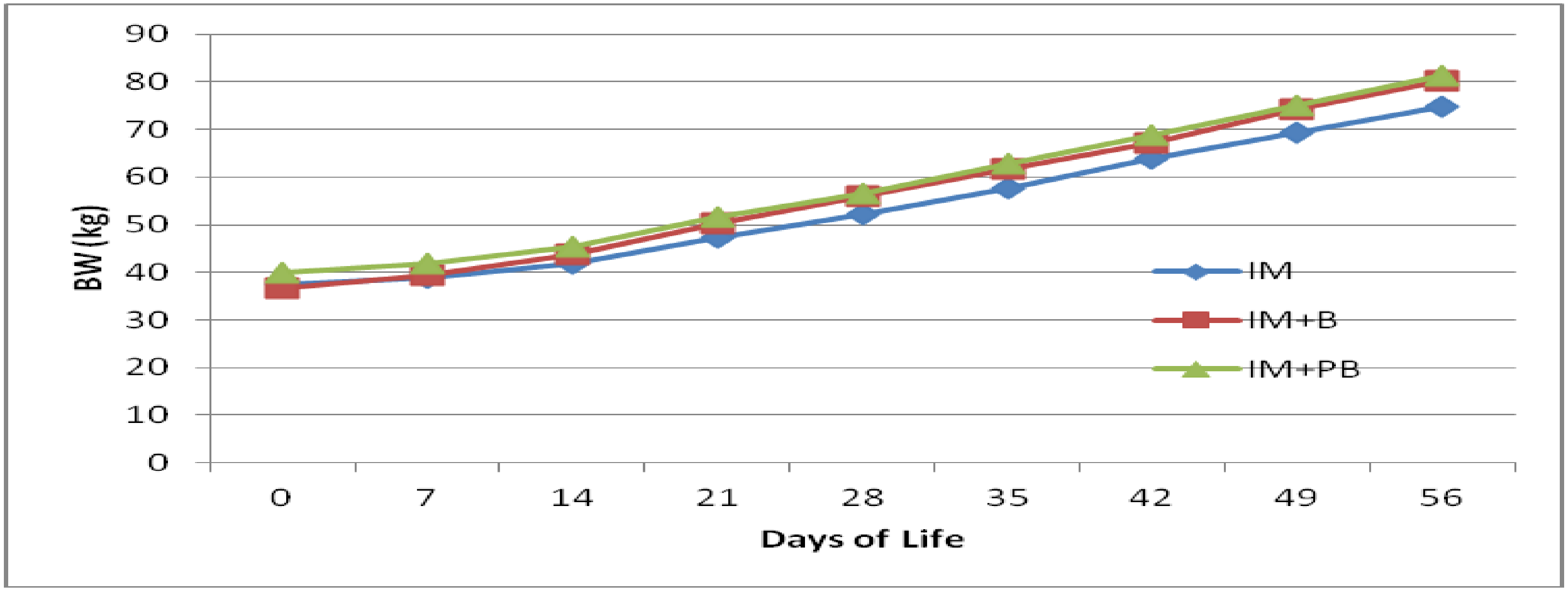
Daily M: 0.45kg milk DM (d 1-56)

Daily M+B: [0.45kg milk DM] + 0.23kg balancer per 0.45kg milk DM (d 1-56)

Daily IM: 0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)

Daily IM+B: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg balancer per 0.45kg milk DM (d 1-56)

Figure 2. Experiment 2 BW through the preweaning period in weeks



Daily IM: 0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)

Daily IM+B: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg all milk balancer per 0.45kg milk DM (d 1-56)

Daily IM+PB: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg protein blend balancer per 0.45kg milk DM (d 1-56)

Table 3. Experiment 1 Growth data preweaning period final day

	Treatment					
	M[2]	M+B[3]	IM[4]	IM+B[5]	SEM	$P \leq$
BW[1], kg	72.1^{a}	75.7^{ab}	75.4^{ab}	84.0^{c}	1.5	<0.0001
HH[1], cm	90.6	91.1	91.4	92.8	2.1	0.05
HW[1], cm	20.7^{a}	21.1^{ab}	21.0^{ab}	21.9^{c}	0.9	0.0017
WH[1], cm	86.0^{a}	86.7^{ab}	86.6^{ab}	88.5^{bc}	1.8	0.0043

