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Effects of Feeding Different Amounts of Milk Replacer on Growth Performance and Nutrient Digestibility in Holstein Calves to 4 Months of Age Using Different Weaning Strategies

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EFFECTS OF FEEDING DIFFERENT AMOUNTS OF MILK REPLACER ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN HOLSTEIN CALVES TO 4 MONTHS OF AGE USING DIFFERENT WEANING STRATEGIES

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of the Requirements for the Degree
Master of Science
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by
Rebecca Naomi Klopp
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Accepted by:
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Dr. Vincent Richards
Dr. William Bridges
ABSTRACT

A 2x2 factorial design (moderate (MOD) or high (HI) milk replacer (MR) feeding rates and abrupt (AB) or gradual (GR) weaning) was utilized to compare how calf performance and nutrient digestibility is affected pre and post-weaning. The 112 d study consisted of a 56 d nursery and a 56 d grower phase. Calves (n=50) were randomly assigned to one of four pre-weaning treatments. Data were analyzed as a completely randomized design with repeated measures when applicable by PROC MIXED in SAS. Calves assigned to MOD-AB were fed 0.66 kg (DM) MR for first 42 d then 0.33 kg for 7 d, MOD-GR were fed 0.66 kg MR for 28 d, 0.33 kg for 14 d, and 0.17 kg for 7 d, HI-AB were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 28 d, and 0.66 kg for 7 d, HI-GR were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 14 d, 0.66 kg for 14 d, and 0.33 kg for 7 d. All calves received the same MR (25% CP, 17% fat) and were given ad libitum access to water and starter (42% starch and 20% CP) during the nursery phase. For the 56 d grower portion of the experiment, calves were grouped into 12 pens based on MR program, 4-5 calves per pen. All calves received ad libitum access to water and the same textured starter blended with 5% chopped grass hay. The results of the nursery phase indicated that HI MR promoted greater BW and FE, GR weaning lead to more starter consumption and lower HH, and HI MR digested readily available nutrients more efficiently but MOD MR resulted in increased fibrous fraction digestibility. The results of the grower phase indicated that HI calves lost their growth advantage over MOD calves after weaning, calves receiving MOD amounts of MR are more adapted to consuming complex nutrients, and GR weaning is beneficial during the weaning transition.
DEDICATION

I would like to dedicate this thesis to my parents, Richard and Denise Klopp. Without their unconditional love, support, and encouragement I would not be where I am today. Thanks, Mom and Dad!
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CHAPTER ONE

LITERATURE REVIEW

Introduction

Since the late 1800s, when dairy nutrition research began, the focus was on the lactating animal and its needs. It was not until the 1970s that there started to be an emphasis on calf nutrition and the development of the calves’ digestive tract (Kertz et al., 2017). When calves are born they are considered non-ruminants because the functionality of the rumen is limited, the abomasum is the primary site of digestion, and the amount of microbes present in the rumen is very limited. Calves are also born with no active immunity so it is important that calves receive colostrum, which is packed with antibodies and nutrients, right after birth to protect them from common diseases until their own immune system can protect them. Not only is it important that calves receive colostrum as soon as possible after birth but it is also crucial that the colostrum is high quality (> 50 mg of IgG/ml and < 100,000 cfu/ml of bacteria) to ensure successful passive transfer of immunity (plasma IgG >10 mg/mL; Godden, 2008).

The volume of colostrum that a calf consumes is vital to ensure successful passive transfer of immunity. Experts determined that calves should be fed at least 100 g of IgG in the first feeding of colostrum, which is the most prevalent immunoglobulin found in colostrum (Godden, 2008). It is recommended, that calves receive 4 L of high-quality colostrum at birth to meet the 100 g of IgG recommendation and then 2 L of high-quality colostrum 12 hours later to maximize the effects of passive immunity (Godden, 2008). After colostrum, calves will be switched to milk/milk replacer (depending on the farm’s
preference and resources) which will serve as the young calf’s main source of nutrients until their rumen starts to develop (Girard et al., 1992). There is debate within the industry regarding how much milk/milk replacer calves should receive. Some experts suggest feeding calves limited milk to encourage them to consume starter as soon as possible, which will start the development of the rumen (Hill et al., 2016c). Others suggest feeding calves elevated amounts of milk to promote high ADG pre-weaning (Jasper and Weary, 2002). Colostrum and milk are digested and absorbed by the only functioning stomach of young calves, the abomasum (Heinrichs and Jones, 2003). This is very different from mature cows who have a very large, functioning rumen with a thriving microbial population. Scientists are asking the question what exactly occurs as a calf transitions from a non-ruminant to a ruminant and what factors affect that transition.

Research shows that solid feed is necessary for a calf to transition from a non-ruminant to a ruminant (NRC, 2001). Calves should be given access to calf starter as soon as possible to start the development of the rumen and the transition to a ruminant animal. Readily fermented carbohydrates in solid feeds are converted to volatile fatty acids (VFAs) in the rumen and then these VFAs, particularly butyrate, stimulate papillae development so nutrients can be absorbed in the rumen (NRC, 2001). Feeding forages to calves when they are still consuming milk and starter is controversial. Some argue that calves in nature start consuming forages at a young age, however, most calving occurs in spring in nature, during this which time grass is immature and higher in sugar and lower in fiber (NRC, 2001). Today most calves do not receive forage at this stage of life because the rumen microbes do not have the capacity to ferment it until the rumen pH is
stabilized (Drackley, 2008). The physical form, for example pelleted, mashed, or
texturized (Bach et al., 2007; Porter et al., 2007; Hill et al., 2008b), carbohydrate source
and levels (Maiga et al., 1994; Khan et al., 2008; Hill et al., 2008b; 2008c), and
processing techniques (Abdelgadir and Morrill, 1995; Lesmeister and Heinrichs, 2005;
Bateman II et al., 2009) of calf starters can all alter its ability to stimulate the
development of the rumen. There is a lot to consider when devising the most effective
diet for young calves.

Another factor that can have a positive impact on rumen development is group
housing and socialization. Studies have shown that calves, who are housed in groups or
pairs, promote socialization and start to consume calf starter sooner than those houses
alone (de Paula et al., 2010; Costa et al., 2015).

Research has shown that healthy calves have higher ADG which has been linked
to decreased age at first breeding, higher milk yield, and reduced incidence of illness. The
first step to a healthy, efficient calf is the proper diet, weaning procedure, and care so
they can transition into ruminants. A vital aspect of this that has been left out of the
discussion, until now is how variations in the microbial population in the rumen can
affect the development of the rumen. Do healthy and sick calves have different microbial
species present in their rumens and can that knowledge then be used to treat sick calves?
That is the focal point of this thesis experiment.
The Newborn Calf

When calves are first born and up until about 2-3 weeks of age, they are considered non-ruminants with minimal microbes in their GI tract. Calves that are reared by their dam acquire anaerobes from her as well as their peers and the environment. Calves that are artificially reared, depend on their feed, housing, and handling situation to acquire anaerobes in their gastrointestinal tracts. (Khan et al., 2016)

Due to calves not possessing a functional rumen at birth, they rely on the abomasum, the only active stomach compartment, and its enzymes to digest the nutrients that are coming from milk or milk replacer (Davis and Drackley, 1998). Young calves have the ability for liquids to bypass the rumen via the esophageal groove and flow directly into the abomasum. The esophageal groove is stimulated by suckling and milk proteins and is formed by muscular folds of the reticulorumen coming together (Heinrichs and Jones, 2003). This creates the necessary acidic environment vital for the initiation of digestion and absorption in the lower gut. (Baldwin et al., 2004; Steele et al., 2016)

They also possess an immature immune system at birth because they do not acquire any immunity from their mother during gestation because antibodies cannot pass through the placental wall to the fetus (Heinrichs and Jones, 2003; Godden, 2008). Calves rely on colostrum (first milk produced after a dry period) to receive passive immunity after they are born. Colostrum is high in nutrients like proteins, fats, vitamins, and minerals (Heinrichs and Jones, 2003; Godden, 2008). Colostrum compared to milk contains a greater percentage of total solids (23.9 vs. 12.9), fat (6.7 vs. 4.0), protein (14.0
Within the first 24 hours after birth, calves can directly absorb intact antibodies into their bloodstream. After this 24-hour window closes, large molecules can no longer be absorbed by the cells lining the intestinal wall. In addition, the secretion of digestive enzymes is low at birth but starts increasing around 12 hours after birth leading to the breakdown of antibodies. This is why getting colostrum to calves as soon as possible after birth is vital for health and survival. (Heinrichs and Jones, 2003; Godden, 2008)

Two factors determine colostrum quality, antibody concentration, and bacteria concentration. The most prominent antibody in colostrum is immunoglobulin G (IgG) (Larson et al., 1980). Colostrum is considered high quality if it contains at least 50 mg/ml of IgG (McGuirk and Collins, 2004). Cleanliness is vital for low bacteria concentrations in colostrum. This means making sure calves are not receiving colostrum from cows with known infections or bloody colostrum. Milkers need to make sure the cow’s teats and udders are clean, sanitized, and dried before they start the milking process and that all the milking equipment is well maintained and working properly. Colostrum with high quality (IgG concentration above 50 mg/ml) and low bacteria counts, will give calves the best chances of absorbing sufficient antibodies to protect them from illnesses (Heinrichs and Jones, 2003; Godden, 2008).

**Gastrointestinal Epithelium**

To understand how ruminants absorb and digest nutrients, the gastrointestinal epithelium needs to be evaluated. A major role of the gastrointestinal epithelium is to
protect the host from the various chemicals, microorganisms, and toxins within the lumen (Gäbel et al., 2002; Steele et al., 2016). The gastrointestinal epithelium also regulates metabolism, nutrient absorption, and the delivery of nutrients to other parts of the body (Cant et al., 1996; Steele et al., 2016).

