FEEDING STRATEGIES DURING THE NURSERY PHASE OF DAIRY CALVES TO PROMOTE INCREASED GASTROINTESTINAL DEVELOPMENT EFFICIENCY AND REDUCED WEANING COSTS

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENTS OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

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JULY 2014

Acknowledgements

I owe a great deal of thanks to many people and organizations who supported me as a scholar and as a person through my journey in graduate school. Above all, I owe my gratitude to Kyle Mathews for supporting my decision to continue my education, and although distance and the stress often seemed unbearable, he continued to be patient, understanding, loving, and supportive.

Sincere thanks goes to my unofficial mentor through undergraduate and graduate school; William Hansen. Without his support and willingness to hire me at the Dairy Cattle Research and Teaching Facility, many doors of opportunity may have never been opened, or friendships created. I hold Mr. Hansen in the highest of esteem for his daily guidance, mentorship, and friendship as his support allowed my success and happiness caring for animals.

I would like to thank the members of my committee, Dr. Noah Litherland, Dr. Anthony Seykora, Dr. Jeremy Schefers, and Dr. Susan McClanahan. Their support and guidance throughout my undergraduate and graduate career have allowed me to pursue my interests and learn about an industry we are all passionate about.

I would be sorely amiss if I did not recognize those who assisted with care of the calves and data collection over the years. I appreciate the willingness of the students who assisted me with the difficulties of caring for calves through winter weather and odd hours, their flexibility, their thirst for knowledge, and constant desire to ensure calves had the utmost attention, care, and love. I would like to acknowledge the following students: Kelly Froehlich, Molly Feiro, Cameron Sorensen, Stefanie Lewison, Jamie Symens, and Michael Burchard.

My family, Peter, Laura, and Camille, also deserve recognition for their support, love, and endless patience and consideration in organizing family time around my schedule. Their help with chores on holidays and in bitter cold is especially appreciated.

Additionally, the support of the faculty and staff from the Department of Animal Science was essential in the idea that it takes a department to raise a graduate student. I am indebted to Milk Specialties Global, Land O'Lakes, and TechMix Global for supplying products and their financial support of my research interests, and openness allowing me to learn about today’s industry in animal science.

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Introduction and Objectives

Effective nutritional strategies to raise neonatal dairy calves are constantly manipulated and explored as gastrointestinal development, growth, health, and cost are considered. The requirement of neonatal calves to consume milk until their gastrointestinal tract is developed sufficiently to survive on solid feed is a unique requirement of the ruminant. 85.9% of dairy heifer raisers feed milk replacer to calves (National Animal Health and Monitoring System, 2011). Feeding strategies include manipulation of milk replacer formulation and amount fed, starter grain formulation, and management. One style of feeding will not fit other operations due to housing styles, breed of animal, management, labor availability, etc. These factors elicit the need for many nutritional strategies for calf rearing. Increasing pressure on the modern dairy producer to raise calves quickly to weaning without sacrificing animal growth or health stems from increasing milk replacer ingredient cost, the need to selectively determine replacement heifers early in life, and often, beef market prices.

The majority of dairy heifer raisers utilize a milk replacer containing between 20-24% crude protein and 20-24% fat on a dry matter basis (National Animal Health and Monitoring System, 2011). However, increasing competition for ingredients such as whey protein concentrate yields increased costs associated with raising calves. Alternative sources of protein and fat continue to warrant research for cost-effective milk replacers which maintain or benefit calf growth and health. Benefits of shorter fatty acid chain length have been recognized (Daniels, 2013), however, which ingredients may potentially provide the most benefits to the calf with the least cost are still being explored.

Strategies for producers feeding an accelerated milk replacer containing greater amounts of protein and fat that conventional milk replacer continue to be explored. It has been demonstrated that feeding such a program yields greater milk production during the first lactation (Drackley et al., 2007), and decreased days to first calving (Raeth-Knight et al., 2009). However, product cost often drives limit feeding milk replacer and relying on early grain intake for growth. Using potentially functional food components in milk replacer formulation may assist calves during the gastrointestinal stress of early grain consumption, or assist the gut to be prepared for development and weaning off a greater amount of milk.

The main objectives of this thesis were:

1. To determine if replacing a portion of a conventional milk replacer (22% crude protein, 20% fat, dry matter basis) with 0%, 10%, or 20% of whey cream affected calf growth, starter grain intake, health, or gastrointestinal maturation.
2. To determine if feeding soy isolate as a portion of protein in conventional milk replacer (22% crude protein, 20% fat, dry matter basis) would result in similar growth and grain intake, with minimal negative health effects.
3. To determine the effects of feeding accelerated milk replacer (26% crude protein, 20% fat) at varying planes of nutrition (1.25% or 2.5% of birth body weight), with or without a medium chain and poly unsaturated fatty acid complex, on calf efficiency of growth, starter grain intake, health, and gastrointestinal maturation.

Chapter 1: Literature Review

Neonatal calf nutrition is multi-faceted and must be tailored to each farm's requirements. The success of each program is reflected in calf growth and health, farm net costs, and ultimately, her lifetime success as a dairy cow. Each feeding program must account for the development of the digestive system to ensure a smooth transition with minimal stress through the weaning period. The formulation and strategies of feeding milk replacer and starter grain are varied to achieve this same goal.

Milk Replacer Formulation

Functional foods

The first step of success of a milk replacer program is milk replacer ingredients. Exploration into why certain ingredients yield benefits as functional foods can open doors to more efficiently formulated products. The high mortality rate of calves to gastrointestinal upset, specifically scours, during the nursery phase (National Animal Health and Monitoring System, 2011), can be largely influenced by milk nutrition, as the first few weeks of life the calf is greatly, if not entirely, dependent on nutrition from milk. This, coupled with society's views of animal care evolving against the use of fed medications, such as antibiotics and antimicrobials in milk replacers, may necessitate the need of milk replacers to address animal health in other ways. Although medicated milk replacers may positively influence calf growth, the efficacy of using milk replacer containing antimicrobials to manage diarrhea is controversial, but a current review of studies suggests is beneficial overall (Constable, 2009). The primary infection sites for the calf are in the blood and the gut (Constable, 2009); targeting calf health through the manipulation of milk replacer results in potentially easier on-farm management of calf diarrhea.

Fructooligosaccharides (FOS) are a fermentable fiber which have been shown to benefit the growth of beneficial bacteria *Bifidobacteria* spp and *Lactobaccillus* spp (Sghir et al., 1998; Kaplan and Hutkins, 2000; Campbell et al., 1997). FOS also increases the amount of small chain fatty acids in the gut and decreases pH, promoting increase in gut microbes and inhibiting the colonization of Clostridium difficile (May et al., 1994). FOS incorporation into milk replacer by Donovan et al. (2002) in combination with probiotics and allicin in a product known as Enteroguard© resulted in similar improvement in growth and health performance as calves fed a milk replacer containing antibiotics, over calves fed a non-medicated milk replacer.

Investigation of other oligosaccharides in milk replacer yielded similar fecal score and body weight response improvements with medicated milk replacers over non-medicated milk replacers (Quigley et al., 1997). Similarly, Heinrichs et al. (2003) fed milk replacers of control, with mannan oligosaccharides, or with antibiotics. Overall, fecal scores were improved for calves fed oligosaccharides or antibiotics in milk replacer, and week 6 feed intake increased for oligosaccharide calves, although a difference in growth was not noted. Mannan oligosaccharides are often derived from the yeast *Saccharomyces cerevisiae*, and have been demonstrated to increase goblet cell numbers in broilers, increasing the potential for mucus secretion in the small intestine (Baurhoo et al., 2009). This, in turn, could provide benefits for neonatal calves during early periods of gut development and stress.

Another functional food of interest that provides additional value to the dairy industry is milk fat globule membrane (MFGM). MFGM composition appears to be species-specific (Bracco et al., 1972), surrounds fat globules in milk, and is mainly composed of membrane-specific proteins: glycoproteins, phospholipids, sphingolipids, as well as mucin 1 (Dewenttinck et al., 2008; Reinhardt et al., 2006). Phospholipids and sphingolipids play a role in regulating cell growth and development, adhesion, and are associated with enhanced health (Astaire et al., 2003; Spitsberg, 2005). 55-70% of phospholipids in milk are located in MFGM (Mather and Keenan, 1998). Mucin 1 is a membrane-bound mucin which is secreted by goblet cells in the small intestine (Kim and Ho, 2010), and thus could benefit the gut health of a calf stressed with scours by providing lubrication to ease tissue stress if present in milk. While MFGM's origins are the apical plasma membrane of the secretory cells, the endoplasmic reticulum, and other intracellular components, the importance during the secretory process of mucins proteins is as of yet unknown (Heid and Keenan, 2005). Bovine MFGM also possesses fatty acid-binding proteins (Fong et al., 2007), which may promote fatty acid transfer within the calf.

Interest in short and medium chain fatty acids (SCFA, MCFA) has recently increased due to health benefits for the calf. Bovine milk fat contains a greater amount of SCFA and MCFA than lard or tallow, the two most common sources of fat in milk replacers. Milk fat is 95-97% digestible compared to digestibility of 87-94% of lard and tallow (Raven, 1970; Toullec et al., 1980). The importance of fatty acid chain length lies in the digestion process. Daniels (2013) and Sun et al. (2002) describe the digestion process: minimal digestion occurs oral and gastrically, but oral and gastric lipase liberate the fatty acid in the sn-3 position, which is almost exclusively a short or medium chain fatty acid (4 - 10 carbons). If this chain is less than 12 carbons, it may be absorbed through the stomach wall, oxidized through the liver, and provide a source of immediate energy. The small intestine digests more fat by use of bile and pancreatic lipase, the activity of which both are limited in the neonate. Most long chain fatty acids (LCFA) must be resynthesized by formation of very low density lipoprotein and chylomicrons, which must lymphatically be transferred to the portal vein. When digestion and absorption is negatively impacted, greater LCFAs can be detected in the portal blood.

Besides the availability of absorption for SCFA and MCFA, the early hydrolysation of milk fat has been shown to have antimicrobial and antibacterial properties. Sun et al. (2002) demonstrated that the 9-42% hydrolysis of 4% bovine milk fat, in the maximum pH expected in a calf stomach, successfully inhibited bacterial growth, with decreasing pH only making the response stronger. Additionally, antiviral properties were demonstrated by feeding a combination of unsaturated LCFA and saturated MCFA (Hristov et al., 2004). The benefits of altered fatty acid profile in milk has been shown with improved fecal score, days treated for illness, and pre and post-weaning average daily gain (Esselburn, 2013).

Functional foods that address the fatty acid profile of milk have been examined with sources other than milk fat, such as vegetable oils, to simultaneously address milk replacer cost. In a study by Mills et al. (2010), coconut oil provided 32% of the FA content in milk replacer and calves had heavier liver weights with 38% more lipid, a deposition presumed to be from the difficulty of metabolizing C14:0. Piot et al. (1999) fed tallow versus coconut oil, and did not find a difference in liver weight, but found 18 times the triglyceride concentration in the liver for calves fed coconut oil over those fed tallow. Hill et al. (2011) fed a blend of butyric acid, coconut oil, and flax oil, which resulted in improved titers of vaccinations for bovine viral diarrhea and respiratory parainfluenza-3, as well as reduced treatments for clostridium, and improved feed efficiency and growth. The literature overwhelmingly points to benefits of altering the fatty acid profile of milk replacer for animal health benefits.

Alternative proteins

Growth in pre-weaned calves occurs largely through muscle and bone growth, both of which rely heavily on protein accumulation, thus, the growth of the calf relies on energy and protein (Drackley, 2008). Milk proteins have historically comprised the majority of proteins in milk replacer, but as demand for these proteins for the human food industry increases and cost increases, alternative proteins have been investigated to maintain calf health and performance, while minimizing milk replacer ingredient cost. Studies evaluating performance of alternative proteins continue to use milk sourced protein as a standard.

Soy is one of the most largely utilized alternative proteins in milk replacers. A wide variety of soy protein sources are available: flours, concentrates, and isolates. Soy contains a very similar essential amino acid profile to milk (Lalles, 1993), which allows it to be a viable protein substitute. Heated soy flour has been found to be inferior as a protein sourced when compared to soy protein concentrate and milk protein (Dawson, 1988). Additionally, full fatted soybean flour tends to have the least apparent total tract digestibility compared to milk protein and soy protein concentrate (Akinyele and

Harshbarger, 1983). Overall, soy flours are considered to be an inferior product due to decreased digestive potential due to trypsin inhibitor, antigenic proteins, and more (Lalles, 1993; Lalles et al., 1995; Drackley, 2008). Further processing of soy flour removes indigestible carbohydrates, glycinin and beta-conglycinin (which may cause allergic reactions in calves), and inactivates antigenic proteins, resulting in soy concentrate, and is the most popular variety of soy protein utilized in calf milk replacers (Drackley, 2008; Quigley, 2001). Soy isolate also requires further refining from soy flour, and is an extensive, and thus expensive, process (Drackley, 2008). Growth performance from soy isolate is varied from a negative response to no response, and may be in part due to inhibited fat digestion of calves fed soy protein, or decreased amino acid digestibility (Lalles et al., 1995; Yuangklang et al., 2004; Khorasani et al., 1989).

Pea protein has been researched for use in veal calf milk replacer, but has been found to contain half the fat of soy protein, and a relatively high amount of starch which may limit its incorporation in milk replacer (Lalles, 1993). Today, very little pea protein is utilized or researched.

A less common plant protein incorporated into milk replacers comes from potatoes. Branco-Pardal et al. (1995) fed milk replacers where protein provided was either from skim milk powder, or 52% of protein was from native gluten or potato concentrate. There appeared to be lower true digestibility from the novel proteins, which resulted in lower apparent total tract digestibility. Feeding potato protein concentrate in milk replacer has also been shown to increase ileal flow of mucin, and has been proposed to account for the lower apparent total tract digestibility of plant proteins, as well as impairing absorption of proteins (Montagne et al., 2000).

Animal based proteins are also available for use in calf milk replacer. Of these, fish protein was more extensively used in the past and can provide similar growth performances to milk protein based replacers when included in levels of up to 70% of the protein. Drackley (2008) noted that the use of fish protein hydrolyses can provide an undesirable smell, and at times the product may be difficult to acquire. Supplemental vitamin E is required in the milk replacer formulation, as nutritional myopathy is observed when fish is the only source of protein (Huber, 1975; Michel et al., 1972).

A more popular animal based protein is spray dried plasma. Studies replacing up to 25% of protein with animal plasma have found similar performance to that of milk sourced proteins (Quigley et al., 1996, 2002). Morrill et al. (1995) found that bovine or porcine sourced plasma increased body weight gain over calves fed milk replacer with whey protein concentrate alone or with a probiotic. Quigley et al. (2003) also compared spray dried plasma from bovine or porcine sources. Compared to whey protein concentrate, including either of these plasma sources at 5% of the formula reduced mortality by 75%, and reduced days scouring. Overall, literature points to the benefits of spray-dried plasma when economical.

Quigley et al. (2000) demonstrated that up to 43% of protein in milk replacer can be replaced with spray dried hydrolyzed red blood cells (SDRBC) and maintain calf growth, feed efficiency, starter intake, and fecal scores. SDRBC are a co-product of plasma production, which may be lacking in the methionine and isoleucine requirements of the calf (Quigley et al., 2000), but in a nursery pig study, adding supplemental isoleucine overcame decreased ADG, feed intake, and gain:feed noticed with increasing SDRBC (Kerr, 2004).

Finally, Touchette et al. (2003) found replacing up to 10% of the milk replacer formula, or 27% of the protein, with liquid egg, successfully achieved similar growth rates and health performance to calves receiving all milk protein. Replacing a portion of milk replacer with liquid egg or spray dried whole egg has only been found beneficial when calves are offered starter grain by seven days of age (Touchette et al., 2003).

The incorporation of alternative proteins in calf milk replacer needs to maintain gastrointestinal health as well as achieving maximum growth potential. Decreased villi height and increased crypt depth has been associated with soy protein, although it was not attributed to increased bacterial counts and was found to be reversible when calves were fed milk protein (Lalles, 1993; Seegraber and Morrill, 1986). Dawson et al. (1998) also demonstrated the improved intestinal mucosal morphology of calves consuming milk protein over soy protein.