$^{a\text{-}c}$Means with differing superscripts (*P* < 0.05)

[1]The average weight and frame measurements were based on final day measurements at weaning (56 d)

[2]Daily M: 0.45kg milk DM (d 1-56)

[3]Daily M+B: [0.45kg milk DM] + 0.23kg balancer per 0.45kg milk DM (d 1-56)

[4]Daily IM: 0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)

[5]Daily IM+B: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg balancer per 0.45kg milk DM (d 1-56)

Table 4. Experiment 1 Growth data postweaning period

	Treatment					
	M[2]	M+B[3]	IM[4]	IM+B[5]	SEM	*P* ≤
BW[1], kg	147.49	148.78	151.21	155.57	4.10	0.37
HH[1], cm	104.65	103.53	105.41	105.92	0.99	0.32
HW[1], cm	27.53	27.25	27.58	28.02	0.46	0.39
WH[1], cm	99.26	98.93	99.75	100.61	1.07	0.36
ADG, kg	1.21	1.19	1.23	1.17	0.04	0.47

[a-c]Means with differing superscripts ($P < 0.05$)

[1]The average weight and frame measurements were calculated from 56 d until 180 d of age

[2]Daily M: 0.45kg milk DM (d 1-56)

[3]Daily M+B: [0.45kg milk DM] + 0.23kg balancer per 0.45kg milk DM (d 1-56)

[4]Daily IM: 0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)

[5]Daily IM+B: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg balancer per 0.45kg milk DM (d 1-56)

Table 5. Experiment 1 Growth data postweaning period final day

	Treatment					
	M[2]	M+B[3]	IM[4]	IM+B[5]	SEM	*P* ≤
BW[1], kg	217.1	217.3	223.1	223.3	5.2	0.5
HH[1], cm	116.8	116.2	117.4	117.3	1.3	0.6
HW[1], cm	33.2	33.1	33.6	33.4	0.5	0.8
WH[1], cm	110.9	110.2	110.8	111.3	1.3	0.7

[a-c]Means with differing superscripts ($P < 0.05$)

[1]The average weight and frame measurements were based on final day measurements at 6 months (180 d)

[2]Daily M: 0.45kg milk DM (d 1-56)

[3]Daily M+B: [0.45kg milk DM] + 0.23kg balancer per 0.45kg milk DM (d 1-56)

[4]Daily IM: 0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)

[5]Daily IM+B: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg balancer per 0.45kg milk DM (d 1-56)

Table 6. Experiment 1 and 2 nutrient analysis average of preweaning calf starter

	Experiment 1	Experiment 2
DM %	88.1 ± 1.0	88.3 ± 1.1
CP, %DM	22.5 ± 1.9	20.9 ± 2.2
NDF, %DM	20.1 ± 2.7	25.5 ± 5.4
ADF, %DM	14.9 ± 2.6	17.4 ± 4.6
NE_L, Mcal/kg	0.77 ± 0.01	0.8 ± 0.04
Ca, %DM	0.84 ± 0.17	0.7 ± 0.10
P, %DM	0.54 ± 0.04	0.5 ± 0.04

Table 7. Experiment 1 Whole milk pasteurization verification per location

	Standard Plate Count, log_{10}[1] (cfu/mL)							
	Lake Wheeler Dairy				Piedmont Research Station			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Pre-Pasteurization	4.6	0.73	3.3	6.6	5.4	0.33	4.9	6.0
Post-Pasteurization	1.8	0.64	0.7	3.3	1.6	0.77	0.0	3.2
After last calf fed	1.88	0.60	1	3.3	2.3	0.62	1.2	3.5