The epithelial lumen is divided into two sections. The reticulo-rumen and omasum are one section and they contain stratified squamous epithelium. The abomasum, small intestine, cecum, and large intestine (the lower gut) make up the second section, which contains columnar epithelium (Steele et al., 2016). The stratified squamous epithelium (SSE) contains papillae that protrude from its surface, this increases the surface area of the epithelial therefore increasing the absorption of minerals and short-chained fatty acids (SCFA) (Lavker and Matoltsy, 1970) as well as the secretion of bicarbonate (Aschenbach et al., 2011). The SSE is made up of 4 different layers. The first layer is the stratum basale followed by the stratum spinosum. Both of these layers contain mitochondria that partake in the metabolism of SCFA to ketones (Baldwin et al., 2004). The next layer is the stratum granulosum, which contains tight junctions that provide strength to the epithelium. The last layer is the stratum corneum. This layer comes into direct contact with the contents of the lumen and it protects the lower strata from coming into contact with the contents of the lumen (Graham and Simmons, 2005). The lower gut does not contain multiple layers it only contains one, the columnar layer. The lower gut encompasses cells that secrete protective substances, enzymes, hormones, and facilitate nutrient absorption (Peterson and Artis, 2014). The lower gut is the site where most
nutrients are absorbed into the blood so they can travel to other tissues in the body (Steele et al., 2016).

**Milk Feeding Programs**

A major goal when raising heifers is to have a feeding program that allows them to efficiently transition from a liquid diet to a solid feed diet without negative effects on growth and rumen development. Studies (Kertz et al., 1979; Baldwin et al., 2004) have looked at calf feeding programs that will decrease the age of weaning because when calves are being fed milk they are at the highest risk for diseases (Khan et al., 2011).

In order to decrease the age of weaning, researchers started to reduce calves’ milk consumption, inducing the newborn calf to fulfill their nutrient requirements by increasing solid feed consumption earlier (Khan et al., 2011). Some disadvantages have been seen with feeding calves a limited amount of milk. This strategy has shown poor weight gains (Flower and Weary, 2001), abnormal behaviors, which scientists believe is due to chronic hunger, as well as lower nutrient intake which can lead to high calf mortality and morbidity (USDA, 2007). Restricted milk feeding can lead to low nutrient availability (Appleby et al., 2001), decreased productivity (Pollock et al., 1993), reduced health (Huber et al., 1984), and depressed behavior (Chua et al., 2002). This led to the approach of feeding calves high rates or even ad libitum milk or MR with the objective to solve the issues seen with restricted feeding. Researchers started examining the effects of feeding calves increased amounts of milk or MR, and found that it can lead to an increase
in ADG (Diaz et al., 2001; Jasper and Weary, 2002) and it improves growth and feed efficiency pre-weaning (Brown et al., 2005; Khan et al., 2007a).

Traditionally, producers in the U.S. feed calves 10% of their body weight in milk/MR (Drackley, 2008). The idea of feeding calves more than 10% of their body weight brought with it the concern of increased scouring. Many studies (Huber et al., 1984; Appleby et al., 2001; Diaz et al., 2001) observed that simply feeding more milk/MR does not cause scouring, it is the presence of pathogens in the environment that cause scouring in calves. However, more milk/MR can cause softer feces.

Research does show that calves fed high rates or ad libitum milk/MR have reduced solid feed consumption because they are not getting hungry and relying on another feed to satisfy their needs. This can cause poor performance (Khan et al., 2007a), reduced ADG (Cowles et al., 2006), and reduced feed efficiency (Hill et al., 2006) post-weaning because rumen development is being delayed leading to decreased rumen function.

Another study (Terré et al., 2007) looked at how the amount of milk replacer fed to calves, in this case, either conventional (CF) or enhanced (EF) affects digestibility at weaning. CF calves received 4 l/d of MR at 12.5% DM dilution rate from d 1 to 28 of the study and 2 l/d (only in afternoon) from d 29-35. EF calves received 4 L/d of MR at 18% DM dilution rate from d 1-7, 6 L/d from d 8-14, 7 L/d from d 15-21, 6 L/d from d 22-28, and 3 L/d (only in afternoon) from d 29-35. They found that the final body weight was greater in EF compared to the CF calves. Starter DMI was greater in CF calves compared to the EF calves during pre-weaning and post-weaning but there was no difference in
total DMI. Apparent DM, OM, NDF, CP, and GE digestibility coefficients were greater in the CF calves compared to the EF calves one week after weaning. They concluded that EF calves had lower nutrient digestibility coefficients compared to the CF calves the week after weaning. This could be due to EF calves having lower starter intakes during the pre-weaning and post-weaning periods causing lower ruminal microbiota and decreased fermentation.

Hill et al. (2016c) investigated how milk replacer program affects calf performance and digestion of nutrients. The milk replacer (28% CP, 20% fat) programs for this study included MOD= 0.66 kg/day for 39 days and then 0.33 kg/day for 3 days; HIMOD= 0.88 kg/day for 5 days, then 1.1 kg/day for 23 days, then 0.66 kg/day for 18 days, and last 0.33 kg/day for 3 days; HI= 0.88 kg/day for 5 days, then 1.1 kg/day 37 days, and then 0.56 kg/day for 7 days. They concluded that feeding more than 0.66 kg/day of MR pre-weaning, did not increase body weight and hip width gain between birth and 4 months of age. Calves fed HIMOD and HI had less starter intake between 3-8 weeks of age and they tended to have looser feces compared to the calves fed MOD. Digestibility of DM, OM, ADF, and NDF at 11 weeks of age was less for HI compared to MOD calves. However, HIMOD calves had similar digestibility estimates to the MOD calves, meaning the HIMOD program allowed calves’ digestive systems to develop similar to the MOD program.

As research continued to show that large amounts of MR leads to increased ADG before weaning but leads to reduced growth post-weaning due to decreased nutrient digestibility, researchers started investigating the effects of supplementing extra nutrients
with a lower amount of milk/MR, to help improve ADG while still promoting rumen
development. Hill et al. (2016b) explored the inclusion of functional fatty acids (NT) to
calves fed MR. Functional fatty acids are those that positively affect health above basic
nutritional requirements. Calves were fed MR at two different rates, moderate (MOD)
and aggressive (AGG), with and without NT. The MOD MR program was fed at a rate of
0.66kg of DM for 49 days and the AGG MR program was fed at a rate of 0.66 kg of DM
for 4 days, 0.96 kg of DM for 4 days, 1.31 kg of DM for 34 days, and 0.66 kg of DM for
the last 7 days. All calves were weaned at 49 days of age. They concluded that
digestibility was less in the calves fed the AGG diet compared to the MOD diet. This
implies that the rumen was less developed in the calves fed the AGG diet. The calves fed
NT had a greater digestibility of DM, OM, ADF, and NDF, more BW gain, and hip width
change compared to the calves that did not receive NT. This would imply NT has the
capability of improving the poor growth post-weaning and reduced nutrient digestibility
in calves fed large amounts of MR.

Stamey et al. (2012) took another approach and investigated how the CP content
of calf starter, when fed with an enhanced MR feeding program, would compare to a
conventional MR feeding program from birth until 10 weeks of age. Their objective was
that feeding higher CP starter to calves on an enhanced MR feeding program would help
to maintain calf growth at weaning and post-weaning. This study included three different
treatment, conventional MR with conventional starter, enhanced MR with conventional
starter, and enhanced MR with high CP starter. When comparing the MR feeding rates,
calves that consumed enhanced MR had greater ADG through 10 weeks of age compared
to the conventional MR calves. This aligns with current research. Calves fed enhanced MR with enhanced starter had a lower ADG and BW slump compared to calves fed enhanced MR with conventional starter, but the effects were small. Starter intake was still lower for calves fed enhanced MR compared to conventional MR, even in those fed enhanced starter. They concluded that regardless of MR program, calves should consume at least 1 kg of starter DM/d prior to weaning to prevent a slump in post-weaning growth.

In summary, feeding calves large amounts of MR has been shown to increase ADG pre-weaning compared to calves fed less MR. However, post-weaning calves experience a slump in ADG and have decreased nutrient digestibility due to an underdeveloped rumen. Calves fed a conventional amount of milk replacer tend to have lower ADG before weaning and some abnormal behaviors due to hunger, but they have increased starter intake pre-weaning. This makes them better equipped post-weaning to digest the nutrients found in calf starter and allows them to reduce the difference in BW to calves fed large amounts of milk replacer. Finding a MR program that optimizes ADG and rumen development pre-weaning, may be the key to maximizing the productivity of calves.

**Calf Starters**

Calf starter, which is the first solid feed calves are exposed to, stimulates the development of the rumen (Warner et al., 1956). Common commercial calf starters come in many varieties, with the two most common forms being pelleted and texturized. A pelleted feed is made by applying heat, moisture, and pressure to a mixture of finely
ground ingredients to form a pellet. Texturized feed is a combination of whole, rolled, and ground grains mixed with some pellets (Khan et al., 2016). Corn is a very common starch source found in calf starters because it promotes increased BW gain compared to barley, whey, and wheat (Maiga et al., 1994). One study showed that oats may be an acceptable substitute for corn because it does not decrease ADG like other corn substitute carbohydrate sources do (Hill et al., 2008c). NRC (2001) recommends that starter feeds contain 20% CP on a DM basis to meet the young animal’s protein needs for growth and development. Soybean meal is a very common protein source found in calf starters (Drackley, 2008) because it is a great source of quality protein and is very digestible. Generally, starters contain only 3-4% fat because higher fat levels can lead to bloat and reduce intake and ADG (Doppenberg and Palmquist, 1991).

When grains undergo mechanical or chemical processing their surface area is increased which improves their total tract starch digestibility. Steam-flaked grains usually have the greatest starch availability and digestibility. Grains that are processed with heat have been shown to increase ruminal propionate production and grains that undergo physical processing have been shown to increase butyrate production (Lesmeister and Heinrichs, 2004). This is because chemical and mechanical alterations increase surface area and improve digestibility (Huntington, 1997).

Lesmeister and Heinrichs (2004) performed a study to determine how different corn processing methods affect growth characteristics, rumen development, and intake in neonatal calves. They concluded that the type of corn processing does affect feed efficiency and intake, growth, blood VFA concentration, and rumen criterion in
developing calves. Calves that received the whole corn or dry-rolled corn consumed more starter but the increased consumption had very little influence on body weight gain, structural growth, or rumen development. Calves that received roasted-rolled corn had increased structural growth and ruminal butyrate production, even with a lower intake compared to the other treatments, but all calves had similar body weight gains, feed efficiency, and rumen development. This indicates that roasted-rolled corn better prepares the rumen for weaning because of its greater capacity to convert nutrients into growth.