Detrimental effects of such novel proteins in the gastrointestinal tract and lower protein efficiencies call for strategic offering of alternative proteins in milk replacer. A higher quality protein, such as spray-dried plasma, SCRBC, or whey protein, may need to be utilized in milk replacers for the first few weeks of life. After the calf has begun grain consumption and the tract is more equipped to handle novel proteins, a lesser quality sourced protein may be utilized, as calves are more equipped to use soy proteins as they age (Akinyele and Harshbarger, 1983).

On-farm Nutritional Strategies: Milk Replacer Planes of Nutrition

Milk replacer programs are influenced by environment, housing style, management strategies, cost of milk replacer, breed, composition of milk replacer, and other factors which vary between farms. Successful planes of nutrition at one dairy do not necessarily have the same outcomes at another farm. Two overlying industry strategies of feeding milk replacer are often discussed: 1) Feed milk at low levels to promote early grain intake, and prepare the calf for weaning; 2) Feed milk at high levels to promote efficient early life growth. The former allows the early weaning strategy to be implemented, although the calf's body must digest a novel protein from grain early in life. The latter may come with an increased initial cost, but can take advantage of a period in life where the calf's growth is highly efficient. Whichever a producer decides to utilize, the importance of management is recognized as it is necessary to maximize upon an animal's genetic potential.

Limit feeding milk to calves occurs primarily for economical, and, for whole milk, availability reasons. Feeding calves around 4-5 liters of milk is fairly common in the US and around the world (NAHMS, 2011; Kristensen et al., 2007; Silper et al., 2014). Calves weighing 45 kilograms require approximately 3 liters of milk per day for maintenance alone (Drackley, 2008). Thus, as the calf grows, it will rapidly approach receiving only enough nutrients from milk for maintenance if additional milk is not provided above 4-5 liters, or if the temperature drops below thermoneutral, and will rely on nutrients from grain intake for growth. Programs feeding low amounts of milk replacer strive to promote early life grain intake for growth and reduced weaning stress.

Low feeding rate programs may not be the most effective use of certain systems, such as automated calf feeders, as restricted fed calves pay more unrewarded visits to the feeder, tying up time for potential use, and increase cross-sucking (Jensen, 2003).

The type of milk replacer offered also plays a role in the feeding rate. Some producers feeding an accelerated milk replacer will attempt to feed it in lower rates. However, Drackley (2008), outlines this strategy results in energy becoming limited, and thus, the excess nitrogen from protein provided will be excreted in urine, a waste.

Feeding calves greater amounts of milk has been increasing in interest recently. Van Amburgh et al. (2008) reported studies where one treatment received at least 50% more calories from milk than the standard rate; this resulted in a milk production increase of 1,700 pounds. Also reported was a benefit of 1,000 pounds of milk produced during the first lactation for every pound of average daily gain increase before weaning. Brown et al. (2005b) manipulated a milk replacer and grain program for low and high planes of nutrition and examined parenchymal tissue. Accelerated milk replacer benefited growth of mammary parenchyma and DNA concentration during weeks 2-8 of life (Brown et al., 2005b). This may contribute to the increased ability of animals to produce greater milk during the first lactation.

Feeding high amounts of milk calls for consideration during weaning of calves. The use of automatic feeders in situations feeding high planes of nutrition from milk may allow gradual weaning to occur over a longer period of time than typical in individually raised calves, and result in better grain intake and weight gain than abrupt weaning (Sweeney et al., 2010).

Conversely to the discussion of limit feeding accelerated milk replacer, Drackley (2008) also notes that providing a conventional milk replacer, where fat and protein levels are similar, in an above standard rate, provides excess fat to the calf with protein becoming limiting, and body tissue deposition occurs as fat. Increasing protein is needed for lean tissue deposition, growth, gain:feed, and more growth factors when metabolizable energy is maintained (Blome et al., 2003; Brown et al., 2005a).

Several studies have compared low and high planes of nutrition from milk replacer, with varied results. The comparison of the feeding rates is often between conventional milk replacers fed at lower rates, and accelerated milk replacers fed at high rates, due to the use of protein by calves (Drackley, 2008). Ballou (2012) fed Holstein and Jersey calves either 0.454 kg of a 20% protein, 20% fat milk replacer, or 0.81 kg during the first week followed by 1.18 kg until weaning of a 28% protein, 20% fat milk replacer. Pre-weaned growth of calves was improved for high planes of nutrition, but post-weaning growth and dry matter intake was not affected. However, dry matter intake per unit of body weight was increased for low plane nutrition calves (Ballou, 2012). Terre et al. (2007) also found a numerical increase in body weight for calves fed a high plane of nutrition from milk replacer, and increased daily starter grain consumption and digestibility at weaning by low plane calves, but no difference in grain intake post-weaning.

Conversely, Hill et al. (2006), fed a 20% protein, 20% fat milk replacer at 0.45 kg daily, compared to a 28% protein, 20% fat milk replacer fed at either 0.68 kg, 1.13 kg, or 1.36 kg daily. Overall, the increased feeding rate was only successful when fed at 0.68 kg, increasing calf weight gain by 55%. The two highest amounts of accelerated milk replacer increased scouring and treatments, and decreased feed intake. Hill et al. (2006) also supplied the conventional milk replacer at 0.68 kg, and found no benefit, indicating that at this level, crude protein was apparently limited for the conventionally fed calves.

Ad libitum milk intake is rare for most systems, but especially for milk replacers. However, benefits of ad libitum milk intake have resulted in increased total body weight gain by weaning and continued through post weaning (Jasper and Weary, 2002). Interestingly, the authors found no difference between treatments for diarrhea, and proposed that management, rather than nutrition, is more likely the source of enteric disease. Producers who implement ad libitum milk intake may find this strategy easiest with an acidified milk group housing system, or automated feeder.

Feeding rates of milk replacers should take milk replacer nutrient formulation into consideration, as well as starter grain management strategy, system and labor opportunities, and environmental conditions, for optimal performance at each farm.

Gastrointestinal Maturation

The bovine neonate is born without the fully developed ruminant system of its adult counterpart. By the point of weaning, the system must be developed enough for the calf to subsist on solid feeds, while maintaining growth and health.

A calf is born with the majority of stomach tissue comprise by the abomasum (~60%), with an underdeveloped reticulorumen, or forestomach (~30%), and undeveloped omasum (~10%) (The Pennsylvania State University, 2003). As development of the ruminal compartments occurs, the size of the stomachs will reach proportions closer to that of an adult's, where the forestomach comprises ~58% of the tissue, ~12% is the omasum, and the remaining ~30% is the abomasum (The Pennsylvania State University, 2003).

The purpose of the underdeveloped tissues at birth lies in the diet composed primarily of milk anticipated by the neonate; the development of the reticulorumen is initiated when solid feed consumption begins. Calves are capable of complete ruminal function only a few weeks after dry feed consumption begins (Lalles and Poncet, 1990). Successful development of the rumen will increase the volume of the stomach compartment, maturation of papillae, and the establishment of the microbial population. The calf must be prepared to ferment feeds and absorb volatile fatty acids (VFAs) as a source of energy, as well as rely on the microbial population for much of its amino acid requirements (Drackley, 2008).

It is widely accepted that calves should begin grain consumption as early as possible to increase health and prepare the calf for weaning. Successful starter programs are often derived from a combination of successful formulation and management.

Starter grain for gastrointestinal maturation: formulation

The importance of fermentation of carbohydrates to volatile fatty acids (VFAs) for ruminal development begins with butyrate, then propionate, and finally acetate. Sander (1959) infused sodium butyrate, sodium propionate, sodium acetate, sodium chloride, and glucose into the rumen and found marked development of the ruminal mucosa by sodium butyrate and propionate. This indicates the end products of fermentation are required for tissue development. However, the esophageal groove in ruminants shunts milk to the abomasum, so supplying a substrate for tissue maturation in milk will be relatively ineffective for the rumen (Orskov et al., 1970). Thus, a highly fermentable feedstuff is crucial for efficient digestion and metabolic requirements for development of tissue. Rationally, the use of grain is preferred over forage to stimulate ruminal development; fermentation products from grain result in a greater proportion of butyrate and propionate over forages, which will ferment more acetate. Grain will thus have a greater impact on maturation of ruminal tissue, as demonstrated in images found in many of Penn State's popular press calf publications. Corn is found in the majority of calf starters as starch is highly fermentable to propionate. Additionally, greater nutrient density of grain over forage allows for greater growth potential. Additional supplementation of VFAs in starter grains may be beneficial. Gorka et al. (2009) supplemented sodium butyrate in both milk replacer and starter grain, which resulted in increased reticulorumen weight compared to the other stomachs, as well as greater papillae development.

With gastrointestinal development and maturation largely dependent on grain intake, formulation should consider for increasing intake. Protein inclusion in the grain should be considered, especially when grain is paired with a milk replacer feeding program. Stamey et al. (2012) demonstrated feeding a starter grain containing 25.5% crude protein (DM basis) compared to 19.6% crude protein, with an accelerated milk replacer program, increased grain intake, particularly around weaning.

Formulation of starter grain must also account for physical form. Franklin et al. (2003) found calves prefer textured starters over pellets, and will consume more total grain, allowing for increased body weight gain during the neonatal period, however, Bateman II (2009), criticized this finding as treatments did not contain similar crude protein or ingredients. Conducting a similar trial, Bateman II et al. (2009), found no difference between pelleted and textured starters, and attributed the finding due to similar bedding and forage offered to calves. This is consistent with findings by Bach et al. (2007), who fed a textured or pelleted starter comprised of the same ingredients; textured starter consumption was greater, but body weight gains were similar, indicating greater efficiency of pelleted starter. Hill et al. (2012) found no difference for textured starter versus pelleted, but increased performance for calves fed textured starter versus mash. Calves also will consume less starter with a greater percentage of fines (Bateman II et al., 2009).

Lesmeister and Heinrichs (2005) studied molasses inclusion level of 12% versus 5% in textured starter, which resulted in decreased dry matter intake, but similar weight gain, and increased blood volatile fatty acid levels associated with increasing molasses inclusion, which allowed for greater papillae development. Similarly, Hill et al. (2008) found that a molasses inclusion level in textured starter of 10% versus 5% resulted in trends for decreased feed intake and average daily gains. Difficulties of handling starter with such high molasses content are often undesirable in production settings.

Studies evaluating using a similar flavor in both starter grain and milk replacer to promote intake through feed association have found to have no difference on feed intake (Thomsen et al., 1980; Montoro et al., 2011; Morrill and Dayton, 1978). However, a preference in each of these studies for either maple or orange flavoring was of note as calves consumed feed at an earlier age.

Starter grain for gastrointestinal maturation: management strategies to maximize intake

Management of starter grain begins with availability. The simplicity of presence equates to the potential of a substrate to develop the ruminal and gastrointestinal tracts. Achieving early grain intake allows for the potential of earlier weaning, as calves may successfully be weaned when consuming 2 pounds of grain for 3 consecutive days (NAHMS, 2011). Many “common sense” recommendations are common in the industry and can mean the difference between good and great neonatal performance.

Most heifer raising operations do not offer grain or water until 6 days of age, however, smaller operations wait until about 10 days, while large operations offer grain and water at 3 days (NAHMS, 2011). Kertz et al. (1984) demonstrated a positive correlation between water intake and grain consumption, where providing no water limited grain intake by 31%, impacting body weight gain by 38%. Pairing water with grain is essential as the rumen microbes require an aqueous environment in which to function (Quigley, 2001).

Beginning calves on grain may require extra labor or thought as to introduction of grain. Offering grain after milk by hand feeding or placing in the bottom of the milk bucket while the calf is still suckling may encourage intake (Jones and Heinrichs, 2007). Nipple bottles designed for grain intake are also available, although starter consumption appears to be similar between bottles and open containers (Hopkins, 1997). Fresh grain is also essential, along with considering the height of the grain bucket; smaller breed calves may require the feeder height to be lowered, or a shallow pan used instead of a bucket. Buckets need to be checked daily and filled when necessary. Leaving an older calf in with younger calves housed in group systems may “teach” younger calves about grain consumption.

Chapter 2: Replacing 10 and 20 percent of dairy calf milk replacer with whey cream yields similar starter intake, growth, and health performance during the nursery phase[1].

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[1] Supported in part by funds and calf milk replacer donation by Milk Specialties Global, Eden Prairie, MN.

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Interpretive Summary

Whey cream (**WC**) is a byproduct of whey protein concentrate production and contains milk nutrients and potentially healthful functional food compounds, such as milk fat globule membrane and short and medium chain fatty acids. To determine if replacing a portion of all milk protein calf milk replacer (**CMR**) with increasing amounts (0, 10, or 20%) of WC affects calf growth and health, 72 Holstein and Holstein-cross dairy calves, male (n=33) and female (n=37), raised in hutches October 2012 to January 2013 were assigned to 1 of 3 CMRs formulated to provide 22% protein and 20% fat; 1) 0% WC (**0WC**); 2) 10% WC (**10WC**); 3) 20% WC (**20WC**). Milk replacer was fed at 1.5% of birth body weight and reconstituted to 13% solids. Birth body weight and serum total protein concentration averaged 40.4 ± 0.7kg and 6.3 ± 0.07mg/dL, respectively. Calves were fed CMR twice daily d 1 to 41, once daily d 42 to 48, weaned d 49, and removed from trial d 56. Starter (19.9 % CP DM basis) and drinking water were offered ad libitum. Daily starter intake, thrice weekly fecal score, and weekly growth (body weight, wither height, hip height, hip width, body length, heart girth) were measured. Six bull calves/treatment (n=18) were euthanized d 56 for gastrointestinal measurements and histology. Data were analyzed using PROC MIXED in SAS as a block design. Starter intake through d 56 averaged 1.0, 1.1, and 1.1±0.08 kg, ADG averaged 0.83, 0.91 0.84± 0.05kg, and gain:feed through d 56 averaged 0.55, 0.58, 0.53±0.03, for 0WC, 10WC, and 20WC, respectively. Calves fed 20WC tended to consume more starter than 0WC by d 49. Calves fed 10WC consumed 500g/d of starter 2.3 ± 1.8 d before 0WC. Average fecal score tended to be less for 10WC over 0WC. Small intestine weight was greater for calves consuming WC, but length did not differ. Cecum weight was greater for 20WC over 0WC. Histology scores of papillae, villi, and duodenal mucosa were similar, yet starter intake by harvested calves (3.5±0.3kg/d) yielded scores of mild-moderate inflammation or thickening. Duodenal goblet cell presence was lower for 20WC than 0WC, and tended to be lower for 10WC than 0WC. Results indicate replacing up to 20% of CMR with WC resulted in some trends for calf growth and health and did not affect gastrointestinal maturation and health. High starter consumed appeared to instigate undesirable inflammatory responses in the small intestine.

Introduction

Whey cream (**WC**) is a byproduct of cheese production, and contains short and medium chain fatty acids and milk fat globule membrane (**MFGM**). Mucin 1 (**MUC1**) is an immune protein present in bovine milk fat globule membrane (**MFGM**) (Reinhardt and Lippolis, 2006), and has protective properties in the intestine, including anti-rotavirus properties (Kvistgaard et al., 2004). MUC1 is also secreted by goblet cells located in the epithelial lining in the small intestine (Kim et al., 2010). Bovine MFGM also possesses fatty acid-binding proteins (Fong et al., 2007), which may promote fatty acid transfer. Due to rising calf milk replacer (**CMR**) ingredient cost, evaluation of more economical sources of milk nutrients, such as whey cream, for calves should be evaluated. WC may be more economically advantageous to use in CMR than whey protein concentrate and isolate, which have been recognized for their flexibility and health benefits in the human food industry. WC may also have an advantage over plant proteins, which may negatively affect the development of the small intestine (Drackley et al., 2006). We are unaware of previous research using whey cream in CMR. Objectives for this study were to replace ten or twenty percent of CMR with WC and evaluate the effects on calf growth, efficiency, health, and starter intake on gastrointestinal growth and morphology. Our hypothesis was increasing amounts of whey cream would increase small intestinal health, resulting in increased growth and efficiency of growth in preweaned calves.