[1]Log 10^X = X

Table 8. Experiment 1 Whole milk pasteurization verification comparison

	Standard Plate Count, $\log_{10}^{1}$ (cfu/mL)				
	Pre-Pasteurization	Post-Pasteurization	After Last Calf Fed	SEM	*P* ≤
Bacterial contamination	4.92^{a}	1.78^{b}	2.07^{b}	0.10	0.01

[1]Log 10^{X} = X

[a-b]Means with differing superscripts ($P < 0.05$)

Table 9. Experiment 1 Colostrum pasteurization verification separated by location

	Standard Plate Count, $\log_{10}{}^{1}$ (cfu/mL)							
	Lake Wheeler Dairy				Piedmont Research Station			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Pre-Pasteurization	5.0	1.3	3	7.7	6.1	0.8	4.5	7.8
Post-Pasteurization	2.1	0.5	1	4.1	2.1	0.7	1.3	4.4

[1]Log 10^X = X

Table 10. Experiment 1 Colostrum IgG concentration before and after pasteurization at both locations

	IgG concentration g/L			
	Mean[1]	SD	Min	Max
Pre-Pasteurization	87.5	28.2	34.4	153.7
Post-Pasteurization	72.7	26.8	19.3	147.5

[1]High quality colostrum: IgG concentration > 50 g/L

Table 11. Experiment 1 Passive Immune Transfer separated by location

	Serum Protein / IgG							
	Lake Wheeler Dairy				Piedmont Research Station			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Serum Protein[1] (g/dL)	6.0	0.7	4.8	8.6	6.2	0.6	5.3	8.0
IgG[2] (g/L)	25.3	10.3	2.5	63.0	25.1	7.9	11.6	53.5

[1]FPT < 5 g/dL

[2]FPT < 10 mg/mL

Table 12: Experiment 2 FE data by week (kg)

FE Tot; G:F	Treatment				
Week	IM[1]	IM+B[2]	IM+PB[3]	SEM	*P* ≤
1	.81	.57	.40	.26	.54
2	.77	.80	.69	.10	.70
3	.88	.84	.80	.07	.74
4	.73	.73	.63	.06	.49
5	.77	.67	.75	.06	.46
6	.81	.62	.65	.07	.12
7	.65	.73	.66	.06	.60
8	.58	.56	.57	.06	.96

[1]Daily IM: 0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)

[2]Daily IM+B: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg all milk balancer per 0.45kg milk DM (d 1-56)

[3]Daily IM+PB: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg protein blend balancer per 0.45kg milk DM (d 1-56)

Table 13. Experiment 2 Starter DMI by week (kg)

Starter DMI	Treatment				
Week	IM[1]	IM+B[2]	IM+PB[3]	SEM	*P* ≤
1	.23	.13	.13	.03	0.4
2	.80	.42	.44	.11	.01
3	1.41	.65	.60	.17	.01
4	1.83	.88	0.74	.22	.01
5	2.60	1.37	1.16	.30	.01
6	3.26	1.76	2.12	.36	.01
7	3.88	2.63	2.94	.45	.01
8	5.21	3.83	3.94	.61	.01

[1]Daily IM: 0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)

[2]Daily IM+B: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg all milk balancer per 0.45kg milk DM (d 1-56)

[3]Daily IM+PB: [0.45kg milk DM (d 1-14); 0.68kg milk DM (d 15-49); 0.34kg milk DM (50-56)] + 0.23kg protein blend balancer per 0.45kg milk DM (d 1-56)

Table 14. Experiment 2 Passive Immune transfer separated by location

	Serum Protein / IgG							
	Lake Wheeler Dairy				Piedmont Research Station			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Serum Protein[1] (g/dL)	6.4	0.6	4.8	9.2	5.8	0.7	4.4	7.7
IgG[2] (g/L)	31.0	10.2	9.2	59.4	20.8	11.2	6.2	56.6

[1]FPT < 5 g/dL

[2]FPT < 10 mg/mL

Table 15. Experiment 2 Whole milk pasteurization verification separated by location