Another study (Khan et al., 2008), investigated the effects that calf starter starch source has on rumen development and nutrient digestibility. The four sources they evaluated were ground corn, ground, barley, ground wheat, and crimped oats. Calves that received the corn and wheat starter diets have greater concentrations of ruminal, acetate, propionate, butyrate, and ammonia, more developed rumens, and longer, more functional papillae. They also have greater solid feed intake, N utilization, and BW gain. These results imply that corn and wheat increase rumen development as well as nutrient digestibility and availability for growth.

Suarez-Mena et al. (2015) investigated the effects that whole vs. ground oats in starter have on reticulorumen fermentation and the development of the digestive system of pre-weaned calves. The diets for these trials were an all-pelleted starter with ground oats in the pellet (G) or pellets plus whole oats (W). In trial 1, calves received grain for 3 weeks and were killed at 5 weeks of age, in trial 2, calves were fed grain for 4 weeks and killed at 6 weeks of age, and in trial 3, calves were given grain for 4 weeks and killed at 7 weeks of age (only received starter at 3 weeks of age rather than 2 weeks like trial 1 and
2). It was concluded that physical form of oats in starter had no effect on rumen pH and had little effect on rumen VFA and ammonia concentrations. They discovered that calves on the G diet tended to have larger reticulorumen weights, rumen papillae, and deeper intestinal crypts, which could be due to greater nutrient availability.

Calf starters that contain small particles can negatively affect rumen epithelial health. This is because, small particles unlike larger particles, have a lower ability to physically remove dying epithelial cells in the rumen. If the cells are not removed they have a tendency to get trapped between the papillae which can lead to rumen parakeratosis, which is the enlargement and hardening of the rumen papillae (Lesmeister and Heinrichs, 2004). This can hinder and have negative effects on rumen development and nutrient absorption.

Calf starter regimen, just like milk replacer, is a balancing act. Calves need a diet that provides them with all the appropriate nutrients for maintenance and growth as well as optimizes rumen development. It also needs to be economically feasible and available for producers. There are many questions that need to be answered regarding calf starter ingredients, processing, and nutrient composition to ensure we are maximizing calf performance.

**Forage Pre-weaning**

Feeding forage to calves during the milk-feeding period was been disputed. Some argue it is more natural because they consumed forage in the wild. Others argue that feeding forage would reduce the concentrate intake and favor the production of acetate
instead of butyrate, delaying rumen papillae development (Drackley, 2008). However, research has shown that controlled particle size hay can alter the rumen environment, increasing intake and feed efficiency. One study (Khan et al., 2012) saw that forage intake and total DMI were greater in heifers that were fed starter and hay previously compared to the heifers that just received starter. There were no differences in the body weight of heifers between the two groups. ADG and apparent feed efficiency were greater for the heifers that just received starter previously compared to the heifers that got starter and hay. Giving forage along with starter to calves early in life improved their intake when they were switched to a high forage diet later in life. It is thought that this is due to the rumen having an increased capability to digested forage. However, this increased forage intake did not lead to increased body weight gains.

Jahani-Moghadam et al. (2015) examined how forage and its physical form in calf starters affect calf performance. They used three treatments which were fed until weaning at 76 days of age. Treatments were 100% semi-texturized starter (CON), 90% semi-texturized starter with 10% chopped alfalfa hay (CH), and 90% semi-texturized starter with 10% pelleted alfalfa (PH). Calves also received 6 L/day of milk replacer between two daily feeding. They concluded that neither forage nor its physical form affected total DMI. They concluded that when calves are fed high levels of milk, forage had little effect on intake, ADG, and efficiency. There were no beneficial differences between chopped and pelleted hay.

Another study (Castells et al., 2013) investigated the effects feeding different forage sources would have on rumen fermentation and GIT development. This project
consisted of three different treatment groups (starter alone, starter with alfalfa hay, and starter with oat hay). The results of this study showed that calves fed starter with oat hay ate about 4% of their total solid intake in oat hay. These calves had a better rumen environment compared to the other two treatments. This was determined because the oat hay calves had increased rumen pH and expression of MCT1 transporters. This transporter, located in the rumen epithelium, is involved in the transport of protons, acetate, and lactate from the rumen into the blood stream. With more MCT1 present in the rumen, more protons are being removed from the rumen, meaning the pH of the rumen would increase, causing an increase in the absorption of short-chain fatty acids. Calves fed oat hay also showed an increase in ruminal passage rate but not an increase in rumen gut fill. A better rumen environment and increased passage rate will allow calves to eat more solid feed.

One meta-analysis looked at the effects of forage provision on calf growth and rumen fermentation. They concluded that starter intake, final body weight, ADG, rumen pH, and the acetate to propionate ratio in the rumen can be improved when forage is fed. Forage has the ability to positively affect starter intake and calf performance but these effects depend on the source, level, and the physical form of the starter (Imani et al., 2017).

Including forage in calf diets pre-weaning may have some benefits in regards to rumen development, and intake later in life. However, more research is needed to definitively state that producers should be offering forage in their pre-weaned calves.
Weaning Method

During the weaning process, the GI tract of the calf transitions from functioning predominantly like a non-ruminant to a ruminant. The process of weaning changes the site of digestion as well as what nutrients are available for absorption. The primary location of digestion in a young calf is the abomasum and digestion in the rumen is minimal. As a calf starts consuming solid feed and is weaned, the primary site of digestion becomes the rumen and the role of the abomasum as it relates to digestion is reduced. The primary sugar found in milk and MR is lactose, which is broken down into glucose and galactose. Prior to weaning glucose is the main energy source for calves. However, post-weaning most of their energy is coming from volatile fatty acids (Baldwin et al., 2004; Steele et al., 2016).

Studies have shown that dietary and management factors such as gradual weaning (Roth et al., 2008; Weary et al., 2008) and grouping (de Paula et al., 2010) promotes the intake of solid feed, therefore, helping to establish the rumen microbiome.

Step-down is one method of weaning calves. It involves initially feeding calves high amounts of MR and then decreasing the amount of MR fed prior to weaning to reduce the stress once the calf reaches the weaning period. It is a way to allow calves to get a lot of milk initially and promote ADG while also reducing the amount of MR closer to weaning to promote solid feed consumption.

One study specifically looking at the effects of the step-down weaning method was (Khan et al., 2007a). They compared conventional milk feeding to step-down milk feeding and how they affected pre and post-weaning performance. The STEP calves
received 20% of their BW in MR for 25d, this was then reduced to 10% of their BW between d 26 to 30, and then they remained on 10% BW in MR for the remaining 15 d until weaning. The conventional calves received 10% of their BW in MR for the entire 45 d until weaning. Both methods were weaned the same by diluting MR with water from d 46 to 50. They found that the conventional calves consumed more starter and hay in the pre-STEP phase (d 1 to 30) compared to the STEP calves but the STEP calves consumed more starter and hay in the post-STEP (d 31 to 50) and post-weaning (d 51 to 90) phases. Calves in the STEP method had higher BW gain, feed efficiency, and DMI compared to the conventional calves. Lower blood glucose levels and higher blood urea levels in the STEP calves at and after weaning could indicate that they have a better functioning rumen than the conventional calves. The conventional calves also had a greater occurrence of diarrhea during 3 and 4 weeks of age compared to the STEP calves. They believe the increase in starter and hay intake in the post-STEP and post-weaning phases of the STEP calves were because they were used to a higher intake of nutrients. When their milk consumption was reduced, they had to find another way to make up for the amount of nutrients they were used to, causing them to eat more starter and hay. They concluded that STEP MR feeding could help improve decreased solid feed intake seen in calves fed high amounts of MR as well as improve reduced BW gain seen pre-weaning in conventional MR feeding methods.

Another study (Roth et al., 2008), investigated the effects of a concentrate-intake-dependent weaning strategy (decreasing milk consumption based on the increasing amount of solid feed consumption) compared to a conventional weaning strategy. Calves
that were weaned on an individual basis (concentrate-intake-dependent) showed an increased growth rate compared to calves weaned conventionally. They concluded that in order to improve a calf’s transition to a ruminant, their ability to consume solid feed should be evaluated on an individual basis, to improve welfare and performance.

Even though research has shown that abrupt weaning has negative impacts, not much has been reported about the duration of gradual weaning and how it affects starter intake and weight gains. Sweeney et al. (2010) performed a study to try and gain more insight on this topic. The study used four different treatment groups, calves weaned abruptly at day 41 and calves weaned gradually over 4 days, 10 days, and 22 days by decreasing the amount of milk fed each day. The results showed that milk intake was lowest for the 22-day gradual weaning followed by the 10-day and 4-day gradual weaning and milk intake was the highest for the abrupt weaning at day 41. Calves weaned over 22 and 10 days had the highest starter intakes, however, it was not enough to compensate for the reduced milk because digestible energy (DE) was lowest for the calves weaned over 22 days and intermediate for 10 d weaned calves. Gradual weaning showed improved starter intake but due to the reduced milk intake, it still resulted in reduced DE intake before weaning and lower BW gain at weaning. The 10-day gradual weaning treatment showed the best results in terms of weight gain during and after weaning. It allowed calves to receive enough milk early on to promote weight gain while also promoting a gradual transition to solid feed and a developed rumen leading to the more effective digestible energy intake.
A recent study by the Provimi calf research group (Dennis et al., 2018), evaluated the effects that the age at weaning and the method in which MR is reduced to weaning has on digestion and performance in dairy calves. This study consisted of four treatments. The first (MOD6) included feeding 0.66 kg of DM/d of MR and weaning calves at d 42, (HIGH6) offered calves up to 1.09 kg of DM/d of MR and weaned at d 42, (HIGH8) offered calves up to 1.09 kg of DM/d of MR and weaned at d 53, and the last (GRAD8) offered up to 1.09 kg of DM/d of MR and gradually weaned from d 35 to 53. At d 56, MOD6 calves weighted 3.4 kg lighter compared to the other treatments. Calves fed MOD6 had the greatest starter intake from d 0 to 56. At d 35, MOD6 calves had the lowest total-tract digestibility of DM, OM, CP, and fat but greater digestibility for starch, NDF, and ADF. By d 84, MOD6 calves had the greatest DM, OM, CP, NDF, ADF, and fat digestibility compared to the other treatments but by d 112 digestibility was similar between treatments. Between 56-112 d, ADG was greatest for MOD6 calves but there were no differences in BW on d 112. They concluded that feeding calves 0.66 kg of DM/d of MR and weaning them at d 42 resulted in similar growth and BW to calves fed 1.1 kg of DM/d of MR. Insinuating that any advantage seen in ADG and BW in calves fed increased amounts of MR is lost by 4 months of age. Also, that a 2-week gradual weaning did not improve the rate of growth.