Materials and Methods

Animals

Seventy two male (n = 34) and female (n = 38) Holstein and Holstein-cross dairy calves were born and housed at the University of Minnesota Dairy Teaching and Research Facility (St. Paul, MN) from October 2012 to January 2013. Care of animals was in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching and approved by the Institute of Animal Care and Use Committee. Calves were removed from dams within one hour of birth, received an ear tag, navel dip, intranasal vaccination (Inforce-3, Zoetis©, Kalamazoo, MI), and fed colostrum within the first 3 hours of birth with a 22% solids or greater, determined using a refractometer (Reichert Rhino VET360, Reichert, Depew, NY). Calves were fed colostrum for two additional feedings within the first 24 hours of life. Animals were moved to individual hutches outside (Polydome, Litchfield, MN) bedded with straw, and jackets (Udder Tech, Inc., Lakeville, MN) were used until d 28. The mean temperature during the study was -0.5°C, mean high was 3.9°C, and mean low was -5°C.

Assignment to treatments

At 2 days of age, calves were assigned to one of three non-medicated CMR treatments based on gender, birth body weight (**BBW**), breed, and total serum protein (**TP**). Calf BBW averaged 40.4 ± 0.7 kg and TP averaged 6.3 ± 0.07 mg/dL. Milk replacers (**Table 1**) were formulated to be all milk protein, non medicated, isonitrogenous, and isocaloric and provided 23.0% protein and 19.5% fat (DM basis)

(**Table 1**). Treatments were: 1) 0% WC (**0WC**); 2) 10% WC (**10WC**); 3) 20% WC (**20WC**). Milk replacer was bucket fed at a rate of 1.5% of BBW in powder and reconstituted to 13% solids, and divided into two feedings at 0630h and 1700h from d 1 to d 42. Morning feeding was discontinued d 42 to d 48, calves were weaned d 49, and remained on trial through d 56. *Ad libitum* fresh water was provided after each CMR feeding and texteurized starter (19.9% CP, DM basis) was fed *ad libitum*.

Feed intake, body measurements, and health scores

Starter grain intake was recorded daily, fecal scores (**FS**) recorded thrice weekly on Monday, Wednesday, and Friday, and body measurements recorded weekly; (body weight (**BW**), wither height (**WH**), hip height (**HH**), hip width (**HW**), body length (**BL**), heart girth (**HG**)). Rectal temperature was taken once weekly. Body measurements and FS were taken by the same person to ensure consistency. Fecal scores were evaluated using the calf health score criteria: 0 = normal, 1 = semi-formed, pasty, 2 = loose, but stays on top of bedding, 3 = watery, sifts through bedding (McGuirk, 2009). We defined scours as FS $\geq$ 2.

Gastrointestinal measurements and tissue analysis

On d 56, six bulls calves per treatment (n = 18), representing the average BBW, TP, and breed, were euthanized by a licensed veterinarian through administration of sodium pentobarbital (Fatal Plus, Vortech Pharmaceuticals, Dearborn, Michigan) via the jugular vein for gastrointestinal measurements. Measurements from harvested calves included: reticulorumen (**RR**), abomasum (**AB**), omasum (**OM**), small intestine (**SMI**), and cecum (**CE**), weighed with and without contents. Length of SMI and CE were recorded. A 2cm x 2cm tissue sample was obtained from the rinsed RR ventral sac, and a 4.4 cm length rinsed section of the duodenum, and were fixed in a solution of 10% neutral buffered formalin. Tissues were processed for routine histology, cut into 4 µm sections, mounted on slides, and stained with hematoxylin and eosin. Goblet cells (**GC**) were counted microscopically using 40X enlargement in a grid at ten points in duodenum tissue. A rumen fluid sample was collected, immediately snap frozen in liquid nitrogen, and later processed for pH and volatile fatty acid (**VFA**'s). VFA concentrations were determined using high performance liquid chromatography (Dairyland Laboratories, Arcadia, WI).

Feed and fecal analysis

Samples of CMRs and starter grain were collected weekly and composited on a wet weight basis for further analysis using wet chemistry methods (Dairyland Laboratories, Arcadia, WI). Ash content was determined using AOAC 942.05 and crude protein was determined using AOAC 990.03. Heat-stable, alpha-amylase-treated and sodium sulfite NDF (**aNDF**) for feed ingredients was determined using an ANKOM 200 fiber analyzer (Ankom Technology, Macedon, NY) based on procedures described by Van Soest et al., (1991). Acid detergent fiber was determined using AOAC 973.18, and ether extract was determined by AOAC 920.39. Starch was determined using an enzymatic method described by Knudsen (1997). Metabolizable energy (**ME**) content was calculated based on NRC (2001) equations. Fecal sample were collected from the rectum on d 49, and on day 56 from harvested bull calves (n = 18) and frozen for apparent total tract digestibility using acid insoluble ash as a marker (Block et al., 1981). Fecal samples were dried at 37.8°C, measured for DM, and ground by a mill through a 1mm sieve for acid insoluble ash analysis.

Blood analysis

A blood sample was collected via jugular puncture into evacuated serum tubes (SST, Beckton Dickenson Vacutainer Systems, Franklin Lakes, NJ) at 24 hours of age, and at 0830 after feeding each week and centrifuged at 2000×*g* for 20 minutes at 2°C. Serum was separated and frozen at -20°C. Serum taken at 24 h of age was immediately analyzed for total serum protein concentration using a refractometer (Reichert Rhino VET360, Reichert, Depew, NY), and all serum was analyzed for non-esterified fatty acids (**NEFA**) using a test kit (Wako Life Sciences, Inc., Richmond VA).

Statistical Analysis

Data were analyzed using the PROC MIXED procedure in SAS (Release 9.3: SAS Institute, Cary, N.C.). Model effects besides treatment, time, and the interactions of treatment by time included in the model were calf gender, breed, BBW, and TP. Calf was considered a random effect nested in treatment. Harvested calves were included in the main trial data set. Data measured over time were subjected to ANOVA by using the REPEATED statement in the MIXED procedure of SAS (Littell et al., 1998). Correlation analysis among variables of interest was conducted using Pearson correlations. Least squares means for treatment effects were separated by use of the PDIFF statement when the overall F-test was $P \leq 0.05$. Trends are indicated when $0.05 < P \leq 0.10$. The largest SEM of main treatment effects are reported.

Results

Starter grain and nutrient intake

Calculated metabolizable energy intake from CMR + starter grain through d 49 for 0WC, 10WC, and 20WC was 24.7, 24.2, and 24.5 Mcals, respectively, and through d 56 was 43.7, 44.8, and 44.9 Mcals, respectively (**Table 3**). Calves fed 20WC had a tendency ($P < 0.08$) for greater total starter grain consumption by weaning on d 49 versus calves fed 0WC, however, total BW gain was not affected and by d 56, total starter consumption, total BW gain, ADG, gain:feed, and feed digestibility were not different (**Table 4**). Calves fed 10WC consumed 500g/d of starter grain before calves fed 0WC ($P < 0.03$) (**Table 4**).

Growth, fecal, and health

There were no significant differences between treatments for gains in hip height, wither height, hip width, or body length measurements (**Table 5**). Heart girth was greater ($P < 0.05$) for calves fed 0WC versus 10WC and 20WC. Average fecal score for the entirety of the trial tended (*P* < 0.09) to be lower for calves fed 10WC over calves fed 0WC, and days scouring did not differ (*P* < 0.28).

First three weeks of life

Table 7 and **Table 8** explore the impact of whey cream during the first three weeks of life for all calves. Digestive upsets are typically noted in the first three weeks of life. During week two of life calves fed 10WC had lower (P < 0.03) fecal scores and tended (*P* < 0.09) to have fewer days scouring versus 20WC. Body temperature during week one was greatest (P < 0.05) for 0WC versus 20WC. Week one BW gain was lower (*P* < 0.004) for 20WC versus 10WC, and tended (*P* < 0.08) to be below 0WC, with week one BW gains being 3.3, 3.4, and 2.3 ± 0.4 kg for 0WC, 10WC, and 20WC, respectively. As expected, as days scouring increased, average fecal score increased (*P* ≤ 0.0001). Higher fecal scores and more days scouring were negatively correlated (*P* < 0.03) with body temperature and weight gain.

Non-esterified fatty acids

No difference (*P* < 0.61) between treatments or treatment by week were observed for serum concentrations of non-esterified fatty acids (**NEFA**) throughout the trial (**Figure 1**). There was a decrease in serum NEFA as calves aged.

Harvested calf measurements

Harvested calves differed slightly in starter grain intake measurements, reported in **Table 9**, than the overall calf data from the trial reported earlier. Calves consuming 20WC tended (*P* < 0.09) to have earlier starter intake of 500g/d than calves fed 0WC, and had earlier intake (*P* < 0.04) for 1000g/d. Similarly, total starter intake by both d 49 and 56 and average daily gain tended (*P* < 0.07) to be greater for 20WC over 0WC. Harvested calves consuming 10WC were intermediate for all discussed measurements, however, the analysis of all 72 calves resulted in no differences for body weight gains or starter intake, with the exception of calves fed 10WC consuming 500g of starter/day earlier than 0WC.

There were no differences in RR or AB empty weight or when expressed as a percentage of BW on day 56 (**Table 10**). Empty OM weight tended (*P* < 0.07) to be greater for 20WC versus 0WC, but did not differ when expressed as a percentage of d 56 BW. Empty SMI weight was greater (*P* < 0.02) for calves fed 10WC and 20WC versus calves fed 0WC, and when expressed as a percent of BW, was greater (*P* < 0.03) for calves fed 10WC and 20WC compared with 0WC. Length of the SMI alone or evaluated as cm/kg of BW was not different. Weight of the CE and CE weight expressed as a percentage of body weight was greater (*P* < 0.02) for calves fed 20WC versus 0WC, however, CE length was not altered (*P* < 0.39) by treatment.

When discussing goblet cells and visual rumen and SMI scores, it should be remembered the samples sizes are extremely small relative to the entire tissue, and that only 6 calves/treatment were collected. There were more (*P* < 0.004) goblet cells in the duodenum sample for 0WC calves over 20WC calves, and tended (*P* < 0.08) to be more in 0WC over 10WC. No treatment differences (*P* < 0.39) of histological scores assigned to the appearance of the rumen and duodenum tissue, duodenal mucosal inflammation, or papillae appearance were found (**Table 11**). All scores, however, approached mild-moderate inflammation, thickening, or bluntness. While duodenal mucosal and villi inflammation scores were not different among treatments, presence of GCs decreased (*P* < 0.10) when starter intake increased.

Total amount of volatile fatty acids (**VFA's**), and concentrations of acetic, propionic, and butyric acid did not differ among treatments, with the exception of propionic acid when expressed as a percentage of total VFA (**Table 11**). The concentration of propionic acid tended (*P* < 0.09) to be greater in rumen fluid from 0WC than 20WC.

Discussion

Minimal differences in growth between treatments in this study demonstrate the success of replacing a portion of CMR with whey cream. Measures of ADG were not significantly different, but intriguing as 10WC ADG was 9.6% and 8.3% greater than

0WC and 20WC, respectively. There was no clear impact of WC on calf structural growth. During the first three weeks of life, decreased body weight gain may be a combination of scours resulting in: fewer nutrients available to the calf as feed exits more rapidly, the need to warm the body from a depressed body temperature, and use of energy to rebuild gastrointestinal tissue. Serum NEFA concentrations did not differ during the study, and were similar to those previously reported (Kuehn et al., 1993; Bartlett, et al., 2006). NEFA concentration decreased as calves aged; Quigley et al. (1990) reported similar declines in plasma NEFA from week 0 to week 6 of life.

Digestion of fatty acids is minimally accomplished in the oral cavity and the abomasum, but what is achieved gives preferential hydrolysis to short and medium chain fatty acids (**FA**) as a faster source of energy (Daniels, 2013; Sun et al., 2002). Most fat digestion occurs in the small intestine and is where long chain FAs are hydrolyzed. Milk fat is highly digestible and is known to contain greater amounts of short and medium chain FA content than today's CMRs. Short chain FA are defined as 2 to 6 carbons, medium chain FA are 8 to 12 carbons, and long chain FA are greater than 14 carbons. Milk FA profile yields a higher concentration of short and medium chain FA content in milk (26.1%) than WC (19.4%), and thus, WC contained more long chain FAs (80.6%) (milk FA profile not shown, WC analysis by Rtech Laboratories, Shoreview, WI). Analysis of CMRs yields the total amount of short and medium FA to be 2.4%, 2.9%, and 4.1% of DM for 0WC, 10WC, and 20WC, respectively. This is greater than the amount found by two studies using CMR formulated with sweet whey by Hill et al. (2007 a&b), (1.67% in a 22% CP, 20% fat CMR, 1.81% in a 20% CP, 20% fat CMR). The total amount of short and medium chain FAs found in **Table 3** is different between treatments and is lowest for 20WC and highest for 10WC, with 0WC being intermediate. This may explain lower fecal scores for calves fed 10WC. We anticipated calves fed WC would have lower fecal scores, and potentially fewer days scouring due to the ability of WC to assist gastrointestinal health. Scours are recognized as the second largest cause of mortality in preweaned heifer calves in the US (NAHMS, 2011). It is known that increasing short and medium chain FAs in CMR increases health, as it is more similar to whole milk. Antimicrobial properties of medium chain FAs have been demonstrated in several species (Sun et al., 2002; Dierick et. al., 2002; Immerseel et. al., 2004). Feedstuffs containing high amounts of short and medium chain FA, such as coconut oil and palm kernel oil have been investigated for inclusion in CMR. However, in a study by Mills (2010), where coconut oil provided 32% of the FA content in CMR, calves had heavier liver weights with 38% more lipid, a deposition presumed to be from esterification rather than oxidation of C14:0.

Because CMR's did not affect calf growth or starter intake, harvested calf data focused on the impact of amount and timing of starter grain intake on gastrointestinal maturation. We hypothesized increased amounts of starter grain intake early in the liquid feeding phase would result in greater gastrointestinal development or maturation compared with delayed starter intake and that greater gastrointestinal development would result in increased calf growth. While treatment did not affect histological scores, overall scores approached mild-moderate inflammation and mucosal thickening in the duodenum and mild-markedly blunted for ruminal papillae. Additionally, 15 of the 18 calves were noted for ruminal papillae fusion and parakeratosis, which can occur when papillae are stimulated to grow quickly by consuming a high amount of grain without forage available for rumen abrasion value (McGavin and Morrill, 1976). Parakeratosis reduces surface area and the quality of surface area available for nutrient absorption. At day 56, calves were consuming 3.5 ± 0.3kg/d of a 38.0% starch starter. Although signs point to subacute ruminal acidosis (**SARA**), ruminal pH fell between 5.7-6.0. Mature cows are considered to have SARA when pH reaches 5.5. Do calves experience acidotic conditions at a different pH than cows? The impact of whey cream on rumen VFA measures is not likely a direct effect as calves were weaned a week prior to harvest and were consuming the same starter grain and supplied *ad libitum* water.

The increased weight of the SMI with no significant difference in length for harvested calves consuming WC may have resulted from increased inflammation from starter consumption. While duodenal mucosal and villi inflammation scores were not different between treatments, presence of GCs decreased when starter increased. The high volume of starter intake may have caused increased sloughing of the epithelial lining and resulted in fewer GCs counted. GCs secrete mucin, and the sloughing of these cells may have compounded the inflammation from starter intake.

Conclusions

Replacing up to 20% of CMR with WC maintained growth performance with some benefits for calf health. The inclusion of greater amounts of short and medium chain FAs and utilizing the MFGM found in by products of cheese production for incorporation into CMRs should be explored and perhaps combined with other lower cost CMR protein options to promote calf growth and health. Promoting early starter intake may result in large amounts of starter consumed by weaning, and successfully introducing forage to maintain rumen and gastrointestinal health should be tailored for various feeding strategies.

Chapter 3: Soy isolate as a partial protein source in conventional calf milk replacer

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Introduction

Rising cost of calf milk replacer (CMR) ingredients drives limit feeding milk to achieve early starter grain intake for a more economical energy source, and potentially earlier weaning and reduced labor costs (Anderson et al., 1987). Earlier grain intake begins fermentation and production of volatile fatty acids in the rumen and results in rumen development to allow for more efficient use of solid feed and potential decreased stress at weaning. However, limit feeding CMR may be detrimental to calf growth and health (Soberon et al, 2011). Use of plant based proteins in milk replacer has been used to lower the nutrient content of milk and thus promote earlier starter intake (Ghorbani et al., 2007). However, antigenic properties found in plant based proteins may stress calves less than three weeks of age with an immune response mounted due to undigested protein amounts in the intestines (Longenback et al., 1998), and some soy proteins, especially flours, appear to result in decreased digestive potential by containing trypsin inhibitors (Lalles, 1993; Lalles et al., 1995). Further processing of soy flour results in soy concentrate, which is widely popular in literature as it removes indigestible carbohydrates and inactivates antigenic proteins, and is more economically feasible than whey protein (Drackley, 2008). Soy isolate, which is refined further than soy concentrate may have benefits of less antigenic proteins due to processing, but literature reports that as soy is processed, including the refined soy product in milk replacer results in decreased fat absorption, which may potentially affect growth (Nitsan et al., 1972; Lalles et al., 1995). However, soy isolate incorporated in milk replacer has only minimally been studied.