	Standard Plate Count, $\log_{10}$[1] (cfu/mL)							
	Lake Wheeler Dairy				Piedmont Research Station			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Pre-Pasteurization	4.2	0.6	3	5.4	6.1	0.8	4	7.2
Post-Pasteurization	2.1	0.6	1.2	3.3	1.6	0.4	1	2.7
After last calf fed	2.4	0.5	1.3	3.5	2.4	0.8	1	4

[1]$\text{Log } 10^X = X$

Table 16. Experiment 2 Colostrum pasteurization verification separated by location

	Standard Plate Count, $\log_{10}{}^{1}$ (cfu/mL)							
	Lake Wheeler Dairy				Piedmont Research Station			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Pre-Pasteurization	5.9	0.4	5.2	6.5	6.2	0.9	4.5	7.8
Post-Pasteurization	2.7	0.5	1.9	3.6	2.1	0.6	1.3	4

[1]Log 10^{X} = X

Table 17. Experiment 2 Colostrum IgG concentration before and after pasteurization at both locations

	IgG concentration g/L			
	Mean	SD	Min	Max
Pre-Pasteurization	85.8	31.2	29.2	168.8
Post-Pasteurization	72.9	29.1	19.8	157.1

[1]High quality colostrum: IgG concentration > 50 g/L

Table 18. Experiment 1 and 2 Formulated 15% cottonseed hulls calf starter calculated analysis (DM)

Crude Protein (% DM)	23.7
UIP (% DM)	9.2
Sol. Protein (% DM)	4.5
ADF Fiber (% DM)	12.2
NDF Fiber (% DM)	21.5
NF Carbohydrate (% DM)	48.3
NE (MCal/kg)	1.7
Fat (% DM)	2.6
Calcium (% DM)	0.72
Phosphorus (% DM)	0.51
Sodium (% DM)	0..39
Magnesium (% DM)	0.24
Sulfur (% DM)	0.29
Potassium (% DM)	1.20
Chlorine (% DM)	0.49

Table 19. Experiment 1 and 2 Formulated 15% cottonseed hulls calf starter ingredients kg/907 kg calculated ration (AF)

Cottonseed Hulls (kg)	135.6
Corn grain – ground (kg)	406.8
48% soybean meal (kg)	309.5
Soybean hulls (kg)	2.26
Dried cane molasses (kg)	30.7
Plain white salt (kg)	4.52
Calcitic limestone (kg)	8.41
NC State VTM pack[1] (kg)	0.45
Dicalcium Phosphate (kg)	5.51
Rumensin 90 (kg)	0.23

[1]calcium carbonate, manganese, sulfate, zinc sulfate, vitamin E supplement, copper sulfate, ferrous sulfate, sodium selenite, zinc oxide, vitamin A supplement, cobalt sulfate, calcium iodate, vitamin D_3 supplement

Table 20. Experiment 1 and 2 Ingredients of 15% cottonseed hull calf starter (% DM)

Ingredients	% of diet DM (88.2%)
Cottonseed Hulls	17
Corn grain - ground	51
48% soybean meal	39
Soybean hulls	0.2
Dried cane molasses	4.0
Plain white salt	0.6
Calcitic limestone	1.0
NC State VTM pack	0.05
Dicalcium Phosphate	0.7
Rumensin 90	0.02

Table 21. Experiment 1 and 2 N.C. State VTM pack guaranteed analysis

Calcium (min)	20.00%
Calcium (max)	22.00%
Zinc (min)	55,000 ppm
Manganese (min)	39,500 ppm
Sulfur (min)	5.20%
Copper (min)	9,000 ppm
Iron (min)	10,164 ppm
Iodine (min)	704 ppm
Selenium (min)	300 ppm
Cobalt (min)	598 ppm
Vitamin A (min)	6,084,070 IU/kg
Vitamin D_3 (min)	143,805 IU/kg
Vitamin E (min)	78,314 IU/kg

Table 22. Experiment 1 and 2 Formulated 30% cottonseed hulls heifer grower calculated analysis (DM)