Research shows that step-down weaning methods could help to improve the decreased solid feed intake pre-weaning seen in calves fed high amounts of MR as well as improve reduced BW gain seen pre-weaning in conventional MR feeding methods (Khan et al., 2007a). Weaning calves individually based on their concentrate intake
consumption could also positively affect a calves’ transition to a ruminant animal by increasing their rate of growth (Roth et al., 2008). The exact age and duration of weaning to optimize growth and development are still unknown but research shows that when comparing a 4, 10, and 22 d weaning program, the 10 d gradual weaning showed the best results in terms of weight gain during and after weaning (Sweeney et al., 2010). However, more research is still needed to fully understand the impacts of gradual weaning.

**Housing, Socialization, and Separation**

Research has shown that housing also has an effect on intake in calves. Calves that are housed together started eating solid feed sooner and ate more solid feed pre-weaning compared to calves housed separately (de Paula et al., 2010). Calves that are housed individually but are commingled before weaning consume more solid feeds than calves that are isolated (Bach et al., 2010). Calves raised in groups were observed ruminating more frequently and for a longer period of time than those individually housed (Phillips, 2004). Social housing has been shown to improve calf performance, reduce behavioral responses to weaning, and reduce neophobia. Group-housed calves tend to have more DMI and increased weaning weights when compared to individually housed calves (de Paula et al., 2010). Many farms house calves separately for the first few weeks and then move them into group pens. However, it was not known at what age contact with peers needs to take place in order to achieve these benefits.
Costa et al. (2015) looked at the effects of early and late pairing on feeding behavior and weight gain before and after weaning. The three treatments were individually reared, early pairing (~d 6), and late pairing (~d 43). Early-paired calves consumed more calf starter and had a higher total DMI compared to the late-paired and individually housed calves. Early-paired calves also gained more weight compared to the other two treatments during the entire experiment period. ADG was not different during the pre-weaning period, but early-paired calves had a higher ADG during the weaning period compared to the other two treatments. Early-paired calves started consuming calf starter sooner than late-paired and individually housed calves. They concluded that grouping has to occur before 6 weeks of age to see these benefits.

Flower and Weary (2001) wanted to evaluate the effects of early and late cow-calf separation in dairy animals. Early separation took place on d 1 and late separation occurred on d 14. This study evaluated 24 cow-calf pairs for the first 24 h after separation. Prior to separation, cow-calf pairs were relatively inactive. After separation, cows in the late separation group had a higher rate of movement and calling compared to the early separation cows. Milk yield during the first two weeks after calving was lower for cows in the late separation group due to calf consumption. Milk yield did not differ between the two groups from d 15-150. They determined that separation response was greater for both cow and calf in the late separation group but late separation calves gained more weight and showed more developed social behaviors compared to the early separation calves.
Allowing calves to socialize with other calves through pair or group housing or keeping them with their dam for an extended period of time, has been shown to increase starter consumption, rumination, and weight gain. Research shows that these impacts are greatest when calves are socialized from a very early age.

**Characteristics of the Rumen and its Development**

A major factor that determines the efficiency of the rumen is its pH (Khan et al., 2016). In general, the pH of rumen fluid is influenced by the absorption of VFAs and the rate of fermentation. Both of these are affected by the passage rate and the buffering capacity of the rumen contents (Williams et al., 1987). Low pH can have major effects on rumen microorganisms like decreased productivity or death in the rumen leading to decreased digestion of food (Penner and Oba, 2009). Increased acidity decreases the motility of the rumen (Krause and Oetzel, 2006) and increases the keratinization of the papillae, which will result in decreased VFA absorption and blood flow to the rumen mucosa (Hinders and Owen, 1965).

Prolonged low pH (pH <5.8 for 3h/d) is referred by some researchers as subacute rumen acidosis (SARA) (Khan et al., 2016). Due to calves low production of saliva which is a natural rumen buffer, calves are more likely to suffer from SARA. Saliva production is influenced by eating and chewing but the salivary glands in calves have little ability to produce saliva before 4 weeks of age (Kay, 1960). This paired with the fact that concentrates usually require less chewing and regurgitation leading to less saliva production compared to forages could also be why calves are at a higher risk for SARA.
Feeding calves forage, a substrate that with reduced fermentation rates, can help balance rumen pH. Even feeding calves a small amount of hay can alleviate rumen acidosis (Laarman and Oba, 2011). This was concluded after the results of this study showed that hay intake is negatively correlated to SARA (breakpoint at 0.08 kg/d). This is believed to be due to increased saliva buffer from the chewing of the hay.

Laarman et al. (2012) performed a study that looked at how substituting dry ground corn (CRN), with high-fiber byproducts, like beet pulp (BP) and triticale dried distiller’s grains (DDGS), in calf starters would affect rumen pH and growth during weaning. They hypothesized that by decreasing the starch content of the calf starters they would increase the rumen pH and decrease SARA. This was not true, DDGS had a greater severity of SARA compared to CRN. They reported no difference in the ADG between CRN and BP or between CRN and DDGS. They concluded that dietary starch concentration of calf starter was not the main factor contributing to rumen pH. Even though DDGS had a greater severity and duration of SARA compared to CRN, the growth of the calves was not affected. More research is needed to determine what the main effect that contributes to rumen pH is.

Khan et al. (2008) performed a study looking at differences in rumen development between calves fed barley, wheat, corn, and oat based diets. They reported that calves on barley and wheat had lower ruminal pH compared to those on corn and oats. This could be due to differences in solid feed intake and the pattern of starch fermentation. The rumen pH in calves is determined by the rate at which VFA and
ammonia build up and the rate of their absorption into circulation or utilization by microbes (Baldwin et al., 2004). Calves on corn and wheat diets had greater ruminal ammonia which was probably due to greater protein intake and its degradation by ruminal microbes. Calves on corn and wheat also had greater ruminal VFA concentration which was probably due to greater solid feed intake, starch consumption, and better fermentation of organic matter by ruminal microbes meaning a more developed rumen. Corn and wheat based diets resulted in calves having a more developed and effective rumen.

The development of the rumen involves the establishment of the microbial ecosystem, vascularization and muscularization of the rumen wall, development of the papillae, and the initiation of rumination. Butyrate is the main VFA stimulator in the development of the rumen epithelium (Flatt et al., 1958) which is why there is so much emphasis on feeding grain-based starters to calves because they promote butyrate production through starch digestion.

In pre-weaned dairy calves, high carbohydrate solid feed diets stimulate the proliferation of rumen microbes and the production of volatile fatty acids, these both help to initiate rumen development. Butyrate is the most stimulatory VFA followed by propionate and then acetate, because it is the most readily absorbed by the epithelium (Heinrichs, 2005). The main source of starch in ruminant diets are cereal grains such as corn, barley, rice, wheat, oat, and sorghum. There are many factors that can change the rate of enzymatic digestion of corn, barley, oat, and wheat starch, such as starch granule size and shape and interactions between surface compounds (fatty acids and protein) and
amylose (Nocek and Tamminga, 1991). These factors can affect the quality and proportion of VFAs in the rumen of neonatal calves which will affect rumen development and the utilization of nutrients (Khan et al., 2008).

Saccharomyces cerevisiae fermentation products (SCFP) is a common feed additive for dairy calves that has been shown to improve calf survival (Magalhães et al., 2008), ADG (Lesmeister et al., 2004), VFA production (Quigley et al., 1992), and rumen morphological development (Brewer et al., 2014). However, little is known about its effect on GI epithelium development and microbial community. Xiao et al. (2016) looked at three different diets, texturized calf starter with no SCFP (control), starter with 0.5% SCFP, and starter with 1% SCFP. The results showed that the two diets that contained SCFP had higher ruminal butyrate concentrations compared to the control group. They also saw higher *Butyrivibrio* and lower *Prevotella* richness in the rumen fluid. *Butyrivibrio* is the bacterial group responsible for the production of butyrate (Bryant and Small, 1956) and *Prevotella* is responsible for the production of propionate and acetate but not butyrate (Matsui et al., 2000). It is logical that there were higher concentrations of butyrate. Calves on the diets that contained SCFP had increased papilla length in the rumen, reduced crypt depth in the jejunum, and an increased ratio of villus height to crypt depth throughout the entire small intestine. These effects were even greater for the diet with 1% SCFP in the texturized calf starter. They concluded that SCFP could improve ruminal morphology by positively manipulating the microbial population and increasing the ruminal production of butyrate.
Studies have shown that concentrate diets result in increased propionate and butyrate molar proportions at the expense of acetate (Schwartzkopf-Genswein et al., 2003). Forage intake, on the other hand, increases acetate molar proportions in the rumen and promotes the growth of cellulolytic microbes (Zitnan et al., 1998). Studies have reported that calves fed chopped hay had more muscle and less musoca in the rumen compared to those fed concentrates (Nocek et al., 1984). It has been reported that monocarboxylate transporter isoform 1 (MCT1) which is involved in absorption of acetate, lactate, and protons in the rumen, had increased expression in calves fed forage (Castells et al., 2013). The implications of these reports are that concentrate based diets promote the production of butyrate and propionate which are vital for rumen development, while forage and hay can negatively affect rumen development. This is why the focus of young calves’ nutrition should be high-quality calf starter not forages.