Thus, we wanted to observe if feeding a 22% crude protein CMR containing 5% of protein from soy isolate, and 17% conventional whey protein at a higher feeding rate could capitalize on preweaning efficiency of growth in dairy calves, and maintain calf health while utilizing a more economically viable feedstuff.

Materials and Methods

Thirty four male (n = 13) and female (n = 21) Holstein and Holstein-cross dairy calves born at the University of Minnesota Dairy Teaching and Research Facility (St. Paul, MN) were enrolled in trial on day 2 of life and housed in calf hutches (Polydome, Litchfield, MN) bedded with straw from February 2013- May 2013. Animal care was in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching, and approved by the Institute of Animal Care and Use Committee.

Assignment to treatments

Calves were assigned to one of two treatments based on gender, birth body weight (**BBW**) which averaged 41.0 ± 1.3 kg, breed, and total serum protein (**TP**) at 24 hours of age which averaged 5.7 ± 0.15 mg/dL. Treatments were: 1) control CMR (**CONTROL**) where protein was derived from whey; 2) soy CMR where 5% of protein was derived from soy isolate, and 17% of protein was from whey protein (**SOY**). CMRs (Milk Specialties Global, Edina, MN) were formulated to provide 22% crude protein and 20% fat (DM basis) and reconstituted to 13% solids (**Table 13**). Treatments were bucket fed at 1.5% of BBW day 1-9 in two daily feedings, 2.0% of BBW day 10-42 in two daily feedings, 1.0% of BBW in one feeding day 43-49. Calves remained on trial through day 56. Warm tap water and texteurized starter grain (19.9% CP, DM basis) (Super Krunch, Hubbard Feeds, New Richmond, WI) were provided ad libitum throughout the trial.

Feed intake, body measurements, and health scores

Starter grain intake was recorded daily. Body measurements were recorded weekly; (body weight, wither height, hip height, hip width, body length, heart girth). Fecal scores (FS) recorded thrice weekly, evaluated using calf health score criteria: 0 = normal, 1 = semi-formed, pasty, 2 = loose, but stays on top of bedding, 3 = watery, sifts through bedding (McGuirk, 2009). Any CMR refusals or treatments were recorded as the trial progressed.

Statistical Analysis

Data were analyzed using the PROC MIXED procedure in SAS (Release 9.3: SAS Institute, Cary, N.C.), with gender, birth weight, and TP included in the model, and calf was a random effect nested in treatment. Data measured over time were subjected to ANOVA by using the REPEATED statement in the MIXED procedure of SAS (Littell et al., 1998). Least squares means for treatment effects were separated by use of the PDIFF statement when the overall F-test was $P \leq 0.05$. Trends are indicated when $0.05 < P \leq 0.10$. The largest SEM is reported.

Results and Discussion

Results rarely differed in this trial. Growth and feed results are shown in **Table 14**. Both treatments more than doubled calf BBW by 56 days of age, had similar ($P < 0.67$) weight gains at weaning on day 49, and at removal from trial on day 56. Comparable weight gains were noticed because calves received the same amount of milk replacer and consumed similar ($P < 0.37$) amounts of starter grain at benchmarks of 49 and 56 days, as well as average daily DMI. As expected, similar weight gains and grain intake yielded similar ($P < 0.79$) gain to feed ratios. Yuangklang et al. (2004), and Lalles et al. (1995) also noted similar growth performance between calves where soy isolate accounted for half or more of CMR protein. However, both of these studies utilized veal calves that were not offered grain, so the use of soy isolate in CMRs fed to dairy heifers also offered grain is extremely limited. It is possible that the nutritional value from the grain helped calves in this study to overcome any inhibitory fat digestion noted with processed soy proteins previously mentioned.

Interestingly, feed intake during week 8 after weaning was similar ($P < 0.90$) (**Figure 2**), but body weight gain was greater ($P < 0.04$) for CONTROL calves (**Figure 3**). These results are puzzling, as no major health events were noted for the SOY calves. We hypothesize that a calf effect contributed to this, where CONTROL calves may have simply adapted to the stress of weaning sooner than SOY calves.

Additional calf growth measurements and health performance is reported in **Table 15**. Gain in body measurements from day 1 to day 56 for wither height, hip width, hip height, heart girth, and body length did not differ ($P < 0.90$), which reflects the similar growth reported. There was a tendency ($P < 0.07$) for milk refusal days to be greater for SOY. However, days treated did not differ ($P < 0.56$). Since the refusals did not result in additional treatments for SOY calves, we conclude that there were not extra management costs for feeding soy isolate in CMR for this study.

In this trial, week 2 of life slump of growth was overcome by increasing the milk feeding rate. Week 2 body weight gain slump or plateau is often noted when calves receive one similar plane of nutrition throughout the preweaning period (Quigley et al., 2006; Timmerman et al., 2005). Increasing feeding rate allows additional protein and energy to be provided from CMR until calves consume enough grain to allow for increased energy for tissue growth.

Conclusion

Feeding conventional milk replacer which contained a protein of the whey protein replaced with soy isolate protein allowed similar growth when fed at higher feeding rates without negative effects on calf health. Milk refusals tended to be greater for calves fed soy, but did not result in more calf treatments. Finally, increased feeding rates allowed calves to overcome a slump in calf growth often seen during week two of life.

Chapter 4: Effects of medium chain fatty acids in dairy calf milk replacer fed at a low or high plane of nutrition on growth, health, and gastrointestinal maturation[1].

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[1] Supported in part by funds and calf milk replacer donation Land O'Lakes, Shoreview, MN.

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Interpretive Summary

Increased nutrition from calf milk replacer (**CMR**) can capitalize on efficiency of growth in pre-weaned dairy calves, and recognition of the value of fatty acid profile is also gaining interest in early life health. To determine the interaction of plane of nutrition and fatty acid profile, 96 male (n = 36) and female (n = 60) Holstein and Holstein-cross dairy calves raised in hutches from September 2013 to March 2014 and fed one of two CMRs (CON, MCFA) at one of two rates based on birth body weights (**BBW**); LOW (1.25% of BBW), HIGH (1.8% of BBW day 1-9, 2.5% of BBW day 10 to 42). Treatments were 1) **LOW CON**; 2) **HIGH CON**; 3) **LOW MCFA**; 4) **HIGH MCFA**. CMRs (28% protein and 21% fat, DM basis) were reconstituted to 13% solids and fed twice daily day 1- 42, once daily day 43-49, and remained on trial through day 56. Calves received free choice water and starter grain (26.3% CP, DM basis) throughout the trial. Daily starter intake, five times weekly health scores, and weekly growth and body structure were measured. Five bull calves/treatment (n=20) were euthanized d 22 for gastrointestinal measurements and histology. Data were analyzed using PROC MIXED in SAS as a 2 x 2 factorial design. Daily starter DMI through d 56 averaged 0.97, 0.58, and 1.0, 0.64 ± 0.05 kg and

ADG averaged 0.80, 0.80, 0.76, 0.88 ± 0.03kg, for LOW CON, HIGH CON, LOW MCFA, and HIGH MCFA respectively. LOW calves consumed more starter, but HIGH calves had greater weight gain and increased wither height, hip height, body length, and heart girth. Plane of nutrition did not impact health scores, but CON had lower average fecal score than MCFA. Harvested calf tissue measurements were similar across treatments, but abomasum tissue weight was greatest for HIGH calves. Total ruminal volatile fatty acids tended to be greater in LOW calves. Harvested calf tissue weights were similar among treatments, but ruminal total VFAs was increased for LOW calves. Starter intake began to differ at 3 weeks of age due to amount of CMR fed, but LOW calves did not equal energy consumption (CMR + grain) of HIGH calves until week 6 of life. Greater plane of nutrition from milk replacer positively impacted growth of calves, while low plane of nutrition resulted in greater grain intake, with minimal benefits from milk fatty acid profile.

Introduction

Increased nutrition from milk for pre-weaned calves is rising in interest due to reports of greater future milk production (Soberon et al., 2011; Van Amburgh et al., 2008). Greater nutrition through milk can be achieved through the use of an accelerated calf milk replacer (**CMR**), which delivers greater protein to the calf for growth, and also benefits the growth of mammary parenchyma and DNA concentration during weeks two to eight of life (Brown et al, 2005). However, accelerated CMR is associated with increased cost, increased fecal score during early life, and decreased grain intake. Thus, some producers feed accelerated CMR at a low plane of nutrition, which may promote earlier grain intake, but results in limiting energy from the CMR, causing excess protein being excreted in the urine (Drackley, 2008), and may stress the gastrointestinal tract by the consumption of grain. Also of current interest are functional foods within CMR which benefit calf health; early life gastrointestinal integrity can be compromised by bacteria and lead to decreased growth and health performance, and death. Specifically, medium chain fatty acids (**MCFA**) are of interest because of the digestibility and potential to be antimicrobial and antibacterial, benefiting gastrointestinal health (Sun et al., 2002; Hristov et al., 2004; Esselburn, 2013). MCFAs in milk replacer has demonstrated increased growth, feed efficiency, and decreased scouring previously when fed in an accelerated form in a step up program (Hill et al, 2007, 2011), but effect of MCFA on plane of nutrition has not been examined. Preventatively accounting for compromised gastrointestinal health through using MCFA in milk replacer may be advantageous when calves are fed a low or high plane of nutrition. Our objectives were to determine if MCFAs at a low or high level of CMR nutrition would increase calf health through decreasing gastrointestinal stress, from large amounts of milk or the calf's early consumption of a novel protein from grain, driven by a low plane of milk nutrition. We also examined the effects of CMR plane and MCFAs on growth and gastrointestinal maturation. We hypothesized that a high plane of nutrition from milk replacer would increase efficiency of growth, delay starter grain intake, result in decreased early life gastrointestinal maturation, but in combination with MCFAs might increase benefits seen by high levels of milk nutrition.

Materials and Methods

Animals

Ninety-six male (n = 36) and female (n = 60) Holstein and Holstein-cross dairy calves were born and housed at the University of Minnesota Dairy Teaching and Research Facility (St. Paul, MN) from September 2013 to March 2014. Animal care was in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching, and approved by the Institute of Animal Care and Use Committee. Calves were removed from dams within one hour of birth, received an ear tag, navel dip, intranasal vaccination (Inforce-3, Zoetis©, Kalamazoo, MI), and fed colostrum within 3 hours of birth with a 22% solids or greater. Colostrum quality was determined using a refractometer (MISCO DD-3 Refractometer, MISCO, Cleveland, OH). Calves received two additional feedings of colostrum within the first 24 hours of life. Calves were housed outside in individual hutches (Polydome, Litchfield, MN) bedded with straw, and fitted with jackets (Udder Tech, Inc., Lakeville, MN) when temperature was below -3.9°C, until day 35. The mean temperature during the trial was -1.8 ± 2.1°C, mean high was 2.9 ± 2.3°C, and mean low was -7.0 ± 2.0°C. The low temperatures throughout the trial likely impacted calf growth as animals needed to spend more energy for maintenance, and may also have resulted in increased grain intake.

Assignment to treatments

At 2 days of age, calves were assigned to one of four non-medicated CMR treatments based on gender, birth body weight (**BBW**), breed, and total serum protein (**TP**). Calf BBW averaged 36.4 ± 1.0 kg and TP averaged 6.3 ± 0.13 mg/dL. Milk replacers were formulated to be, isonitrogenous, isocaloric, and provided 28% protein and 21% fat (DM basis) (**Table 16**). Calves received a low or high feeding rate, where CMR was reconstituted to 13% solids and bucket fed twice daily (0600h, 1700h): 1) fed at 1.25% of BBW d 1-42, fed at 0.625% d 43-49; 2) fed at 1.8% of BBW d 1-9, fed at 2.5% of BBW d 10-42, fed at 1.25% of BBW d 43-49. Within the low and high feeding rates, calves received one of two milk replacers: 1) commercially available accelerated all milk protein CMR with lard as a fat source (Cold Front©, Land O'Lakes, Shoreview, MN), 2) CMR containing fatty acids from a combination of lard and MCFA triglycerides. This two by two factorial arrangement resulted in four treatments: 1) low plane of nutrition (**LOW CON**); 2) low plane of nutrition with MCFA fatty acid complex (**LOW MCFA**); 3) high plane of nutrition (**HIGH CON**); 4) high plane of nutrition with MCFA fatty acid complex (**HIGH MCFA**). Milk replacer fatty acids profiles can be found in **Table 17**, where MCFA CMR contained 16.8% or fatty acids of 12 carbons or less, while CON CMR contained only 2.6% of fatty acids as 12 carbons or fewer. Fresh water was provided *ad libitum* after each CMR feeding and texteurized starter (26.3% CP, DM basis) was offered free choice to provide 25% refusals daily (**Table 16**).

Feed intake, body, health, and gastrointestinal measurements

Starter intake was recorded daily, fecal scores (**FS**) and respiratory scores (**RS**) were recorded once daily five times a week, and body measurements recorded weekly; (body weight (**BW**), wither height (**WH**), hip height (**HH**), hip width (**HW**), body length (**BL**), heart girth (**HG**)). Body measurements were taken by the same individual, and FS were observed by two trained people to ensure scoring consistency. Health scores were evaluated using the calf health score criteria: FS: 0 = normal, 1 = semi-formed, pasty, 2 = loose, but stays on top of bedding, 3 = watery, sifts through bedding; RS: 0 = none, 1 = induced single cough, 2 = induced repeated coughs or occasional spontaneous cough, 3 = repeated spontaneous coughs (McGuirk, 2009). Scours were defined as FS ≥ 2. A fecal sample was collected rectally on d 43 and frozen for apparent total tract digestibility using insoluble ash as a marker (Block et al., 1981). On d 22, five bull calves per treatment (n = 20), representing the average BBW, TP, and breed, were harvested 6 hours post morning feeding by a licensed veterinarian through administration of sodium pentobarbital (Fatal Plus, Vortech Pharmaceuticals, Dearborn, Michigan) via the jugular vein for gastrointestinal measurements. Measurements from harvested calves included weights of reticulorumen (**RR**), abomasum (**AB**), omasum (**OM**), small intestine (**SMI**), and cecum (**CE**), with and without contents. Length of SMI and CE were recorded using a ruler. Tissue samples from the rinsed RR ventral sac and duodenum and were placed in a solution of 10% neutral buffered formalin for further analyses and processed for routine histology (Comparative Pathology Shared Resource, St. Paul, MN). Tissue samples were cut into 4 μm sections, mounted on slides, and stained with hematoxylin and eosin. Villi height, crypt depth were evaluated by measuring each at twenty separate points in the tissue microscopically and averaged, and papillae height was evaluated similarly at fifteen points and averaged (Lesmeister et al., 2004). Fourteen milliliters of rumen fluid and duodenum digesta samples were collected and immediately snap frozen in liquid nitrogen for further analysis of VFA profile by high performance liquid chromatography and pH (Dairyland Laboratories, Arcadia, WI).

Feed and fecal analysis

Samples of CMRs and starter grain were collected weekly and composited on a wet weight basis for analysis using wet chemistry methods (Dairyland Laboratories, Arcadia, WI). Ash content was determined using AOAC 942.05 and crude protein was determined using AOAC 990.03. Heat-stable, alpha-amylase-treated and sodium sulfite NDF (**aNDF**) for feed ingredients was determined using an ANKOM 200 fiber analyzer (Ankom Technology, Macedon, NY) (VanSoest et al., 1991). Acid detergent fiber (**ADF**) was determined using AOAC 973.18, and ether extract was determined by AOAC 920.39. Starch was determined using an enzymatic method described by Knudsen (1997). Metabolizable energy (**ME**) content was calculated based on NRC (2001) equations. Fecal samples were dried at 37.8°C, measured for DM, and ground through a 1mm sieve for acid insoluble ash (**AIA**) analysis for use in apparent total tract digestibility procedures (Block et al., 1981).