Crude Protein (%)	16.6
UIP (%)	6.8
Sol. Protein (%)	3.0
ADF Fiber (%)	20.8
NDF Fiber (%)	33.4
NF Carbohydrate (%)	42.9
NE (MCal/kg)	1.6
Fat (%)	2.7
Calcium (%)	0.65
Phosphorus (%)	0.36
Sodium (%)	0.29
Magnesium (%)	0.18
Sulfur (%)	0.19
Potassium (%)	0.90
Chlorine (%)	0.37

Table 23. Experiment 1 and 2 Formulated 30% cottonseed hulls heifer grower ingredients kg/907 kg calculated ration (AF)

Cottonseed Hulls (kg)	271.2
Corn grain - ground (kg)	418.4
48% soybean meal (kg)	196.2
Plain white salt (kg)	4.5
Calcitic limestone (kg)	10.17
NC State VTM pack[1] (kg)	0.59
Biofos 9072 - Renai (kg)	2.80
Rumensin 90 (kg)	0.13

[1]calcium carbonate, manganese, sulfate, zinc sulfate, vitamin E supplement, copper sulfate, ferrous sulfate, sodium selenite, zinc oxide, vitamin A supplement, cobalt sulfate, calcium iodate, vitamin D_3 supplement

Table 24. Experiment 1 and 2 Ingredients of 30% cottonseed hull heifer grower (% DM)

Ingredients	% of diet DM (88%)
Cottonseed Hulls	34
Corn grain - ground	52
48% soybean meal	25
Calcitic limestone	1.2
Plain white Salt	0.5
Biofos 9072 - Renai	0.35
NC State VTM pack[1]	0.07
Rumensin 90	0.02

[1]calcium carbonate, manganese, sulfate, zinc sulfate, vitamin E supplement, copper sulfate, ferrous sulfate, sodium selenite, zinc oxide, vitamin A supplement, cobalt sulfate, calcium iodate, vitamin D_3 supplement

Table 25. Nutrient analysis average of postweaning heifer grower Experiment 1

DM %	88 ± 1.3
CP, %DM	20.1 ± 2.6
NDF, %DM	32.0 ± 5.8
ADF, %DM	23.4 ± 4.9
NE_L, Mcal/kg	0.74 ± 0.04
Ca, %DM	0.86 ± 0.22
P, %DM	0.42 ± 0.06

Table 26. Hydralyte ® Guaranteed Analysis per 163.4 g Packet

Sodium (min) %	3.2
Sodium (max) %	3.6
Potassium (min) %	1.4
Chloride (min) %	1.8
Each liter of solution contains:	
Potassium (mEq)	30
Sodium (mEq)	85
Acetate (mEq)	60
Chloride (mEq)	45
Citrate (mEq)	10
Glycine (mM)	16
Glucose (mM)	368
Total Osmolarity (mM)	614
Total Metabolizable Energy (Kcal)	296

[1]Dairy Tech Pasteurizer model DT30G serial number 1898 - Dairy Tech Incorporated, Windsor, CO USA: USA Patents #6,276,264, #7,401,546 and patents pending

[2]Land O'Lakes Pasteurized Milk Balancer (for use with pasteurized milk) Dairy Herd & Beef Calf Milk Replacer – Land O'Lakes Animal Milk Products Co., Shoreview, MN 55126-2910: Patent No. 5,571,542

[3]Chantelle Gosselin, Department of Veterinary Microbiology, 52 Campus Drive, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, Canada S7N 5B4, Phone: 306-227-6108 (Chantelle) 306-230-2985 (Ron)

[4]8350 Sherrills Ford Rd, Salisbury, NC 28147 (704) 278-2624

[5]PO Box 669, Maugansville, MD 21767 | 14515 Industry Drive, Hagerstown, MD 21742 | 1.800.CVASLAB

[6]Fisher scientific model Marathon 8K, serial 71100127, cat. 04-977-8K

[7]Standard operating procedure for radial immunodiffusion assay for detecting Bovine IgG levels