In summary, grain-based starters are vital for rumen development; the establishment of the microbial population, development of the papillae, and the initiation of rumination. Grain-based feeds promote the production of VFAs through starch digestion. The most stimulatory VFA is butyrate because it is the most readily absorbed in the rumen. The development of the rumen allows calves to transition from using glucose from milk replacer as their major energy source to using VFAs from solid feed through starch digestion.
Enzymatic Changes

Young calves’ main source of energy is glucose which they acquire from lactose in milk/milk replacer being digested. As calves mature, the amount of glucose extracted from lactose fails to meet the calves’ glucose demand so they must rely on glycogenolysis and gluconeogenesis (Girard et al., 1992). As calves transition from nonfunctioning ruminants to ruminants, carbohydrate metabolism is drastically changed. Ruminants are only capable of absorbing small amounts of glucose from the intestinal tract but are capable of absorbing considerable amounts of acetate, propionate, and butyrate across the forestomach (Herdt, 1988).

The liver plays a major role in the maintenance of plasma glucose levels by balancing hepatic glucose utilization and glucose production (Duran-Sandoval et al., 2005). Pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK) are rate-limiting enzymes involved in gluconeogenesis in the liver; they are needed to convert pyruvate to glucose (Girard et al., 1992). The liver undergoes gluconeogenesis when the plasma glucose levels are low. Acetyl-CoA carboxylase is a rate-limiting enzyme in fatty acid biosynthesis (Kim, 1997). These enzymes are all vital for lipid and carbohydrate metabolism.

Haga et al. (2008) looked at the expression and activity levels of three rate-limiting enzymes involved in hepatic gluconeogenesis, pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK), and fatty acid biosynthesis, acetyl-coenzyme A (CoA) carboxylase (ACC). They reported that the activity of the gluconeogenic enzymes (PC, PEPCK) decreased with age, implying that gluconeogenesis
in the liver of calves, decreases as the rumen starts to develop. They also reported that plasma lactate, a precursor for gluconeogenesis that can be catalyzed by PC to pyruvate, decreased with age, especially after weaning. This is logical because as calves’ rumens develop they switch from glucose as their main energy source to VFA so their need for gluconeogenic enzymes decreases. Acetyl CoA activity also decreased with age and its expression was very scarce in the liver, implying that the liver might not play as big of a role in fatty acid biosynthesis in ruminants as originally thought. They concluded that as the function of the rumen starts to develop in calves, hepatic gluconeogenesis decreases.

**Microbial Profile of Gastrointestinal Tract**

How diet, weaning, housing, and rumen development affect the GIT microbial profile is still relatively unknown. (Malmuthuge et al., 2014) which was the first study of its kind, looked at the bacterial composition throughout the GIT (rumen, jejunum, ileum, cecum, and colon) of 3-week old calves. Bacteria from a total of 83 genera from 13 phyla distributed along the GIT were observed. A majority of the bacteria were from three phyla; *Firmicutes, Bacteroidetes, and Proteobacteria*. The rumen contained the most diverse bacteria population and the prevalence of each bacterium differed depending on the location in the GIT. More research is needed to fully understand the microbial diversity within the GIT but doing so is critical to determining how it affects calf growth and health.

Another study (Jami et al., 2013), looked at the ruminal bacterial populations of four different age groups from 1-day old calves to 2-year-old cows. The groups were 1-3
day old calves, 2-month old calves, 6-month old heifers, and 2-year-old lactating cows. They detected 15 different phyla of bacteria between the 4 groups. The three most prominent in all the groups were Firmicutes, Bacteroidetes, and Proteobacteria but the ratio and composition of each varied among the groups. In the calves that were 1-3 days old, the most abundant phylum was Firmicutes while the most abundant phylum in the other three groups was Bacteroidetes. These results lead to the conclusion that each sampled age group has its own distinct rumen microbial population that is influenced by diet and age.

To shed some light on how weaning strategy can impact the rumen and fecal microbiome during weaning, (Meale et al., 2016) assigned calves to either gradual (step-down) or abrupt weaning. Abruptly weaned calves received 9 L/d of MR until d 48 and then MR was reduced to 0 L on d 49. Gradually weaned calves received 9 L/d of MR until d 36, MR was then reduced to 4.5 L/d until d 48, and then finally to 0 L/d on day 49. Fecal and rumen samples were collected pre-weaning (d 36) and post-weaning (d 54). They concluded that weaning strategy did not impact fecal and ruminal microbiome development so this may not be a contributing factor to the reduced gain and intake seen during weaning.

Bacterial composition differs depending on its location in the GIT, calf age is a major determinant in the ratio and composition of the bacterial phylum within the rumen, and the calf’s microbiome might not be a contributing factor in intake and gain reduction seen during weaning. There is still much to be discovered regarding the role of microbes
in the development of the rumen and how calf diet and its environment might affect the microbes present in the rumen.

**Diet Post-weaning**

The diet of calves post-weaning is just as important as pre-weaning to ensure an efficient ADG and growth. (Chester-Jones et al., 1991) evaluated the performance of Holstein calves fed grower diets containing either whole corn (WC) or rolled corn (RC) at a 3:1 ratio with a pelleted supplement. During the first 28 days of the study, calves fed whole corn gained weight 6.3% faster compared to the calves fed rolled corn. This was thought to be due to an increase in salivary flow with the whole corn which enhanced DMI or more energy being supplied to the rumen epithelium caused by increased butyrate production. Over the entire 127-day study, calves fed WC had an ADG 6.7% higher compared to the calves fed RC. WC led to increased calf performance and growth compared to RC.

(Terré et al., 2013) wanted to evaluate the cause of increased performance seen in calves fed chopped grass hay, was it due to the increased NDF or the inclusion of chopped grass hay. This study consisted of 4 different treatments, a low NDF starter (18%), with and without chopped oat hay, and a high NDF starter (27%), with and without chopped oat hay. Prior to weaning, starter intake did not differ between treatments but low NDF treatments tended to have greater ADG compared to high NDF treatments. After weaning, the provision of forage increased starter intake and ADG, this could be due to the greater ruminal pH observed in the forage fed calves. Due to the
increased ADG seen in low-NDF fed calves prior to weaning and the increased starter intake and ADG seen in forage fed calves post weaning, they recommend feeding calves low NDF starter during the pre-weaning phase and feeding chopped hay right after weaning to improve calf performance.

Another study (Hill et al., 2016a), including two 56 day trials, used weaned Holstein calves around 60 days of age. The treatments for trial 1 included a texturized diet with high starch (52% starch, 13% NDF) and a pelleted diet with low starch (20% starch, 35% NDF). The three treatments for trial 2, were pelleted diets based on soybean hulls, wheat middlings, or corn with varying concentrations of starch (13, 27, and 42% starch and 42, 23, and 16% NDF). In trial 1, they reported greater ADG, hip width change, and feed efficiency in calves fed the high starch diet. In trial 2, calves fed corn (highest starch) had greater ADG, BCS change, and hip width change compared to the soyhulls and wheat middling diets. They observed that the low starch (high NDF) diets, were poorly digested by the calves compared to the high starch diets, resulting in lower ADG and fewer changes in hip widths. These results support the claim that it is energy, not protein that limits ADG (NRC, 2001).

The period in a calf’s life after weaning until breeding has been majorly overlooked in the nutritional research field for years. The limited research available indicates that calves perform better when they are fed whole corn, they should be given access to forage right after weaning, and that their greatest limitation in ADG is the amount of energy they are consuming not the amount of protein. With future research
hopefully, more light can be shed on a calf’s needs after weaning until they are sexually mature and able to be bred.

**Long-term Effects of Healthy, Efficient Calves**

One long-term effect of healthy calves is a higher growth rate, which has been shown to reduce the age at first breeding (Raeth-Knight et al., 2009). Another long-term effect of increased growth rates in calves is a higher milk yield once they are mature (Radcliff et al., 2000; Moallem et al., 2010; Soberon et al., 2012). Healthier calves also mean less money spent by farmers on vet bills and labor.

A meta-analysis performed at the Pennsylvania State University (Gelsinger et al., 2016), evaluated the effects that calf nutrition and growth pre-weaning has on first-lactation performance. The analysis included data from 9 studies and 21 treatments. The effect of study explained 98% of the variance in 305-d milk, 85% in milk fat, and 96% in milk protein. This indicates that other management aspects are more important than pre-weaning intake and growth rate to determine first-lactation performance. However, they did find a synergistic relationship between starter DMI and pre-weaning liquid for improving the production of milk, fat, and protein. They also found a positive relationship between pre-weaning ADG and lactation performance. This analysis concluded that providing calves with enough nutrients from liquid and solid feed to maintain an ADG greater than 0.5 kg/d pre-weaning, in combination with adequate post-weaning practices, can increase first lactation performance.
Conclusion

Even though there are many questions still left to answer, calf nutrition has made lots of progress over the last 100 years. We know the basics behind what a calf needs in order to efficiently transition from a non-ruminant to a ruminant and to be a healthy, productive lactating cow in the future, it is just a matter of optimizing calves feeding programs pre and post-weaning. This includes what is the best liquid diet, weaning system, starter content, forage intake, and housing method. Once we can figure out all those variables we can optimize calf health. The only way to know which methods are more efficient than others is through research and science. For example, my project looked into the microbial population present in a calves’ developing rumen to determine which ones are involved in positively developing the rumen and which ones suppress that development.
CHAPTER TWO
NURSERY PHASE

Introduction

There is an increasing interest in the amount of milk or milk replacer (MR) that should be fed to dairy calves to ensure a healthy animal with the ability to efficiently produce milk later in life. Flower and Weary (2001) called into question early separation in dairy cow/calf pairs and how it might influence calf growth. They reported that calves separated from their dam at 2 weeks vs. 1 day had increased weight gains, most likely associated with increased milk consumption. This started the trend of looking at feeding calves increased milk/MR early on to promote ADG. A report by (Diaz et al., 2001) evaluating the equations in the 1989 Dairy NRC and the 1996 Beef NRC for a calf’s energy and protein requirements for growth. They reported that the current recommendations for calf nutrient requirements are inadequate. This helped to further the idea of feeding calves large amounts of milk/MR after the study indicated that calves fed an increased amount of MR had increased average daily gain (ADG) as well as increased carcass growth. A concern with feeding calves an increased amount of milk or MR is, even though an increased ADG is reported up until weaning, a significant loss in body weight (BW) over the next few months post-weaning. This has been suggested to be caused by an underdeveloped rumen from the lack of solid feed (factor that affects rumen papillae, muscularization, and vascularization development), which leads to a slump in growth due to low starter digestibility as a calf adapts to a solid diet (Terré et al., 2007; Hill et al., 2010; 2016c). Bach et al. (2013) reported that calves fed 8 L/d of MR (25%
CP, 19% fat with 12.5% solids), had a BW at 52 d that was 3.7 kg greater than calves fed 6 L/d. However, BW at d 73 (weaning) and d 228 were similar between the two MR rates. This indicates that calves fed greater amounts of MR have increased growth early in life but this advantage is lost around and after weaning. This aligns with the studies mentioned previously.