Blood analysis

Blood samples were collected 30 minutes post morning feeding via jugular puncture into evacuated serum tubes (SST, Beckton Dickenson Vacutainer Systems, Franklin Lakes, NJ) at 24 h of age and each week after, centrifuged at 2000×*g* for 20 minutes at 2°C, and serum was separated and frozen at -20°C for further analyses. Serum collected at 24 h of age was immediately analyzed for total serum protein concentration using a refractometer (MISCO DD-3 Refractometer, MISCO, Cleveland, OH). Serum from weeks one to eight was analyzed for non-esterified fatty acids (NEFA) using a commercially available test kit (Wako Life Sciences, Inc., Richmond, VA).

Statistical Analysis

Data were analyzed using the PROC MIXED procedure in SAS (Release 9.3: SAS Institute, Cary, N.C.). Gender, birth weight, and TP concentration were included in the model, and calf was a random effect nested within treatment. Breed was not significant (*P* < 0.61). Model effects besides treatment, time, and the interactions of treatment by time, with a *P* > 0.50 were not included in the final model. Data from calves harvested on day 22 of age were not included in the overall trial analysis. Data measured over time were subjected to ANOVA by using the REPEATED statement in the MIXED procedure of SAS (Littell et al., 1998). Least squares means for treatment effects were separated by use of the PDIFF statement when the overall F-test was $P \leq 0.05$, and trends are indicated when $0.05 < P \leq 0.10$. The largest SEM is reported.

Results

Calves on low planes of nutrition from milk more than doubled (*P* <0.0001) starter dry matter intake (**DMI**) over high plane of nutrition calves by day 43, and had more (*P* < 0.0001) total starter DMI by day 56 (**Table 18**). Average daily starter DMI was also greater (*P* < 0.0001) for calves fed low amounts of milk. Supplementing MCFA did not alter (*P* < 0.36) starter intake. By day 43 and 56 calves fed high planes of nutrition from milk had greater (*P* < 0.02) total BW gain and greater ADG. Day 43 gain tended (*P* < 0.07) to be greater for HIGH MCFA over HIGH CON, and day 56 gain and ADG was increased (*P* < 0.04) for HIGH MCFA calves over all other treatments. Feed efficiency was also increased (*P* < 0.001) on both day 43 and 56 for calves receiving a higher plane of CMR nutrition. Dry matter digestibility on day 43 was significantly (P < 0.005) higher for calves receiving a high plane of nutrition.

Compared with a low plane of nutrition, high plane of nutrition increased (*P* < 0.02) gains in WH, HH, BL, and HG, with no (P < 0.59) change in HW gain (**Table 19**). CMR offered did not affect (*P* < 0.54) structural growth measurements. Plane of nutrition had no impact (*P* < 0.19) on milk refusal days, days treated, average FS, days scouring, or average respiratory score. Interactions of plane of nutrition and MCFAs resulted in CON MR tending (*P* < 0.06) to have a lower average FS than MCFA MR and for LOW CON to have a lower (*P* < 0.05) respiratory score than HIGH CON.

Weekly starter intake began to differ (*P* < 0.001) during week 3 of life, when LOW calves consumed more than HIGH calves (**Figure 4**). Weekly BW gain (**Figure 5**) was increased by a greater plane of nutrition (*P* < 0.05), and average weekly FS (**Figure 6**) was lowest (*P* < 0.06) for LOW CON. Although calves on low planes of nutrition had double the starter intake of calves on a high plane of nutrition, they did not consume similar amounts of energy until week 5-6 (**Figure 7**). During week 7, LOW calves continued to increase their energy consumption, while high plane nutrition calves decreased, due to energy intake from starter not yet equaling the CMR feeding which was removed during this time. Serum NEFA concentration did not differ (*P* < 0.14) between treatments, or have interactions throughout the trial, but tended (P < 0.07) to decrease as calves aged (**Figure 8**).

Harvested calf weekly and total BW gain was greater (*P* < 0.05) for HIGH calves (**Table 20**), and gain decreased for all treatments during week 2 (**Figure 9**). Average daily SI increased (*P* < 0.001) as calves aged. Average weekly FS and days scouring were similar (*P* < 0.21). Average weekly serum NEFA tended (*P* < 0.10) to be greater for HIGH CON over HIGH MCFA. There was no difference (*P* < 0.97) for RR, OM, or CE measurements. Empty AB weight was greater (*P* < 0.005) for calves receiving the high plane of nutrition, and empty SI weight tended (P < 0.08) to be greater (**Table 21**).

Total ruminal VFA (acetic, propionic, and butyric) tended (*P* < 0.07) to be greater for calves fed a low plane of nutrition (**Table 22**). Individual ruminal VFA concentration, duodenum total VFAs, and pH were similar (*P* < 0.23) among treatments, with the exception of propionic acid, which tended (*P* < 0.08) to be greater for calves receiving a low plane of nutrition. There was a MR interaction for duodenum digesta pH to be greater (*P* < 0.07) for calves receiving MCFA. Calves receiving CONTROL had significantly (*P* < 0.02) greater concentration of duodenal lactic acid than MCFA, and HIGH CON had the greatest (*P* < 0.04) concentration. Butyric and isobutyric concentrations were not detectable in the duodenum digesta, and thus not reported. Average villus length and crypt depth did not differ (*P* < 0.38). There was a tendency (*P* < 0.09) for a plane by CMR interaction for HIGH CON to have greater papillae length than HIGH MCFA.

Discussion

The MCFA CMR contained 16.8% of fatty acids as 12 carbons or less, while the CONTROL CMR only had 2.1%. The majority of the difference lies in caprylic ($C_{8:0}$) and capric ($C_{10:0}$), which were present in higher amounts in MCFA CMR. Oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) were greater in CONTROL CMR.

As expected, calves receiving a low plane of nutrition from CMR greatly increased starter intake and began consuming greater amounts of grain earlier in life than calves fed more CMR (**Figure 4**). Average weekly BW gain was maintained during week 2 of life for calves fed high plane of nutrition from CMR, while calves fed low amounts of CMR experienced much lower BW gain (2.3 vs 4.9 ± 0.5 kg) during this time, likely because their grain intake was not significantly different from high plane nutrition calves at this time, and their energy intake was lower. However, when CMR was reduced to once daily during week 7, calves receiving low plane of nutrition excelled over calves fed high plane of nutrition, gaining 7.2 and 5.2 ± 0.6 kg, respectively. This was most likely because low plane of nutrition calves were accustomed to consuming greater amounts of grain, which is reflected in total energy intakes (**Figure 8**). Interestingly, by post-weaning during week 8, weekly BW gains were similar at 7.8 ± 0.6 kg/week. Thus, although there were considerable growth differences during the neonatal period between the planes of nutrition, no post weaning effects on growth were observed. Calves receiving high amounts of CMR were more efficient in feed conversion on day 43 primarily because more of their energy was derived from milk, which calves use more efficiently for energy than solid feed (Brown et al., 2005b). The increase in many of the growth measurements for calves fed a high plane of nutrition is reflected in the literature (Soberon et al., 2011).

Interestingly, CONTROL had a lower average FS than MCFA. During weeks 2 and 3, LOW CONTROL maintained the lowest FS, which may be a combination to the low amount of milk received and the small amount of grain being consumed. The tendency for LOW CONTROL to have a lower respiratory score than HIGH CONTROL appears to be negligible as the scores recorded were low. The increased average weekly

FS during week 8 for LOW MCFA in combination with lower energy intake may explain why these calves did not increase weekly BW gain, as LOW CON did. Serum NEFA concentration generally decreases as age increases (Kmicikewycz et al., 2012; Luchini et al., 1992; Kahn et al., 2007), and this trial slightly followed this trend, with treatments having similar weekly values. Serum NEFA levels may not have decreased as much as in other studies due to the extreme cold stress resulting in elevated blood NEFAs in this trial. During week 7 of life, NEFA serum values spiked for HIGH MCFA, which may be a result of decreased weekly BW gain during this time compared to other treatments, as calves received less energy from CMR.

Harvested calves largely represented the data from the collective calf data. The increase in the abomasum tissue weight was expected as calves were consuming twice the milk as low plane of nutrition calves. No tissue difference for the other forestomachs is also logical due to minimal starter intake differences at this time, and maturation of these systems is likely in the beginning stages. Duodenum histology results also followed this pattern, with no significance noted, likely due to calves on low planes of nutrition consuming minimal amounts of grain, with week 3 beginning to show differences in intake (**Figure 4**). The tendency for ruminal papillae to be longer for LOW MCFA over HIGH MCFA may be a result of low plane nutrition calves beginning to consume more grain as harvest approached (**Table 21**), and thus increased total ruminal VFAs (**Table 22**). An increase in total ruminal VFAs was also expected as calves fed a low plane of nutrition are consuming more grain to ferment. The tendency for increased duodenal pH for calves fed the MCFA may be due to limited calves sampled and that calves had access to grain and may not be a true representative of the effect of MCFA on intestinal pH.

Conclusion

A high plane of nutrition from calf milk replacer increased early life growth and efficiency of growth and low plane of nutrition increased grain intake, but energy intakes were not similar until about 6 weeks of age. This study found little to no effect on health of calves fed milk replacers containing increased medium chain fatty acids at these feeding amounts, but some positive growth effects were recorded when fed in combination with a high plane of nutrition from milk replacer. Harvested calves yielded few tissue differences at 3 weeks of age; likely due to minimal grain consumption. This study yielded that medium chain fatty acids benefits in milk replacer might best be capitalized on when fed at a high feeding amount.

Overall Conclusions

Neonatal calf performance is multifaceted, and factors affecting performance vary from farm to farm. Success of a calf program relies on tailoring it to fit each farm's facilities and capabilities of animal care. Milk replacer programs should take into account ingredient formulation and nutrient composition. Early weaning may be achieved in many instances through using grain formulation and feeding strategies to promote gastrointestinal maturation. My research yielded some insights on functional food components benefiting health and growth in both conventional and accelerated milk replacer, as well as grain intake impacts on growth and gastrointestinal health and maturation. As production systems evolve, feeding strategies will continue to require evaluation on functional food components to increase gastrointestinal development efficiency.

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Tables

Table 1. Nutrient composition of milk replacer with 0% whey cream (WC), milk replacer with 10% or 20% replaced with WC, and starter grain.

	Milk replacer (0% WC)	Milk replacer (10% WC)	Milk replacer (20% WC)	Starter grain
	% DM			
Dry Matter	96.7	96.7	96.7	88.0
Crude protein, %	21.6	23.8	23.7	19.7
Ether extract, %	19.5	19.7	19.7	3.9
Starch, %	.	.	.	38.0
ME, Mcal/kg	0.39	0.39	0.39	0.36
ADF	0.01	0.01	0.01	10.3
aNDF, %	0.07	0.10	0.23	20.9
Ash, %	9.5	9.33	9.3	6.3

Table 2. Fatty acid composition 22:20 milk replacers with 0%, 10%, or 20% replaced with whey cream fed to nursery calves.

	Milk replacer (0% WC)	Milk replacer (10% WC)	Milk replacer (20% WC)
Total Fatty Acid % of DM	18.1	18.5	17.7
tUFA[1]	60.4%	59.1%	57.0%
PUFA[2]	19.1%	18.3%	16.6%
MUFA[3]	41.3%	40.8%	40.4%
Fatty Acid		g/100g of fatty acids	
C12:0, 14:0	2.37	2.77	3.71
C14:1	0.10	0.14	0.35
C16:1	26.74	27.46	28.25
C17:0	0.48	0.51	0.56
C18:0	9.37	9.4	9.41
C18:1	40.75	40.29	39.71
C18:2	18.05	17.29	15.68
C18:3	0.73	0.68	0.64
C20:1	0.40	0.39	0.35
C20:3	0.33	0.29	0.24
Unidentified	0.68	0.78	1.09

[1] tUFA = total unsaturated fatty acids
[2] PUFA = polyunsaturated fatty acids
[3] MUFA = multiunsaturated fatty acids

Table 3. Metabolizable energy (**ME**) consumption and total fatty acid consumption for calves fed milk replacer replaced with 0, 10, or 20 percent whey cream.

	0WC[1]	10WC[2]	20WC[3]	SEM	*P*-value
ME from calf milk replacer	24.3	23.8	24.0	0.73	0.56
Total ME	43.4	44.4	44.4	1.3	0.51
Short and medium chain fatty acid consumption from milk replacer (g)	271[a]	329[b]	170[c]	7.9	<0.001
Long chain fatty acid consumption from milk replacer (g)	1083	1098	1063	33.0	0.39

[1] 0WC = 22:20 CMR, 0% whey cream inclusion
[2] 10WC = 22:20 CMR, 10% whey cream inclusion
[3] 20WC = 22:20 CMR, 20% whey cream inclusion

Table 4. Least square means for days to consume varying amounts of starter/day, total starter consumed by day 49 and 56, average starter consumption, body weight gain by day 49 and 56, average daily gain, and gain:feed ratio for calves fed non-medicated calf milk replacers with various inclusions of whey cream.

	Treatment[1]				
	0WC	10WC	20WC	SEM[2]	*P*-value
Days to consume					
250 (g)	18.0	15.5	15.3	1.9	0.15
500 (g)	23.2[a]	19.4[b]	20.6[ab]	1.8	0.03
1000 (g)	32.3	30.8	30.0	1.8	0.20
2000 (g)	45.0	44.4	44.1	1.2	0.45
Total starter consumed (kg)					
Day 49	39.0	43.4	45.0	3.3	0.08
Day 56	61.1	66.1	67.0	4.2	0.17
Average starter consumed/day (kg)	1.0	1.1	1.1	0.07	0.17
Total body weight gain (kg)					
Day 49	37.8	39.7	39.0	1.9	0.30
Day 56	46.4	49.8	48.9	2.4	0.12
Average daily gain (kg)	0.83	0.91	0.84	0.03	0.13
Gain:feed					
Day 49	0.60	0.60	0.57	0.03	0.74
Day 56	0.55	0.57	0.55	0.03	0.91
Analyzed feed digestibility, day 49 (%)	76.7	73.4	75.6	3.1	0.37

[a.b] Main effects of diet in the same row with different superscripts differ ($P \leq 0.05$)
[1] Treatments: 0WC = 22:20 CMR, 0% whey cream inclusion; 10WC = 22:20 CMR, 10% whey cream inclusion; 20WC = 22:20 CMR, 20% whey cream inclusion
[2]The largest standard error of measurement (**SEM**) is reported.

Table 5. Least square means for growth gain from day 56 measurements compared to day 1 measurements for wither height, hip height, hip width, body length, and heart girth for calves fed non-medicated calf milk replacers with various inclusions of whey cream.

	Treatment[1]				
	0WC	10WC	20WC	SEM[2]	*P*-value
Total body weight gain (kg)	46.4	49.8	48.9	2.4	0.12
Average daily gain (kg)	0.83	0.91	0.84	0.05	0.13
Wither height gain (cm)	12.7	12.0	12.9	1.3	0.56
Hip height (cm)	13.6	14.1	13.0	0.96	0.21
Hip width (cm)	6.0	5.9	6.4	0.48	0.36
Body length (cm)	20.8	18.6	18.8	1.7	0.14
Heart girth (cm)	25.6[a]	22.6[b]	22.8[b]	1.4	0.05

[a.b] Main effects of diet in the same row with different superscripts differ ($P \leq 0.05$)
[t] = tendency ($0.05 < P \geq 0.10$) for 20WC above 0WC.
[1] Treatments: 0WC = 22:20 CMR, 0% whey cream inclusion; 10WC = 22:20 CMR, 10% whey cream inclusion; 20WC = 22:20 CMR, 20% whey cream inclusion
[2]The largest standard error of measurement (**SEM**) is reported.

Table 6. Least square means for average fecal scores, scour days, days treated, and milk refusal days from day 1 to 56 for calves fed non-medicated calf milk replacers with various inclusions of whey cream.