1. Dissolve 1.0 agarose in 100.0mL phosphate buffered in saline in a 250mL Erlenmeyer flask – place foam plug in flask to boil in microwave oven (3 minutes)
2. Cool solution to 56˚C using water bath – add 100μL of 10% sodium azide to solution
3. Dispense 24.4mL of solution into 50mL Falcon tubes
4. Add 0.625mL of rabbit anti-immunoglobulin G into each tube and mix well (homogenous mixture without bubbles)
5. Select empty immunodiffusion plate and place on level Table – pour melted agar into plate – allow agar to solidify at room temperature for 15 minutes

6. Place plate in humidified chamber (9x16” container with moistened towels) – incubate for 20 minutes to 12 hours at 20-25˚C
7. Using 2.0mm gel punch, RID template and vacuum pump (8-10” Hg), cut/aspirate wells in agar separated by 13mm
8. Store in humid chamber <12hrsat 20-25˚C until reagent/sample application
9. Prepare serial 2 fold dilutions (1/4 – 1/32) of bovine IgG standard to obtain immunoglobulin concentrations of approx. 8.5, 4.25, 2.13, and 1.06 g/L using PBS (pH 7.25)
10. Prepare dilutions of controls in PBS: Reference bovine serum (1/4 dilution) + reference colostrum (1/15 dilution)
11. Prepare samples for test serial (6.0g of product + 20.0mL water (heated prior to 55˚C) = approx. 24.7mL) – to reconstitute, add 20mLs of water to 6g of dried colostrum in 50mL centrifuge tubes (cap and shake tubes to dislodge powder – uncap tube and mix completely by briefly immersing end of macerator mixer into solution 4-6 times) – tube must be inverted to mix prior to removing sample for dilution
12. Prepare 1/15 dilution of the reconstituted test serial colostrum using PBS – test werums are diluted to ¼ in PBS except for pre-suckle serum with are applied undiluted
13. Dispense using single tip p10 pipette, 5 replicates of 4μL of the diluted reference colostrum and reference serum on the plate
14. Dispense 2 replicated of 4μL of the diluted test serial/test serums on plate
15. Incubate all plates at room temperature for 18-24hrs in humid chamber
16. Using plate reader measure/record the ring diametes for the preceipitin rings surrounding the wells

17. Using the results obtained for each of the 2 fold dilutions of the bovine IgG standard, generate a regression line for the variables R vs. log 10
18. The plate is accepTable if the R^2>0.97 for the standard curve and the mean values obtained for the reference colostrum/serum is the expected values (within 10%)
19. Ig concentration for the test serial/serums is determined using the regression line of the bovine IgG standard obtained for each plate and the average of the observed ring diameters for the sample
20. A regression line for determining IgG concentrations is determined using the measurements of the zones of precipitation of a standard diluted to known IgG concentrations diluted 1:4 (855mg/dL), 1:8 (427.5mg/dL), 1:16 (213.75mg/dL), 1:32 (106.875mg/dL) – the log of the concentrations of the standards is used to create a linear relationship to the precipitin ring diameters (y=mx+b) – where y=the diameter of the ring of precipitin; m=slope of the line; x=the log of the IgG concentration (mg/dL); b=y intercept. To solve for x = inv log ((y-b/m))
21. The measurement of the diameter of the ring of precipitation obtained with each replicate of the test colostrum is inserted into the regression equation to obtain a value for the concentration of IgG in the tested sample (mg/dL)
22. The sample dilution (1/15) is accounted for by multiplying the value obtained by 15

[8]Nasco Bovine Colostrometer: part No. C30687N

1. Collect only 1st or 2nd milking colostrum into a clean and dry container. Make sure the udder is clean and that no foreign debris is allowed into the collection container.
2. Transfer approx. 250mL of standard room temperature (21˚C) colostrum into the included 250 mL plastic jar