Another factor to consider for an optimal transition from pre to post-weaning is an adequate adaptation to a completely solid diet. Weaning can be a very stressful time for a calf, so ensuring a smooth transition to a completely solid feed diet can be vital for development and growth. Combining gradual weaning with higher MR rates could reduce the growth slump and low starter digestibility seen in calves fed higher MR, by enticing calves to consume starter slowly versus abruptly. Hill et al. (2016c) reported that high MR-fed calves weaned gradually (stepped down over 3 weeks), had increased post-weaning digestion and growth compared to high MR-fed calves weaned via step down over 1 week.

In the present experiment, we evaluated these two major factors by assessing MR amount and weaning strategy to determine how they influence growth and nutrient digestibility of young dairy calves. This study incorporated two MR amounts (moderate, MOD and high, HI) as well as two weaning methods (gradual, GR and abrupt, AB) in a 2x2 factorial design. Growth parameters and nutrient digestibility were recorded to compare treatments. We hypothesized that calves on MOD MR intake (MOD-AB and MOD-GR) to have a more developed rumen because of greater solid intake and therefore greater nutrient digestion. Whereas, calves on the HI MR intake (HI-AB and HI-GR) to
experience greater ADG prior to weaning but this advantage lost post-weaning. Finally, we expect calves on the GR weaning program (MOD-GR and HI-GR) to have increased starter consumption because of the earlier reduction in MR. We expect that the treatment with the best overall performing animals will be MOD-AB.

Materials and Methods

Animals, Facilities, and Treatments

All animals were cared for as described in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010). Fifty Holstein bull calves, 2-3 days of age, were obtained from Fair Oaks Dairy (Fair Oaks, IN) and traveled 3.5 hours to Provimi’s Nurture Research Center (New Paris, OH) on April 11th, 2017. Before arriving at the Nurture Research Center, calves were housed in individual hutches with wire pens. They were fed 3 L of fresh colostrum within one hour of birth followed by another 3 L 12 h later. Calves then received 2 L of pasteurized whole milk twice daily until they left Fair Oaks Dairy. Upon arrival at the Nurture Research Center, calves were sorted in individual pens (1.2 m x 2.4 m) with a rock tile-drained base, bedded with wheat straw inside the facility with no added heat, natural ventilation, and curtain sides. Calves were fed 0.66 kg MR (DM) the first PM and the next AM. Then, calves were randomly assigned to one of four treatments: moderate MR with an abrupt weaning period (MOD-AB), moderate MR with a gradual weaning period (MOD-GR), high MR with an abrupt weaning period (HI-AB), and high MR with a gradual
weaning period (HI-GR; Figure 2.1). The MOD treatments had 13 calves each and the HI treatments had 12 calves each.

The day after the calves arrived, they were weighed (initial BW = 42 ± 1.3 kg) and a blood sample was taken intravenously. The blood was centrifuged at 3000 x g (VWR, Batavia, IL) at 20°C for 15 minutes to separate the serum and then serum protein concentration was estimated using an optical refractometer (ATAGO U.S.A., Inc., Bellevue, WA). All calves received the same common MR (25% CP, 17% fat DM basis; 14% solids; Table 2.1), which was formulated with added synthetic amino acids (Hill et al., 2008a) and fatty acids (Hill et al., 2011). MR was fed in equal amounts twice daily at 0630 and 1530 h. Calves were given ad libitum access to a texturized calf starter (20% CP, 42% starch; DM basis; Table 2.1) and water during the nursery phase (first 56 d). Starter consumption was recorded daily. Feces were scored daily on a scale of 1 to 5, with 1 being normal and 5 being watery (modified from Kertz and Chester-Jones, 2004). Medical treatments were also reported daily. All calves were weighted on days 0, 7, 14, 21, 28, 35, 42, 49, and 56, between approximately 11am-1pm. Body condition scores (BCS; 1 being thin and 5 being obese, (modified from Wildman et al., 1982) and hip widths (HW) were recorded every other week (d 0, 14, 28, 42, 56) during the nursery phase. Hip height was measured at the beginning of the trial (d 0) and at the end of the trial (d 56). All calves were weaned on 5/31/17 (day 49) but remained in individual pens indoors for another week.
The nursery portion of the trial took place from April 12th to June 7th, 2017. The average temperature during the 56-day nursery phase was 19°C, with a range from 2-35°C. The average humidity during this period was 69%, with a range from 12-100%.

**Digestibility Estimates**

During the nursery phase of the study, five calves were randomly selected from each treatment (20 total) for fecal sampling to estimate diet apparent digestibility coefficients (dC). Sampling was performed on days 26-30 and 45-49 (indicated as d 26 and 45) and they were composited by calf for each sampling period to estimate total tract diet digestibility as described by (Hill et al., 2016b). Fecal grab samples were taken from the 5 calves randomly selected per treatment on d 26 to 30 and 45 to 49, frozen daily, combined on an equal wet weight basis, and subsampled for analysis. Fecal samples were systematically collected such that a total of 12 fecal samples were collected over the 4 d to represent every 2 h in a 24-h period. Acid-insoluble ash was used as an internal marker for feed and fecal samples taken during both periods (Van Keulen and Young, 1977).

**Feed and Digestibility Analyses**

Every other bag of MR and starter was sampled and composited for nutrient analysis. Feeds and feces were analyzed according to AOAC International (2000) for DM (oven method 930.15), ash (oven method 942.05), CP (Kjeldahl method 988.05), fat (alkaline treatment with Roese-Gottlieb method 932.06 for MR; diethyl ether extraction method 2003.05 for starters and hay), NDF with ash (Van Soest et al., 1991) without sodium sulfite or alpha-amylase, ADF with ash (Robertson and Van Soest, 1981), starch
(α-amylase method; (Hall, 2009), sugar (colorimetric method; (DuBois et al., 1956), and acid insoluble ash (Van Keulen and Young, 1977).

**Statistical Analyses**

Data for the 56-d nursery phase were analyzed as a completely randomized design in a 2x2 factorial arrangement with repeated measures when applicable to identify changes over time by PROC MIXED in SAS (version 9.4; SAS Institute., Cary, NC). A statistical model was developed for the analysis that included terms for the fixed factor of milk replacer rate (Mi; i = 1, 2), weaning strategy (Sg; g = 1, 2), and period (Pj; j = 1 to 8); MS(ig) is the interaction of M and S, PM(ji) is the interaction of P and M, PS(jg) is the interaction of P and S, and PMS(jig) is the interaction of P, M, and S; Ck(ig) is the random effect of calf within treatment (M and S), and e(igjk) is the residual error. The model was represented as follows:

\[ Y_{igjk} = \mu + M_i + S_g + P_j + MS(ig) + PM(ji) + PS(jg) + PMS(jig) + C_k(ig) + e_{igjk} \]

where \( Y_{igjk} \) is a continuous, dependent response variable and \( \mu \) is the overall mean. Calf was the experimental unit (n=50 for the growth measurements and n=20 for digestibility). BW and intake data were grouped by week, and HW and BCS data were grouped by 14-day periods. The digestibility data were analyzed by individual 5-d collection periods as well as for the entire 56-d nursery phase. Analysis of Variance followed by Fisher’s Protected Least Significant Difference Test were used to evaluate the terms in the model. P-values, least squares means, and standard errors of least squares means are presented in tables. Statistical significance was declared at \( P \leq 0.05 \) and trends discussed at \( 0.10 \geq P \geq 0.05 \).
Results and Discussion

Starter and MR chemical composition are presented in Table 2.1. Treatments were planned to differ mainly in MR intake rate and weaning strategy. Thus, nutrient composition was the same among experimental treatments. No significant differences were reported between the four treatments for fecal score, abnormal fecal days, or medical days (Table 2.2). This indicates that MR feeding rate and weaning method had no effect on calf health.

Milk Replacer Feeding Rate Effect

Growth Performance

Initial measurements (serum protein, body weight (BW), hip width (HW), hip height (HH), and body condition score (BCS)) did not differ between calves (Table 2.2). This indicates that all calves started the study with the same structural measurements. As expected, starter consumption was greater for calves consuming a MOD amount of MR compared to those consuming a HI amount ($P<0.01$). Decreased pre-weaning starter intake, in increased MR-fed calves, has been reported in many studies (Terré et al., 2007; Hill et al., 2016c; Dennis et al., 2018). This trend is believed to be due to HI calves fulfilling a high amount of their nutrient requirements from MR, therefore reducing starter consumption. Figure 2.2 outlines starter consumption for each treatment by week. From week 4-6 of the study, MOD calves consumed significantly greater starter compared to HI calves. For the remainder of the study, MOD-GR had significantly greater starter consumption compared to the other treatments, MOD-AB and HI-GR consumed the same, and HI-AB consumed significantly less starter compared to the other
treatments. This high carbohydrate calf starter initiates rumen development by stimulating the proliferation of microbes within the rumen and through the production of VFAs (Suárez et al., 2006). VFAs are produced through the microbial fermentation of carbohydrates. VFAs, especially butyrate will then stimulate rumen papillae and epithelial development, allowing the rumen to become even more effective at digesting solid feeds and absorbing nutrients (Tamate et al., 1962). Feed efficiency (gain, kg; feed, kg; FE), ADG, final HH, final BW, and BCS change were greater ($P<0.05$) for HI calves compared to MOD calves. Many other studies have reported this similar increase in ADG in calves fed increased amounts of MR (Terré et al., 2007; Hill et al., 2007; 2010; Dennis et al., 2018). Terré et al. (2007) did not report effects on FE, HH, or BCS, but they observed greater BW at weaning in calves fed less MR. This could be because they only started feeding calves different amounts of MR around 20 d of age and those first 2-3 weeks of age are vital for enhanced growth. They almost completely missed that period to optimize calf growth pre-weaning. This is evident in a report by (Kertz et al., 1979) which showed that calves have the ability to achieve enough growth and development to be weaned at 28 d of age. Dennis et al. (2018) also reported greater FE and ADG for calves receiving greater amounts of MR through d 56. Calves on HI MR having increased final HH, final BW, and BCS change is logical due to their increased ADG which can all be an effect of the increased feed efficiency and intake of highly digestible sources of nutrients such as MR. These results indicate that HI MR intake promoted higher BW and growth performance before weaning (Table 2.2). This could be due to increased milk
consumption causing higher nutrient availability (Khan et al., 2007b), allowing calves to utilize greater quantities of nutrients for growth and development.