	Treatment[1]				
	0WC	10WC	20WC	SEM[2]	*P*-value
Average fecal score[3]	0.4	0.3	0.44	0.09	0.09
Scour days[4]	2.84	1.93	2.31	0.92	0.28
Days treated[5]	1.71	1.92	2.2	0.52	0.35
Milk refusal days[6]	0.67	0.96	0.94	0.38	0.39

[a.b] Main effects of diet in the same row with different superscripts differ ($P \leq 0.05$)
[1] Treatments: 0WC = 22:20 CMR, 0% whey cream inclusion; 10WC = 22:20 CMR, 10% whey cream inclusion; 20WC = 22:20 CMR, 20% whey cream inclusion
[2]The largest standard error of measurement (**SEM**) is reported.
[3]Average fecal score = Based on calf health scoring criteria where 0 = normal fecal appearance, 1 = semi-formed, pasty, 2 = loose, but stays on top of bedding, 3 = watery, sifts through bedding.
[4]Scour days = Days a calf received a fecal score of 2 or 3. Because fecal scores were taken thrice weekly, each scoring day received 2 scour days for each scour score.
[5]Days treated = Number of days calf received medication and/or electrolytes.
[6]Milk refusal days = Number of days a calf refused a portion or all of a milk feeding.

Table 7. Least square means for first three weeks of trial for fecal scores, scour days, body temperature, and body weight gain by week for calves fed non-medicated calf milk replacers with 0,10, or 20 percent inclusion of whey cream.

	Treatment[1]				
	0WC	10WC	20WC	SEM[2]	*P*-value
Average fecal score[3]					
Week 1	0.87	0.65	0.97	0.14	0.12
Week 2	0.81[ab]	0.61[a]	1.0[b]	0.14	0.03
Week 3	0.27	0.46	0.34	0.13	0.27
Scour days[4]					
Week 1	0.88	1.1	1.0	0.30	0.61
Week 2	1.4[ab]	0.79[b]	1.5[a]	0.29	0.09
Week 3	0.40	0.42	0.17	0.26	0.51
Temperature (°C)					
Week 1	38.8[a]	38.7[ab]	38.6[b]	0.06	0.05
Week 2	38.6	38.8	38.7	0.05	0.12
Week 3	38.6	38.6	38.6	0.05	0.95
Weekly Body Weight Gain					
Week 1	3.3[a]	3.8[ab]	2.3[a]	0.38	0.004
Week 2	2.3	2.4	2.8	0.38	0.43
Week 3	4.3	4.4	4.3	0.38	0.84

[a.b] Main effects of diet in the same row with different superscripts differ ($P \leq 0.05$)
[1] Treatments: 0WC = 22:20 CMR, 0% whey cream inclusion; 10WC = 22:20 CMR, 10% whey cream inclusion; 20WC = 22:20 CMR, 20% whey cream inclusion
[2]The largest standard error of measurement (**SEM**) is reported.
[3]Average fecal score = Based calf health scoring criteria where 0 = normal fecal appearance, 1 = semi-formed, pasty, 2 = loose, but stays on top of bedding, 3 = watery, sifts through bedding.
[4]Scour days = Days a calf received a fecal score of 2 or 3. Fecal scores were taken thrice weekly, thus, each scoring day received 2 scour days for each scour score.

Table 8. Correlations for the first three weeks between average fecal score, scour days, weekly body weight gain, and rectal temperature.

	Average fecal score	Body temperature	Weekly body weight gain
Scour days	0.81*	-0.21***	-0.31*
Weekly body weight gain	-0.03*	-0.02	
Body temperature	-0.16**		

* $P \leq 0.0001$
** $P \leq 0.03$
*** $P \leq 0.004$

Table 9. Least square means for days to consume targeted amount of starter grain/day, total starter consumed by day 49 and 56, average starter consumption, body weight gain by day 49 and 56, average daily gain, and gain:feed ratio for harvested bull calves fed calf milk replacers with various inclusions of whey cream.

	Treatment[1]				
	0WC	10WC	20WC	SEM[2]	*P*-value
Days to consume					
250g	20.8	18.7	17.7	2.7	0.41
500g	27.2	22.8	21.0	2.4	0.09
1000g	34.7[a]	31.5[ab]	28.2[b]	2.0	0.04
2000g	46.0	44.3	41.5	1.9	0.11
Total starter consumed (kg)					
Day 49	34.1	41.3	47.9	4.7	0.06
Day 56	55.6	63.9	71.1	5.5	0.07
Average starter consumed (kg/d)	1.0	1.1	1.1	0.07	0.17
Total body weight gain (kg)					
Day 49	37.0	39.8	40.3	2.4	0.34
Day 56	45.7	50.6	51.7	2.7	0.14
Average daily gain (kg)	0.99	1.14	1.27	0.10	0.07
Gain:feed					
Day 49	0.59	0.58	0.53	0.03	0.14
Day 56	0.54	0.55	0.52	0.02	0.31

[a.b] Main effects of diet in the same row with different superscripts differ ($P \leq 0.05$)
[1] Treatments: 0WC = 22:20 CMR, 0% whey cream inclusion; 10WC = 22:20 CMR, 10% whey cream inclusion; 20WC = 22:20 CMR, 20% whey cream inclusion
[2]The largest standard error of measurement (**SEM**) is reported.

Table 10. Least square means for gastrointestinal and organ measurements and average goblet cell count (from the duodenum microscopically at 40X) for calves fed a 22% protein, 20% milk replacer with 0, 10, or 20 % composed of whey cream.

	Treatment[1]				
	0WC	10WC	20WC	SEM[2]	*P*-value
Reticulorumen					
Empty weight (kg)	1.65	1.68	1.72	0.09	0.61
% of BW	1.82	1.83	1.82	0.08	0.89
Omasum					
Empty weight (kg)	0.39	0.43	0.49	0.03	0.07
% of BW	0.47	0.50	0.46	0.04	0.57
Abomasum					
Empty weight (kg)	0.49	0.52	0.54	0.05	0.46
% of BW	0.54	0.57	0.58	0.04	0.52
Small Intestine					
Empty weight (kg)	2.38[a]	2.98[b]	3.14[b]	0.16	0.02
% of BW	2.63[a]	3.27[b]	3.37[b]	0.21	0.05
Length (cm)	2160.3	2445.9	2308.6	139.2	0.31
Length of BW (cm/kg)	24.0	26.7	25.0	1.6	0.45
Cecum					
Empty weight (kg)	0.75[a]	0.89[ab]	1.08[b]	0.09	0.02
% of BW	0.83[a]	0.96[ab]	1.11[b]	0.08	0.02
Length (cm)	368.4	303.5	298.1	41.8	0.42
Length of BW (cm/kg)	4.0	3.2	3.2	0.46	0.39
Goblet Cells (#)	12.7[a]	10.4[b*]	8.6[b]	0.86	0.004

[a.b] Main effects of diet in the same row with different superscripts differ ($P \leq 0.05$)
[1] Treatments: 0WC = 22:20 CMR, 0% whey cream inclusion; 10WC = 22:20 CMR, 10% whey cream inclusion; 20WC = 22:20 CMR, 20% whey cream inclusion
[2]The largest standard error of measurement (**SEM**) is reported.

Table 11. Histological scores given to duodenum appearance and mucosa, ruminal mucosa and papillae by a licensed veterinarian for calves harvested at 56 days of age.

Score	Treatment[1]			SEM[2]	*P*-value
	0WC	10WC	20WC		
Duodenum appearance[3]	1.67	1.33	1.67	1.48	0.61
Duodenal inflammation[4]	1.33	1.83	1.50	0.55	0.38
Ruminal appearance[5]	1.67	1.83	2.12	0.68	0.48
Papillae score[6]	1.67	2.00	1.83	0.47	0.63

[1] Treatments: 0WC = 22:20 CMR, 0% whey cream inclusion; 10WC = 22:20 CMR, 10% whey cream inclusion; 20WC = 22:20 CMR, 20% whey cream inclusion

[2]The largest standard error of measurement (**SEM**) is reported.

[3] Duodenum appearance: duodenal tissue was evaluated by the naked eye by placing the slide on a white piece of paper for mucosa thickness and assigned a score of: 0 – normal, 1 – mildly thickened, 2 – moderately thickened, 3 – markedly thickened.

[4] Duodenal inflammation: Duodenal tissue (villi) was examined microscopically for the presence of lymphocytes and inflammatory cells and assigned a score of: 0 – no inflammation, 1 – mild inflammation, 2 – moderate inflammation, 3 – marked inflammation.

[5] Ruminal appearance: rumen papillae tissue was evaluated by the naked eye by placing the slide on a white piece of paper for appearance and assigned a score of: 0 – normal, 1 – mildly thickened, 2 – moderately thickened, 3 – markedly thickened.

[6] Papillae score: papillae were examined microscopically for appearance and fusion and assigned a score of: 0 – tall and slender, 1 – mildly blunted, 2 – moderately blunted, 3 – markedly blunted.

Table 12. pH and acetic, propionic, butyric, and ammonia concentrations for bull calves harvested at 56 days old and fed a milk replacer with 0, 10, or 20% inclusion of whey cream until day 49.

	Treatment[1]				
	0WC	10WC	20WC	SEM[2]	*P*-value
pH	5.71	6.02	5.91	0.21	0.31
Total VFA (mmol/L)	111.1	93.5	97.1	13.2	0.36
Acetic					
mmol/L	53.3	46.9	50.0	5.5	0.42
%	48.1	51.1	52.9	2.0	0.11
Propionic					
mmol/L	48.6	39.4	36.8	7.1	0.26
%	43.6	38.8	36.5	2.8	0.09
Butyric					
mmol/L	9.2	8.1	10.4	2.5	0.52
%	8.3	10.1	10.6	2.2	0.46
Ammonia (mmol/L)	0.016	0.019	0.012	0.003	0.06

[a, b] Values with differing superscripts in the same row differ ($P \leq 0.05$)
[1] Treatments: 0WC = 22:20 CMR, 0% whey cream inclusion; 10WC = 22:20 CMR, 10% whey cream inclusion; 20WC = 22:20 CMR, 20% whey cream inclusion
[2]The largest standard error of measurement (**SEM**) is reported.

Table 13. Nutrient composition of milk replacer with all milk protein (CON) or protein where 5% was from soy isolate and 17% was from milk protein (SOY), and starter grain.

	Milk replacer (CON)	Milk replacer (SOY)	Starter grain
		% DM	
Dry Matter	96.7	96.7	88.0
Crude protein, %	21.6	21.6	19.7
Ether extract, %	19.5	19.5	3.9
Starch, %	.	.	38.0
ME, Mcal/kg	0.39	0.39	0.36
ADF	0.01	0.01	10.3
aNDF, %	0.07	0.07	20.9
Ash, %	9.5	9.5	6.3

Table 14. Treatment response for body weight gain, dry matter intake from starter grain, and gain:feed for calves fed one of two milk replacers (CONTROL or SOY).

	Treatment			
	CONTROL	SOY	SEM[1]	*P* - value
Body weight gain (kg)				
Day 49	36.3	38.0	2.8	0.67
Day 56	44.3	45.4	1.8	0.66
Average daily gain	0.79	0.81	0.03	0.66
Dry matter intake (kg)				
Total, day 49	31.8	37.0	3.2	0.26
Total, day 56	51.5	57.9	4.9	0.37
Average daily DMI	0.92	1.03	0.09	0.37
Gain:feed				
Day 49	0.54	0.53	0.02	0.79
Day 56	0.52	0.49	0.01	0.25

[1]The largest standard error of measurement (**SEM**) is reported.

Table 15. Least square means body measurements from day 56 measurements compared to day 1, average fecal score, milk refusal days, and day treated for calves fed non-medicated calf milk replacers with protein from either whey (CONTROL), or soy isolate and whey (SOY).

	Treatment			
	CONTROL	SOY	SEM[1]	*P*-value
Wither height gain (cm)	13.0	11.4	1.3	0.39
Hip height gain (cm)	12.3	12.5	1.2	0.90
Hip width gain (cm)	6.5	6.3	0.7	0.84
Body length gain (cm)	19.9	20.7	1.4	0.67
Heart girth gain (cm)	23.4	24.3	1.3	0.63
Average fecal score	0.20	0.46	0.14	0.19
Milk refusal days	0.08	1.1	0.34	0.07
Days treated	0.18	0.29	0.14	0.56

[1]The largest standard error of measurement (**SEM**) is reported.

Table 16. Milk replacer and starter grain nutrient composition fed to calves to examine medium chain fatty acid inclusion on calf health and growth.

	Control[1]	MCFA[2]	Starter
Dry Matter (%)	96.7	96.8	87.9
Crude Protein (% DM)	28.1	27.7	26.3
Fat (% DM)	21.3	21.0	3.7
Starch (% DM)	.	.	30.5
ME, Mcal/kg DM	4.83	4.80	3.24
ADF	.	.	10.4
aNDF	0.02	0.08	19.2
Ash (% DM)	10.28	10.80	7.2

[1]Control : Milk replacer using lard as a fat source.
[2]FA: Milk replacer with a fat source from a combination of lard and medium chain triglycerides.

Table 17. Milk replacer fatty acid composition fed to calves to examine medium chain fatty acid inclusion on calf health and growth.

	Control[1]	MCFA[2]
Total fatty acid % of DM	22.55	21.53
Saturated fat (%)	9.42	10.49
Omega 3 fat (%)	0.20	0.21
Omega 6 fat (%)	3.63	2.94
Trans fat (%)	0.27	0.24
Cis-monounsaturated fat (%)	8.02	6.59
Cis-polyunsaturated fat (%)	3.83	3.15
Fatty Acid (%)		
$C_{4:0}$	0.3	0.4
$C_{6:0}$	0.2	0.2
$C_{8:0}$	0.8	8.6
$C_{10:0}$	0.9	7.1
$C_{12:0}$	0.4	0.5
$C_{14:0}$	2.5	2.3
$C_{14:1}$	<0.1	0.1
$C_{15:0}$	0.2	0.2
$C_{16:0}$	24.5	20.6
$C_{16:1}$	1.6	1.4
$C_{17:0}$	0.4	0.4
$C_{17:1}$	0.2	0.2
$C_{18:0}$	13.2	10.9
$C_{18:1}$	35.8	30.8
$C_{18:2}$	16.1	13.8
$C_{18:3}$	0.8	0.8
$C_{20:0}$	0.2	0.2
$C_{20:1}$	0.6	0.5
$C_{20:2}$	0.5	0.4

$C_{20:3}$	<0.1	0.1
$C_{20:4}$	0.3	0.2
$C_{21:0}$	0.2	0.1
Unidentified FAs	0	0.2

[1]Control : Milk replacer using lard as a fat source.
[2]FA: Milk replacer with a fat source from a combination of lard and medium chain triglycerides.

Table 18. Growth performance, grain intake, and efficiency for calves fed one of two milk replacers (**MR**) containing with or without added medium chain fatty acids at a low or high plane of milk nutrition.

	Treatment[1]					*P*-value		
	LOW CONTROL	HIGH CONTROL	LOW MCFA	HIGH MCFA	SEM[2]	Plane	MR	Plane* MR
Starter DMI (kg)								
Day 43	26.9	10.8	26.4	11.7	1.6	<0.001	0.88	0.68
Day 56	54.5	32.3	56.0	35.9	3.0	<0.001	0.38	0.74
Average daily intake	0.97	0.58	1.0	0.64	0.05	<0.001	0.37	0.76
Body weight gain (kg)								
Day 43	29.2	30.9	27.0	33.0	1.2	0.001	0.99	0.07
Day 56	44.0[a]	44.6[a]	42.1[a]	49.2[b]	1.7	0.02	0.39	0.05
Average daily gain	0.80[a]	0.80[a]	0.76[a]	0.88[b]	0.03	0.03	0.49	0.04
Gain:feed								
Day 43	0.60	0.74	0.56	0.73	0.02	<0.001	0.20	0.35
Day 56	0.59	0.65	0.56	0.67	0.01	<0.001	0.67	0.12
Day 43 DM digestibility (%)	84.8	89.0	83.4	90.6	2.1	0.005	0.99	0.43

[1] Treatment: LOW CONTROL: Control milk replacer fed at 1.25% of birth body weight (**BBW**) from day 1-42, 0.625% of BBW once daily 43-49, remained on trial until day 56; HIGH CONTROL: Control milk replacer fed at 1.8% of BBW from day 1-9, 2.5% of BBW day 10-42, 1.25% of BBW day 43-49, remained on trial until day 56; LOW MCFA: Milk replacer formulated with medium chain fatty acids fed at 1.25% of BBW from day 1-42, 0.625% of BBW once daily 43-49, remained on trial until day 56; HIGH CONTROL: Milk replacer formulated with medium chain fatty acids fed at 1.8% of BBW from day 1-9, , 2.5% of BBW day 10-42, 1.25% of BBW day 43-49, remained on trial until day 56

[2] The largest standard error of measurement (**SEM**) is reported.