3. Make sure the colostrum is filled to the top of the 250 mL plastic jar, and then remove all remaining foam and scum from the surface
4. Gently lower the colostrometer into the 250mL plastic jar filled with colostrum, allow excess colostrum to overflow the cylinder, until the instrument floats freely
5. With the instrument floating freely in the 250mL plastic jar full of colostrum, determine the quality of the colostrum by reading the color coded scale immediately above the unsubmerged portion of the instrument

[9]Fisher Scientific Traceable Waterproof Thermometer with probe/cable (˚F/˚C), iso 17025 calibrated, cat. No. 15-077-9D

[10]Fisher brand Exact Temp Oven Thermometer with bottle, model no. 151715, vermiculite magnesium aluminosilicate

[11]Petrifilm Aeroic count plate

1. Store unopened packages at <8˚C. use before expiration date on package. In areas of high humidity where condensate may be an issue, it is best to allow packages to reach room temperature before opening
2. To seal opened package, fold end over and tape shut
3. Keep resealed packages at <25˚C and <50%RH. Do not refrigerate opened packages. Use petrifilm plates within one month after opening
4. Prepare a 1:10 or greater dilution of sample. Weigh or pipette product into an appropriate container such as a stomacher bag, dilution bottle, whirl-pak bag, or other sterile container

5. Add appropriate quantity of one of the following sterile diluents: butterfield's phosphate buffer, 0.1% peptone water, peptone salt diluents, buffered peptone water, saline solution, bisulfate-free letheen broth, or distilled water
6. Blen homogenized sample per current procedure
7. Place petrifim plate on level surface – lift top film
8. With pipette perpendicular to petrifilm plate, place 1mL of sample onto center of bottom film
9. Release top film – allow it to drop. Do not roll top film down
10. With ridge side up, place spreader on top film over inoculums
11. Gently apply pressure on spreader to distribute inoculums over circular area – do not twist or slide the speader
12. Lift spreader – wait at least 1 minute for fel to form
13. Incubate plates clear side up in stacks of no more than 20. It may be necessary to humify incubator to minimize moisture loss
14. Petrifilm plates can be counted on a standard colony counter or other magnified light source
15. Colonies may be isolated for further identification – lift top film and pick the colony from the gel

[12] Diversey, inc., in Sturtevant, WI, 531771964

Table 27: All Milk Balancer Product

DM fed			All salable milk										
	($)	balancer	65	66	67	68	69	70	71	72	73	74	75
	Milk cwt	Daily ($)	.65	.66	.67	.68	.69	.70	.71	.72	.73	.74	.75
3.8 L 0.67 8 kg DM	20	1.60	2.25	2.26	2.27	2.28	2.29	2.30	2.31	2.32	2.33	2.34	2.35
	21	1.68	2.33	2.34	2.35	2.36	2.37	2.38	2.39	2.40	2.41	2.42	2.43
	22	1.76	2.41	2.42	2.43	2.44	2.45	2.46	2.47	2.48	2.49	2.50	2.51
	23	1.84	2.49	2.50	2.51	2.52	2.53	2.54	2.55	2.56	2.57	2.58	2.59
	24	1.92	2.57	2.58	2.59	2.60	2.61	2.62	2.63	2.64	2.65	2.66	2.67
		Daily ($)	.98	.99	1.01	1.02	1.04	1.05	1.07	1.08	1.10	1.11	1.13
5.7 L 1.02 kg DM	20	2.40	3.38	3.39	3.41	3.42	3.44	3.45	3.47	3.48	3.50	3.51	3.53
	21	2.52	3.50	3.51	3.53	3.54	3.56	3.57	3.59	3.60	3.62	3.63	3.65
	22	2.64	3.62	3.63	3.65	3.66	3.68	3.69	3.71	3.72	3.74	3.75	3.77
	23	2.76	3.74	3.75	3.77	3.78	3.80	3.81	3.83	3.84	3.86	3.87	3.89
	24	2.88	3.86	3.87	3.89	3.90	3.92	3.93	3.95	3.96	3.98	3.99	4.01

Assumptions:

1. $65-75 per 22.6 kg bag of Land O'Lakes All-Milk Pasteurized Milk Balancer Product
2. $20-24 cwt milk
3. 3.8 L = 3.6 kg
4. 0.226 kg balancer/3.8 L

Table 28: Milk Replacer Comparison

	Milk Replacer ($)	70	71	72	73	74	75	76	77	78	79	80
DM fed												
0.678 kg	Daily ($)	2.10	2.13	2.16	2.19	2.22	2.25	2.28	2.31	2.34	2.37	2.40
1.02 kg	Daily ($)	3.15	3.20	3.24	3.29	3.33	3.38	3.42	3.47	3.51	3.56	3.60

Assumption:

1. $70-80 per 22.6 kg bag of Milk Replacer

Table 29: Protein Blend Milk Balancer Alternative

			All salable milk										
	($)	balancer	55	56	57	58	59	60	61	62	63	64	65
	Milk cwt	Daily ($)	.55	.56	.57	.58	.59	.60	.61	.62	.63	.64	.65
3.8 L 0.678 kg DM	20	1.60	2.15	2.16	2.17	2.18	2.19	2.20	2.21	2.22	2.23	2.24	2.25
	21	1.68	2.23	2.24	2.25	2.26	2.27	2.28	2.29	2.30	2.31	2.32	2.33
	22	1.76	2.31	2.32	2.33	2.34	2.35	2.36	2.37	2.38	2.39	2.40	2.41
	23	1.84	2.39	2.40	2.41	2.42	2.43	2.44	2.45	2.46	2.47	2.48	2.49
	24	1.92	2.47	2.48	2.49	2.50	2.51	2.52	2.53	2.54	2.55	2.56	2.57
		Daily ($)	.83	.84	.85	.86	.87	.88	.89	.90	.91	.92	.93
5.7 L 1.02 kg DM	20	2.40	3.23	3.24	3.25	3.26	3.27	3.28	3.29	3.30	3.31	3.32	3.33
	21	2.52	3.35	3.36	3.37	3.38	3.39	3.40	3.41	3.42	3.43	3.44	3.45
	22	2.64	3.47	3.48	3.49	3.50	3.51	3.52	3.53	3.54	3.55	3.56	3.57
	23	2.76	3.59	3.60	3.61	3.62	3.63	3.64	3.65	3.66	3.67	3.68	3.69
	24	2.88	3.71	3.72	3.73	3.74	3.75	3.76	3.77	3.78	3.79	3.80	3.81

Assumptions:

1. $55-65 per 22.6 kg bag of Land O'Lakes Protein-Blend Pasteurized Milk Balancer Product
2. $20-24 cwt milk
3. 3.8 L = 3.6 kg
4. 0.226 kg balancer/3.8 L

Table 30. Experiment 1 Liquid feed price comparison and lactation requirements

	Treatments			
	M	M+B	IM	IM+B
Milk price[1,2]/calf ($)	108	108	138	188
Balancer price[3]/calf ($)	-	39	-	50
Calf starter[4]/calf ($)	13.30	10.30	9.70	8.00
Total price/calf ($)	121	157	148	196
Required increase in milk production from M[1], kg	-	67.8	50.9	141

[1]Milk cwt assumption $24

[2]Using 100% salable milk

[3]All-milk milk balancer product assumption $70/ 22.6 kg bag

[4]Calf starter assumption $380/ 907 kg

Table 31: Experiment 2 Liquid feed price comparison and lactation requirements

	Treatment		
	IM	IM+B	IM+PB
Milk price[1,2]/ calf ($)	148	148	148
Balancer[3,4]/calf ($)	-	54	45
Calf starter[5]/calf ($)	8.1	4.90	5.10
Total price/calf ($)	156	207	198
Required increase in milk production from IM[1] (kg)	-	96.1	79.1

[1]Milk cwt assumption $24

[2]Using 100% salable milk

[3]All-milk milk balancer product assumption $70/ 22.6 kg bag

[4]Protein-blend milk balancer product assumption $60/ 22.6 kg bag

[5]Calf starter assumption $380/907 kg