Figure 2.3 outlines BW change for each treatment by week. Up until week 3, there was not a significant difference in BW based on MR feeding rate or weaning strategy. It is logical that we did not see a significant difference in BW during this period because calf MR rates were not different for the first week of the study and it was not until week 3 that HI MR calves started to receive almost double the amount of MR as the MOD calves. The HI treatments were designed to allow calves to become acclimated to that higher amount of MR so their MR amounts were increased over two weeks from 0.66 kg to 1.1 kg MR (DM). At week 3, which is the first week that HI calves began receiving 1.1 kg MR (DM), there is a significant difference between the MOD and HI treatments with HI calves having greater BW. This same trend is seen through week 6. Stamey et al. (2012) reported similar results in BW, as early as 2-3 weeks of age, high MR calves had a significantly greater BW compared to low MR calves. This trend continued up through wk 10. During weeks 6, 7 and 8, we see HI-AB has significant greater BW compared to all other treatments. This is logical because HI-AB calves are consuming the greater amount of MR compared to the MOD treatments, as well as consuming greater MR for a longer period compared to HI-GR. This increased MR consumption leads to increased nutrient availability which the animal can then use for growth and development (Khan et al., 2007b).
**Diet Digestibility**

Table 2.3 outlines the apparent dC of nutrients at d 26 and 45 combined, to identify overall digestibility differences for the 56 d nursery phase. Apparent dC of DM, OM, and fat were greater \((P<0.05)\) for HI vs. MOD calves. Apparent dC of NDF, ADF, and sugar were greater \((P<0.05)\) for MOD vs. HI. There were no significant differences between CP or starch apparent dC based on treatment. Terré et al. (2007) also reported greater apparent dC for NDF in calves fed lower amounts of MR, they did not report on ADF, fat, starch, or sugar dC. However, they also observed greater apparent dC for DM and OM in calves fed a lower amount of MR, which is the opposite of what we report in the present experiment. They reported greater apparent CP dC in lower MR fed calves, while we did not see any differences between treatments. These differences could be because they only measured digestibility once, the week after weaning and we measured it twice during the nursery phase and the second time point was a week prior to weaning. A calves’ digestible tract goes through many changes at weaning which could be the cause of this variation. Our results suggest that calves receiving HI MR pre-weaning, digest readily available nutrients more efficiently but MOD MR, results in increased fibrous fractions digestion. These results tie into those reported by (Khan et al., 2007b), who concluded that calves consuming more milk \((20 \text{ vs. } 10\% \text{ BW})\) have higher nutrient availability leading to greater gain and body measurements and early consumption of starter by calves leads to a more metabolically developed rumen, allowing them to more efficiently digest and absorb fibrous feed fraction. Another possible explanation for the
increased ADF and NDF digestibility seen in MOD MR fed calves is a more prominent rumen population of fibrolytic bacteria.

Table 2.3 shows the diet digestibility measurements for d 26. Apparent dC of DM ($P<0.01$), OM ($P<0.01$), CP ($P<0.01$), and fat ($P=0.05$) were greater for HI vs. MOD. Apparent dC of NDF ($P<0.01$), ADF ($P<0.01$), and sugar ($P=0.03$) were greater for MOD vs. HI. Dennis et al. (2018) evaluated digestibility on d 35 and reported greater apparent dC for DM, OM, CP, and fat in HI MR fed calves as well as greater apparent dC for NDF in MOD calves, they did not report on sugar digestibility. These results support what we reported. At 4-5 weeks of age, calves receiving greater amounts of MR are more efficient at digesting DM, OM, CO, and fat while calves receiving a MOD feeding rate are more efficient at digesting some fibrous fractions. As mentioned earlier, (Khan et al., 2007b) reported similar findings, calves with greater starter consumption, similar to our MOD treatment, led to earlier rumen development and increased fermentation capabilities allowing for the digestion and absorption of fibrous feed fractions. Greater milk consumption, similar to our HI treatment, led to increased nutrient availability and digestion capacity, which is linked to greater gain and body measurements.

Table 2.3 shows the diet digestibility measurements for d 45. Apparent dC of NDF ($P<0.01$) and ADF ($P=0.01$) were greater for MOD vs. HI. This indicates that MOD calves have a more developed rumen compared to HI calves, allowing them to have enhanced fiber dC. Apparent dC of fat was greater for HI vs. MOD ($P=0.05$). This could be due to HI calves having greater nutrient availability from increased MR consumption. Dennis et al. (2018) reported digestibility on d 49 and also observed greater apparent
NDF and ADF digestibility for MOD vs. HI calves but did not see any differences in fat digestibility.

Throughout the 56-day nursery period, NDF and ADF digestibility remained greater for MOD vs. HI calves. On d 26, sugar apparent dC was also greater for MOD vs. HI calves but by d 45, this significant difference was lost. On d 26, DM, OM, CP, and fat digestibility were all greater for HI vs. MOD calves, but on d 45, only fat digestibility remained significantly greater for HI calves. This would indicate that early on in the nursery phase, when HI calves are consuming a greater amount of MR compared to the MOD calves, they are more efficient at digesting DM, OM, CP, and fat. However, later on during the nursery phase, once MOD calves start to consume calf starter at greater rates, HI calves lose their advantage of being more efficient at digesting DM, OM, and CP. Calves consuming MOD MR were better at digesting NDF and ADF throughout the entire nursery phase. Consequently, with less MR consumption we see an increased starter consumption, leading to the earlier development of the rumen and greater fibrous fraction digestion. With greater MR consumption, we see increased digestion of more readily available nutrients.

**Weaning Strategy Effect**

**Growth Performance**

Starter consumption was greater for GR weaned calves compared to AB ($P<0.01$). Sweeney et al. (2010) also reported that GR weaned calves consumed more starter compared to AB weaning. Final HH was greater for AB compared to GR calves ($P=0.04$). This could be due to AB calves receiving higher amounts of MR for a longer
period compared to GR calves. Khan et al. (2007b) saw the opposite effect on HH when comparing conventional (fed 10% BW in MR for 44 d) vs. STEP (fed 20% BW in MR for 23 d, reduced to 10% from d 24 to 28, remained 10% for next 16 d) weaning. This inconsistency could be due to treatment effect. The STEP diet is similar to our HI-GR treatment and the conventional to our MOD-AB. The combination of high MR with a gradual weaning could be the key to increasing both starter consumption and growth. Hill et al. (2012) also compared abrupt (0.66 kg/d for 39 d then 0.33 kg/d from d 40 to 42) and gradual (0.82 kg/d from d 0-5, 0.96 kg/d from d 6-28, 0.82 kg/d from d 29-32, 0.55 kg/d from d 33-36, 0.41 kg/d from d 37-39, and 0.21 kg/d from d 40-42) weaning. They reported greater BW and ADG, greater starter DMI from d 43-56, and greater FE from d 43-56 in gradually weaned calves. As planned, our AB calves were allowed to consume a greater amount of MR for a longer period of time, which might have induced a greater structural growth such as greater HH. Calves weaned GR, lead to more starter consumption and lower structural growth (HH). There were no interactions between weaning strategy and MR feeding rate for growth performance. This increased starter consumption seen in GR vs. AB calves could be the solution for low starter intake seen in calves fed high amounts of MR.

Diet Digestibility

Table 2.3 shows the diet digestibility measurements for d 26 and 45 combined, to identify overall digestibility differences for the 56 d nursery phase. Apparent dC of DM ($P=0.05$) was greater for AB vs. GR, there was a trend for greater OM ($P=0.06$) dC for AB vs. GR, and the apparent dC of ADF ($P=0.01$) was greater for GR vs. AB. No
interactions were observed between weaning strategy and MR feeding rate for diet
digestibility over the entire 56 d period. Greater ADF dC for GR weaning means those
calves are better equipped to digest fibrous feeds compared to AB weaning. This is
logical because GR calves also consumed more starter and research shows solid dry feed
intake promotes microbial development and increases ruminal metabolic activity
(Anderson et al., 1987), leading to a rumen that is more effective at digesting more
complex nutrients like fiber.

Table 2.3 shows the diet digestibility measurements for d 26. Apparent dC of
ADF ($P=0.01$) was greater for GR vs. AB weaning. This would indicate that calves
weaned gradually are more efficient at digesting some fibrous fractions compared to
those weaned abruptly. This could be due to GR weaning having greater starter
consumption leading to a more developed rumen with a capability to digest fiber more
efficiently. Dennis et al. (2018) reported no differences at d 35 between calves weaned
abruptly (0.87 kg of DM/d for 4 d, 1.09 kg for 42 d, and 0.54 kg for 7 d) and those
weaned gradually (0.87 kg of DM/d for 4 d, 1.09 kg for 35 d, 0.87 kg for 4 d, 0.66 kg for
4 d, 0.44 kg for 3 d, and 0.22 kg for 3 d). This would indicate that nutrient digestibility
prior to d 35 is not affected by weaning strategy.