Table 19. Growth measurements, health, and fecal responses for calves fed one of two milk replacers (**MR**) containing with or without added medium chain fatty acids at a low or high plane of milk nutrition.

	Treatment[1]					*P*-value		
	LOW CONTROL	HIGH CONTROL	LOW MCFA	HIGH MCFA	SEM[2]	Plane	MR	Plane *MR
Wither height gain (cm)	13.3	13.8	12.2	14.5	0.6	0.02	0.75	0.13
Hip height gain (cm)	12.6	15.1	12.2	15.5	0.6	<0.001	0.96	0.48
Body length gain (cm)	19.5	21.3	18.0	21.5	1.0	0.02	0.56	0.42
Hip width gain (cm)	5.9	5.6	5.8	5.9	0.3	0.59	0.54	0.35
Heart girth gain (cm)	25.0[ab]	25.2[ab]	23.5[a]	26.7[b]	0.7	0.02	0.99	0.05
Milk refusal days[3]	0.19	0.18	0.06	0.12	0.10	0.80	0.34	0.72
Treated days[4]	0.48	0.42	0.69	0.60	0.21	0.73	0.35	0.95
Average fecal score	0.30[a]	0.39[a]	0.42[b]	0.46[b]	0.05	0.19	0.06	0.56
Days scouring	2.3	2.7	3.2	3.0	0.5	0.83	0.22	0.60
Average respiratory score	0.0004[a]	0.08[b]	0.04[ab]	0.02[ab]	0.03	0.35	0.70	0.05

[1] Treatment: LOW CONTROL: Control milk replacer fed at 1.25% of birth body weight (**BBW**) from day 1-42, 0.625% of BBW once daily 43-49, remained on trial until day 56; HIGH CONTROL: Control milk replacer fed at 1.8% of BBW from day 1-9, 2.5% of BBW day 10-42, 1.25% of BBW day 43-49, remained on trial until day 56; LOW MCFA: Milk replacer formulated with medium chain fatty acids fed at 1.25% of BBW from day 1-42, 0.625% of BBW once daily 43-49, remained on trial until day 56; HIGH CONTROL: Milk replacer formulated with medium chain fatty acids fed at 1.8% of BBW from day 1-9, , 2.5% of BBW day 10-42, 1.25% of BBW day 43-49, remained on trial until day 56

[2] The largest standard error of measurement (**SEM**) is reported.

[3]Milk refusal days were recorded day 1 – 49 when milk was offered.

[4]Treated days were recorded day 1 – 56 while animals were on trial.

Table 20. Harvested calf (day 22) weekly response for daily starter intake (**SI**), body weight (**BW**) gain, average weekly fecal score (**FS**), days scouring, and serum non-esterified fatty acids (**NEFA**) for calves fed one of two milk replacers (**MR**) at two varying amounts.

	Treatment[1]					*P*-value				
	LOW CONTROL	HIGH CONTROL	LOW MCFA	HIGH MCFA	SEM[2]	Plane	MR	Week	Plane* MR	Plane*MR* Week
SI (kg)	0.22	0.15	0.21	0.14	0.04	0.16	0.79	<0.001	0.94	0.03
BW gain (kg)	3.1	5.5	4.0	5.0	0.51	0.005	0.61	0.003	0.16	0.10
Average weekly FS	0.56	0.88	0.74	0.76	0.19	0.36	0.89	0.21	0.40	0.67
Days scouring/week	0.60	0.91	0.33	0.93	0.32	0.19	0.69	0.71	0.64	0.37
Serum NEFA (uEq/L)	127	161	128	108	17.5	0.66	0.11	0.24	0.10	0.56

[1] Treatments: LOW CONTROL: Control milk replacer fed at 1.25% of birth body weight from day 1-22; HIGH CONTROL: Control milk replacer fed at 1.8% of BBW from day 1-9, 2.5% of birth body weight day 10-22; LOW MCFA: Milk replacer formulated with medium chain fatty acids fed at 1.25% of birth body weight from day 1-22; HIGH CONTROL: Milk replacer formulated with medium chain fatty acids fed at 1.8% of BBW from day 1-9, 2.5% of birth body weight day 10-22

[2] The largest standard error of measurement (**SEM**) is reported.

Table 21. Tissue measurements from calves harvested on day 22 of age and fed one of two milk replacers (**MR**) at a low or high plane of milk nutrition.

	Treatment[1]					*P*-value		
	LOW CONTROL	HIGH CONTROL	LOW MCFA	HIGH MCFA	SEM[2]	Plane	MR	Plane *MR
BW gain (kg)	7.5	15.6	11.3	13.2	2.4	0.05	0.75	0.21
Reticulorumen								
Full (kg)	2.7	1.9	2.6	2.5	0.4	0.33	0.52	0.44
Empty (kg)	0.48	0.43	0.51	0.49	0.06	0.55	0.47	0.84
% of body weight	1.0	0.79	1.0	0.89	0.11	0.14	0.72	0.64
Omasum								
Full (kg)	0.15	0.12	0.17	0.14	0.02	0.18	0.32	0.83
Empty (kg)	0.11	0.10	0.13	0.13	0.01	0.85	0.12	0.91
% of body weight	0.22	0.19	0.26	0.23	0.02	0.27	0.16	0.82
Abomasum								
Full (kg)	1.18	1.33	1.19	1.7	0.31	0.29	0.54	0.55
Empty (kg)	0.29	0.35	0.29	0.34	0.02	0.004	0.98	0.77
% of body weight	0.61	0.65	0.58	0.64	0.05	0.31	0.78	0.77
Small intestine								
Full (kg)	2.5	2.7	2.8	3.0	0.33	0.35	0.68	0.99
Empty (kg)	1.3	1.7	1.6	1.8	0.15	0.08	0.30	0.72
% of body weight	2.8	3.1	3.1	3.3	0.29	0.38	0.41	0.99
Length (cm)	2003.0	2081.0	1898.0	2103.0	103.0	0.68	0.19	0.54
Cecum								
Full (kg)	0.68	0.74	0.66	0.67	0.08	0.61	0.50	0.74
Empty (kg)	0.36	0.42	0.41	0.44	0.05	0.45	0.48	0.72
% of body weight	0.78	0.77	0.81	0.81	0.10	0.97	0.69	0.99
Length (cm)	264.0	285.0	272.0	260.0	19.0	0.81	0.58	0.38

[1] Treatment: LOW CONTROL: Control milk replacer fed at 1.25% of birth body weight from day 1-22; HIGH CONTROL: Control milk replacer fed at 1.8% of BBW from day 1-9, 2.5% of birth body weight day 10-22; LOW MCFA: Milk replacer formulated with medium chain fatty acids fed at 1.25% of birth body weight from day 1-22; HIGH CONTROL: Milk replacer formulated with medium chain fatty acids fed at 1.8% of BBW from day 1-9, 2.5% of birth body weight day 10-22

[2] The largest standard error of measurement (**SEM**) is reported.

Table 22. Calf pH and volatile fatty acid values from rumen fluid and duodenum digesta, papillae and villus height, and crypt depth, from calves harvested on day 22 of age and fed one of two milk replacers (**MR**) at a low or high plane of milk nutrition. The largest standard error of measurement (**SEM**) is reported.

	Treatment[1]					*P*-value		
	LOW CONTROL	HIGH CONTROL	LOW MCFA	HIGH MCFA	SEM[2]	Plane	MR	Plane *MR
Rumen								
pH	5.27	5.46	5.48	5.54	0.18	0.47	0.41	0.70
Total VFA (mmol/L)	136.7	111.6	152.1	108.5	19.0	0.07	0.73	0.61
Acetic acid (mmol/L)	67.0	74.5	58.0	58.9	7.1	0.11	0.58	0.66
Propionic acid (mmol/L)	49.8	37.7	63.9	35.5	11.7	0.08	0.59	0.46
Butyric acid (mmol/L)	19.8	16.0	13.7	14.2	4.0	0.70	0.36	0.61
Lactic acid (mmol/L)	0.9	0.4	0.9	0.8	0.50	0.59	0.63	0.70
Papillae height (µm)	880	1008	990	848	76	0.75	0.93	0.09
Duodenum								
pH	6.30	6.60	6.26	6.74	0.21	0.80	0.07	0.67
Total VFA (mmol/L)	2.6	4.8	3.3	4.2	1.4	0.25	0.97	0.62
Acetic acid (mmol/L)	3.5	4.3	6.6	3.7	2.0	0.55	0.48	0.32
Propionic acid (mmol/L)	0.67	0.56	0.46	0.50	0.14	0.79	0.34	0.59
Lactic acid (mmol/L)	6.0[a]	9.2[b]	5.7[a]	4.7[a]	1.0	0.27	0.02	0.04
Villus height (µm)	213	221	207	220	26	0.69	0.89	0.93
Crypt depth (µm)	98	91	87	94	8	0.59	0.98	0.38

[1] Treatment: LOW CONTROL: Control milk replacer fed at 1.25% of birth body weight from day 1-22; HIGH CONTROL: Control milk replacer fed at 1.8% of BBW from day 1-9, 2.5% of birth body weight day 10-22; LOW MCFA: Milk replacer formulated with medium chain fatty acids fed at 1.25% of birth body weight from day 1-22; HIGH CONTROL: Milk replacer formulated with medium chain fatty acids fed at 1.8% of BBW from day 1-9, 2.5% of birth body weight day 10-22

[2] The largest standard error of measurement (**SEM**) is reported.

Figures

Figure 1. Least square means of serum non-esterified fatty acids (NEFA) concentration (uEq/L) for 8 weeks of life for calves fed milk replacers replaced with 0, 10, or 20 percent whey cream. Treatment had no effect (*P* < 0.61), treatment by week was not significant (*P* < 0.79), and week was significant (*P* < 0.001).

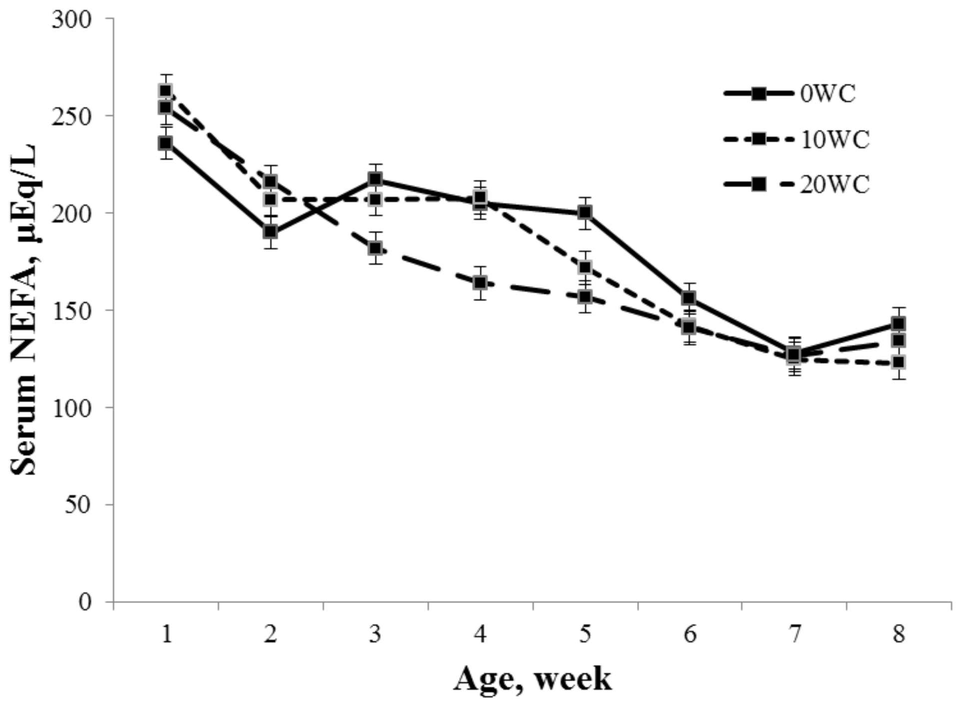

Figure 2. Weekly total starter intake (as-is) for calves fed non-medicated calf milk replacers with protein from either whey (CONTROL), or soy isolate and whey (SOY).

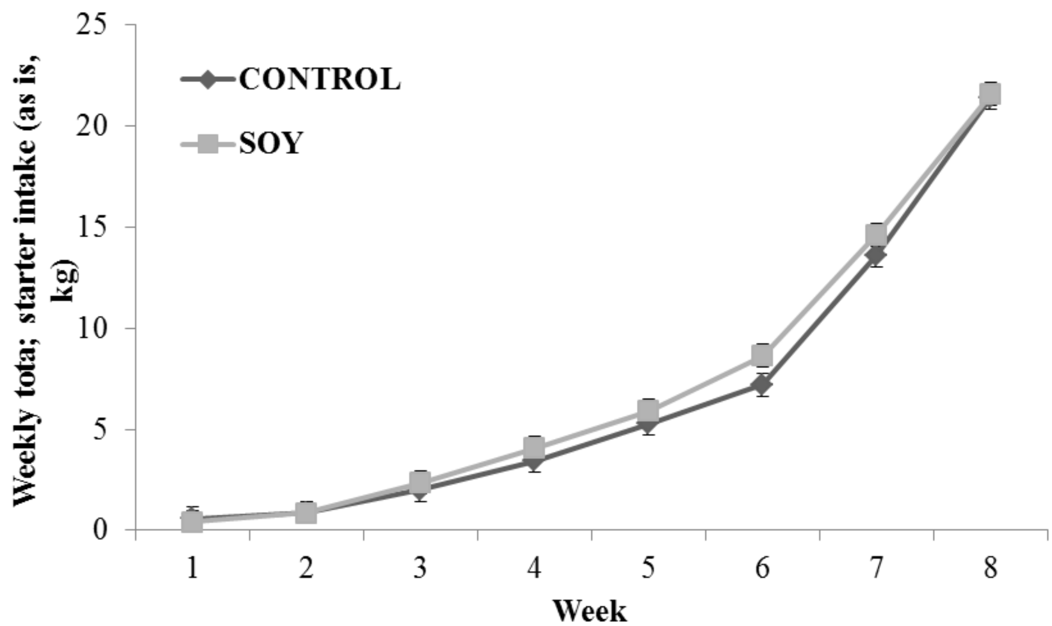

Figure 3. Weekly body weight gains for calves fed non-medicated calf milk replacers with protein from either whey (CONTROL), or soy isolate and whey (SOY).

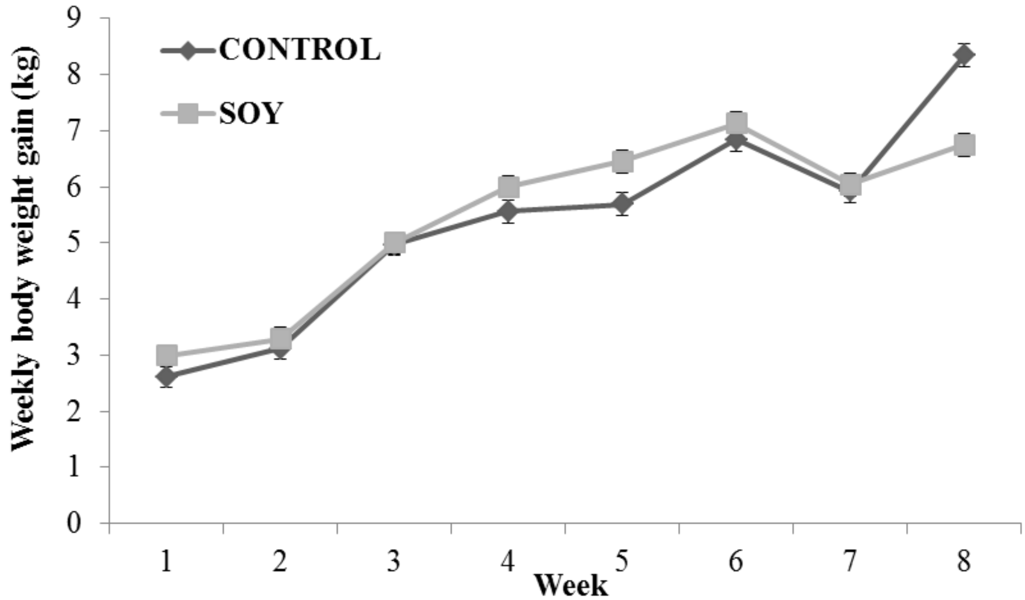

Figure 4. Average daily starter intake by week for calves receiving one of two milk replacers (**CON** or **MCFA**) fed at either a **LOW** (1.25% of birth body weight (**BBW**) day 1-42, 0.645% of BBW day 43-49, remain on trial until day 56) or **HIGH** (1.8% of birth body weight (**BBW**) day 1-9, 2.5% of BBW day 10-42, 1.25% of BBW day 43-49, remain on trial until day 56) plane of nutrition. Standard error was ± 0.09 kg. Beginning week 3 of life LOW calves consumed more grain ($P < 0.0001$) than HIGH calves. Milk replacer formulation did not affect consumption ($P < 0.56$). There was no plane by milk replacer interaction ($P < 0.75$). As week increased, daily starter intake increased ($P < 0.001$).

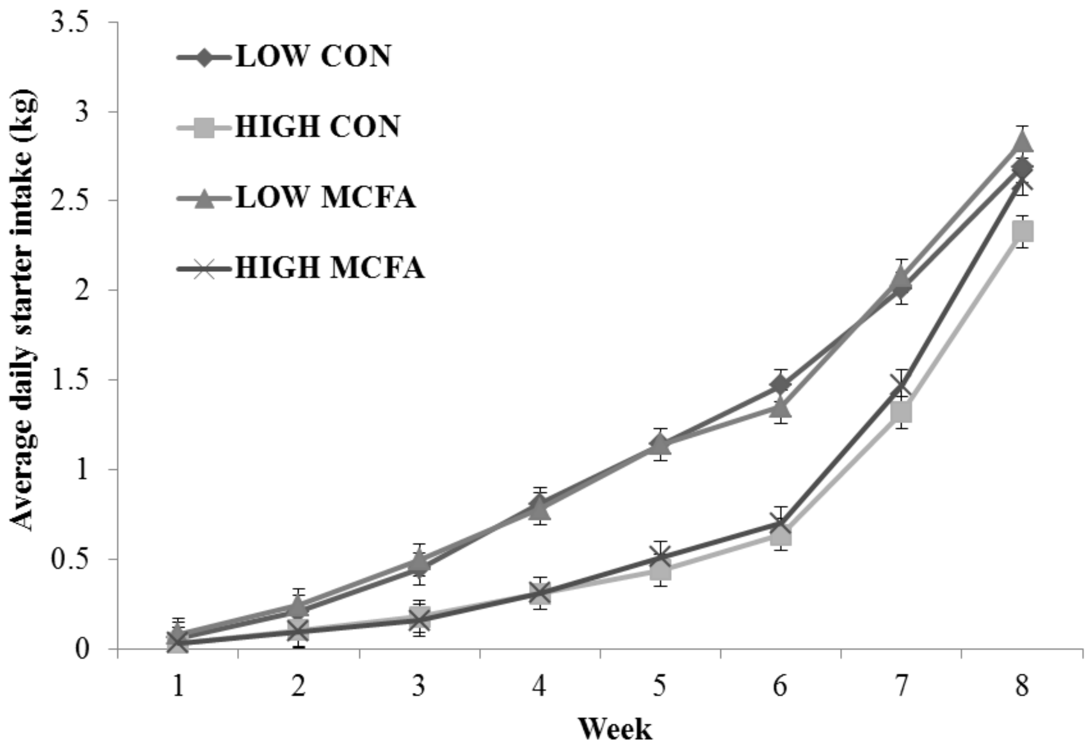

Figure 5. Weekly body weight gain for calves receiving one of two milk replacers (**CON** or **MCFA**) fed at either a **LOW** (1.25% of birth body weight (**BBW**) day 1-42, 0.645% of BBW day 43-49, remain on trial until day 56) or **HIGH** (1.8% of birth body weight (**BBW**) day 1-9, 2.5% of BBW day 10-42, 1.25% of BBW day 43-49, remain on trial until day 56) plane of nutrition. Standard error was ± 0.52 kg. HIGH calves had greater ($P < 0.05$) weekly body weight gain than LOW calves. Milk replacer formulation did not affect growth ($P < 0.58$). There was no plane by milk replacer interaction ($P < 0.55$). There was a week interaction ($P < 0.001$).

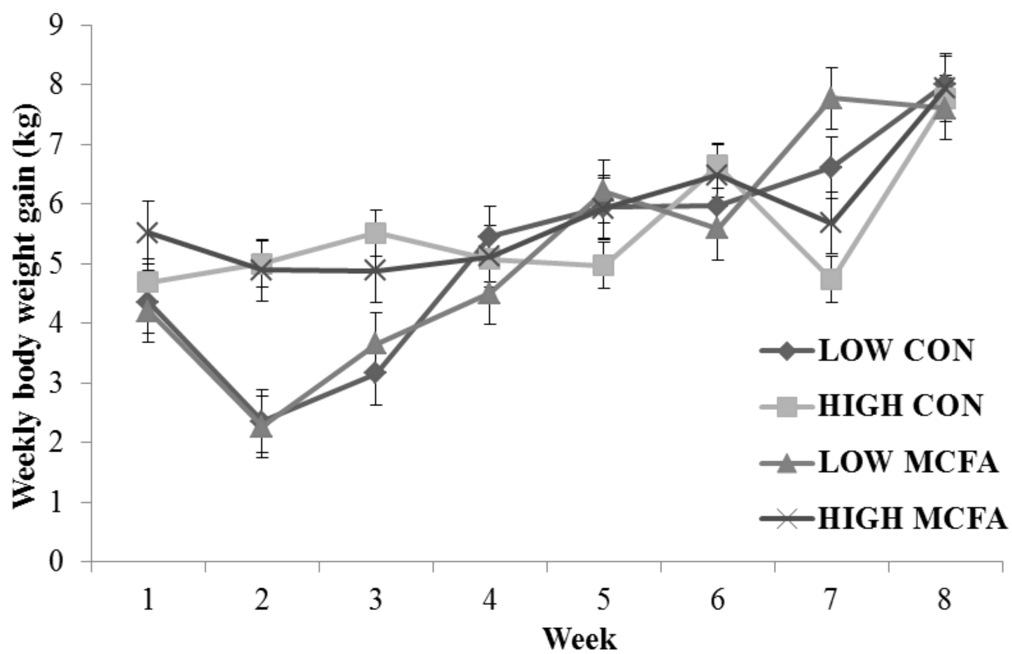

Figure 6. Weekly average fecal score for calves receiving one of two milk replacers (**CON** or **MCFA**) fed at either a **LOW** (1.25% of birth body weight (**BBW**) day 1-42, 0.645% of BBW day 43-49, remain on trial until day 56) or **HIGH** (1.8% of birth body weight (**BBW**) day 1-9, 2.5% of BBW day 10-42, 1.25% of BBW day 43-49, remain on trial until day 56) plane of nutrition. Standard error was ± 0.08 score. There was a week interaction ($P < 0.001$). Plane of nutrition, milk replacer formulation, and their interaction was not significant ($P < 0.13$).

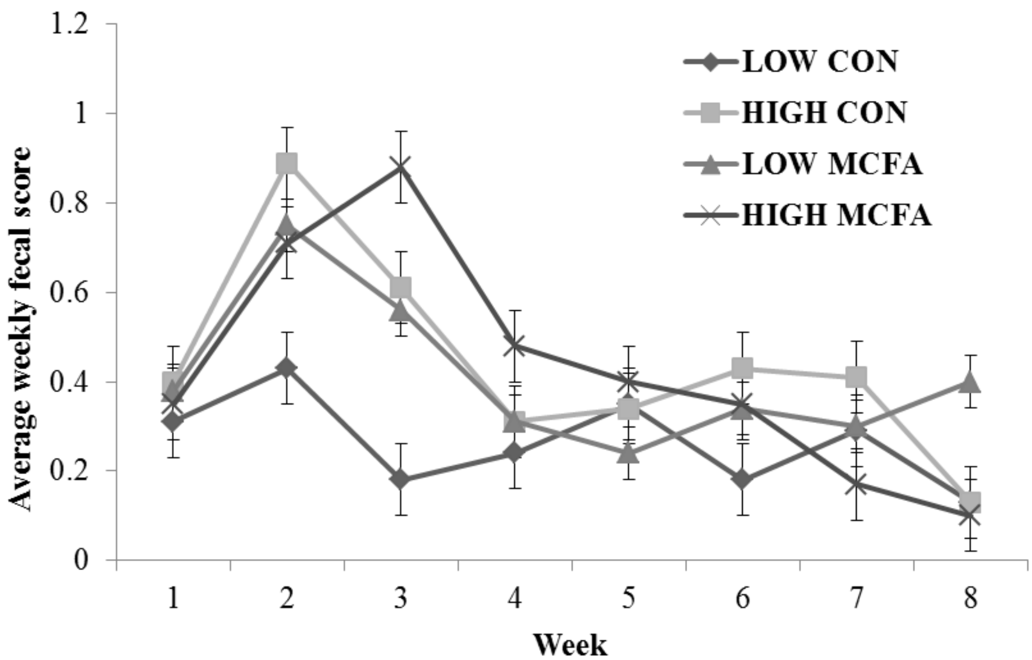

Figure 7. Weekly energy consumption (Mcal) from milk replacer plus starter grain for calves receiving one of two milk replacers (**CON** or **MCFA**) fed at either a **LOW** (1.25% of birth body weight (**BBW**) day 1-42, 0.645% of BBW day 43-49, remain on trial until day 56) or **HIGH** (1.8% of birth body weight (**BBW**) day 1-9, 2.5% of BBW day 10-42, 1.25% of BBW day 43-49, remain on trial until day 56) plane of nutrition. Standard error was ± 2.0 Mcal. HIGH calves had greater ($P < 0.0001$) energy consumption than LOW calves. Milk replacer formulation did not affect energy consumption ($P < 0.92$). There was no plane by milk replacer interaction ($P < 0.57$). There was a week interaction (P <0.0001).

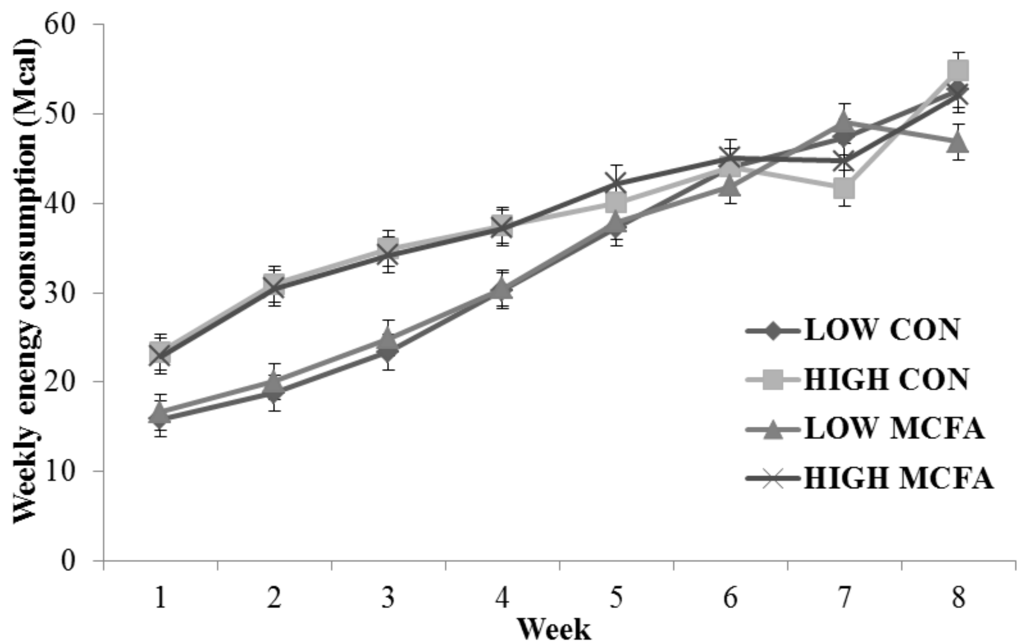

Figure 8. Weekly serum non esterified fatty acid (NEFA, uEq/L) for calves receiving one of two milk replacers (**CON** or **MCFA**) fed at either a **LOW** (1.25% of birth body weight (**BBW**) day 1-42, 0.645% of BBW day 43-49, remain on trial until day 56) or **HIGH** (1.8% of birth body weight (**BBW**) day 1-9, 2.5% of BBW day 10-42, 1.25% of BBW day 43-49, remain on trial until day 56) plane of nutrition. Standard error was ± 20.4 µEq/L. Plane of nutrition, milk replacer formulation, and their interaction was not significant ($P < 0.14$). Week tended to be significant ($P < 0.07$). There were no week by plane by milk replacer interactions ($P < 0.52$).

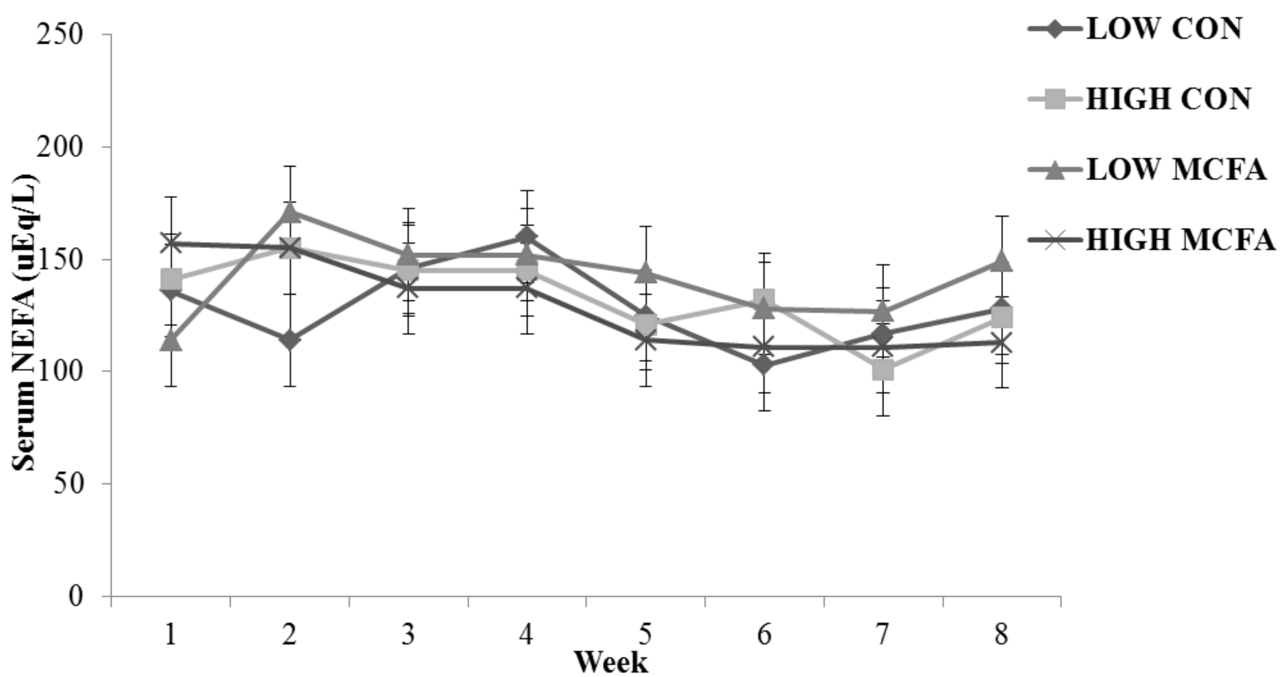

Figure 9. Weekly average body weight gain for calves receiving one of two milk replacers (**CON** or **MCFA**) fed at either a **LOW** (1.25% of birth body weight (**BBW**) day 1-22) or **HIGH** (1.8% of birth body weight (**BBW**) day 1-9, 2.5% of BBW day 10-22) plane of nutrition, harvested at 22 days of age. Standard error was ± 1.1 kg. HIGH calves had greater (*P* < 0.003) weekly body weight gain than LOW calves. Milk replacer formulation did not affect growth (*P* < 0.58). There was no plane by milk replacer interaction (*P* < 0.30).