Table 2.3 shows the diet digestibility measurements for d 45. Apparent dC of DM
($P=0.02$) and OM ($P=0.03$) were greater for AB vs. GR and this was influenced by HI-
AB being numerically greater than the other measurements (rate by weaning interaction,
$P = 0.06$). In addition, apparent dC of fat was greater for AB vs. GR weaning ($P=0.05$).
Just prior to being completely weaned (d 49) calves experiencing an abrupt weaning are
more efficient at digesting DM, OM, and fat. There was also a rate by weaning interaction seen for starch dC, HI-AB tended to have a greater value compared to the other treatments. This is logical because GR-AB calves were offered a greater amount of MR for a longer period, which might have aided in increased nutrient availability and digestion efficiency (Khan et al., 2007b).

**Conclusion**

During the nursery phase, calves consuming a MOD amount of MR had increased starter consumption compared to HI, which is what we hypothesized. However, we also hypothesized a further developed rumen in MOD MR calves, due to increased starter consumption, leading to greater nutrient digestion. This was the case for NDF, ADF, and sugar but not the case for DM, OM, and fat, which had greater apparent dC in HI calves. Calves consuming HI MR also had increased FE, ADG, and growth parameters compared to MOD, which we expected to see. Calves weaned abruptly also had lower starter intake vs. calves weaned gradually, which we also expected to see. This would imply that increased MR promotes growth early in life but a more conventional amount of MR promotes rumen development and therefore the digestion of fibrous feed fractions. MOD-AB was not the treatment with the best performing animals, likely due to it having the lowest DM intake.
Table 2.1 Chemical composition of nursery phase experimental feeds

<table>
<thead>
<tr>
<th>Item</th>
<th>MR</th>
<th>Starter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter, % as fed</td>
<td>96.8 ± 1.10</td>
<td>87.9 ± 2.10</td>
</tr>
<tr>
<td>DM basis, % Ash</td>
<td>6.0 ± 0.62</td>
<td>6.3 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>24.9 ± 2.12</td>
<td>20.7 ± 2.25</td>
</tr>
<tr>
<td>Fat</td>
<td>17.8 ± 1.99</td>
<td>3.5 ± 0.34</td>
</tr>
<tr>
<td>ADF</td>
<td>.</td>
<td>7.5 ± 0.97</td>
</tr>
<tr>
<td>NDF</td>
<td>.</td>
<td>14.2 ± 1.96</td>
</tr>
<tr>
<td>Starch</td>
<td>.</td>
<td>42.4 ± 0.54</td>
</tr>
<tr>
<td>Sugar</td>
<td>.</td>
<td>6.7 ± 0.82</td>
</tr>
</tbody>
</table>

Table 2.2 Growth performance of calves during the nursery phase fed moderate (MOD) or high (HI) rate of milk replacer using an abrupt (AB) or gradual (GR) weaning strategy

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment 1</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>MOD-AB</td>
<td>MOD-GR</td>
</tr>
<tr>
<td>Initial serum protein, mg/dL</td>
<td>5.42</td>
<td>5.42</td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td>42.44</td>
<td>42.01</td>
</tr>
<tr>
<td>Final BW, kg</td>
<td>74.63</td>
<td>73.76</td>
</tr>
<tr>
<td>Initial hip width, cm</td>
<td>17.35</td>
<td>17.13</td>
</tr>
<tr>
<td>Final hip width, cm</td>
<td>21.00</td>
<td>20.81</td>
</tr>
<tr>
<td>Initial hip height, cm</td>
<td>71.23</td>
<td>69.96</td>
</tr>
<tr>
<td>Final hip height, cm</td>
<td>82.96</td>
<td>81.08</td>
</tr>
<tr>
<td>Initial BCS</td>
<td>2.12</td>
<td>2.10</td>
</tr>
<tr>
<td>Final BCS</td>
<td>2.19</td>
<td>2.27</td>
</tr>
<tr>
<td>MR intake, kg/d</td>
<td>0.54</td>
<td>0.43</td>
</tr>
<tr>
<td>Starter intake, kg/d</td>
<td>0.60</td>
<td>0.79</td>
</tr>
<tr>
<td>Total DM intake, kg/d</td>
<td>1.14</td>
<td>1.22</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>0.57</td>
<td>0.57</td>
</tr>
<tr>
<td>Feed efficiency²</td>
<td>0.51</td>
<td>0.46</td>
</tr>
<tr>
<td>Hip width change, cm</td>
<td>3.65</td>
<td>3.67</td>
</tr>
<tr>
<td>Hip height change, cm</td>
<td>11.73</td>
<td>11.12</td>
</tr>
<tr>
<td>BCS change</td>
<td>0.08</td>
<td>0.17</td>
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<tr>
<td>Fecal Score³</td>
<td>2.17</td>
<td>2.18</td>
</tr>
<tr>
<td>Abnormal fecal days</td>
<td>0.31</td>
<td>0.85</td>
</tr>
<tr>
<td>Medical days</td>
<td>0.69</td>
<td>1.31</td>
</tr>
</tbody>
</table>

¹Calves assigned to MOD-AB were fed 0.66 kg (DM basis) MR for first 42 d then 0.33 kg for last 7 d. MOD-GR were fed 0.66 kg MR for 28 d, 0.33 kg for 14 d, and 0.17 kg for the last 7 d, HI-AB were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 28 d, and 0.66 kg for the last 7 d, HI-GR were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 14 d, 0.66 kg for 14 d, and 0.33 kg for the last 7 d
²BW gain divided by milk replacer plus starter intake
³1 to 5 point system with 1=thick, 2=less thick, 3=abnormal like batter, 4=abnormal, watery with color, 5=abnormal, watery with little color.
Table 2.3 Apparent digestibility coefficients (dC) of nutrients in calves during the nursery phase of the trial as influenced by the four treatments

<table>
<thead>
<tr>
<th>Digestibility, %</th>
<th>Treatments$^1$</th>
<th>P-value</th>
<th>Rate</th>
<th>Weaning</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MOD-AB</td>
<td>MOD-GR</td>
<td>HI-AB</td>
<td>HI-GR</td>
<td>SEM</td>
</tr>
<tr>
<td>d 26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>93.1</td>
<td>92.5</td>
<td>95.5</td>
<td>95.5</td>
<td>0.65</td>
</tr>
<tr>
<td>OM</td>
<td>93.7</td>
<td>92.9</td>
<td>96.0</td>
<td>95.8</td>
<td>0.65</td>
</tr>
<tr>
<td>NDF</td>
<td>49.4</td>
<td>56.4</td>
<td>23.2</td>
<td>24.7</td>
<td>4.46</td>
</tr>
<tr>
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$^1$Calves assigned to MOD-AB were fed 0.66 kg (DM basis) MR for first 42 d then 0.33 kg for last 7 d, MOD-GR were fed 0.66 kg MR for 28 d, 0.33 kg for 14 d, and 0.17 kg for the last 7 d, HI-AB were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 28 d, and 0.66 kg for the last 7 d, HI-GR were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 14 d, 0.66 kg for 14 d, and 0.33 kg for the last 7 d.
Figure 2.1 Milk replacer amounts for the four experimental treatments

- 0.33 kg = 2.25 L as-fed, 5% BW
- 0.66 kg = 4.5 L as-fed, 10% BW
- 1.1 kg = 7.5 L as-fed, 16.5% BW

Figure 2.2 Starter intake by week for each treatment

Weaning strategy effect, $\Phi = P < 0.05$
Milk replacer rate effect, $* = P < 0.05$
Figure 2.3 BW change by week for each treatment

Weaning strategy effect, $\bar{Q} = P < 0.05$
Milk replacer rate effect, $* = P < 0.05$
Figure 2.4 Apparent nutrient digestibility coefficients (dC) during the pre-weaning phase

Weaning strategy effect, $Q = P < 0.05$, $QQ = P < 0.10$
Milk replacer rate effect, $* = P < 0.05$, $** = P < 0.10$
Interaction between weaning strategy and MR feeding rate, I= $P < 0.05$, II= $P < 0.10$
CHAPTER THREE
GROWER PHASE

Introduction

There is an increasing interest in the amount of milk or milk replacer (MR) that should be fed to dairy calves to ensure a healthy animal with the ability to efficiently produce milk later in life. An issue with feeding calves large quantities for milk or MR is that the increased pre-weaning average daily gain (ADG) and greater body weight (BW) at weaning (Diaz et al., 2001; Appleby et al., 2001; Jasper and Weary, 2002), is usually lost by 4 months of age when compared to moderate MR feeding regimen (Hill et al., 2016b; 2016c). This is due to an underdeveloped rumen caused by lower starter consumption pre-weaning, calves are not adequately adapted to solid feed intake causing a growth stagnation and allowing traditionally fed calves to converge (Terré et al., 2007; Hill et al., 2010; 2016c). Bach et al. (2013) reported that calves fed 8 L/d of MR (25% CP, 19% fat with 12.5% solids), had a body weight (BW) at 52 d that was 3.7 kg greater than calves fed 6 L/d. However, BW at d 73 (weaning) and d 228 were similar between the two MR rates.

There are conflicting reports regarding the effect that increased MR feeding rate has on post-weaning ADG. Some studies report decreased ADG (Hill et al., 2007; Hill et al., 2010) while others reported no effect (Rosenberger et al., 2017). A reduction is structural growth post-weaning has been observed (Hill et al., 2016b; 2016c). This decreased ADG and structural growth could be due to the reduction in starter digestion. Concurrently, it has also been reported that intensive MR regimens reduces the digestion
of starter post-weaning (Terré et al., 2007; Hill et al., 2010), not allowing all the available nutrients to be absorbed and utilized by the body for growth and development, resulting in lower BW gains and structural growth.

Another factor to consider for an optimal transition during weaning is an adequate adaptation to a completely solid diet. Weaning can be a very stressful time for a calf, so ensuring a smooth transition to a completely solid feed diet can be vital for development and growth. Combining gradual weaning with higher MR rates could be a possible solution to reduce the growth stagnation and low starter digestibility seen in calves post-weaning. Reducing high amounts of MR gradually could lead to increased starter consumption and adequate development of the rumen to prepare the calf for weaning. Many studies have evaluated the effects post-weaning of pairing a high MR feeding program with gradual weaning, however, they did not report diet digestibility (Hill et al., 2007; Sweeney et al., 2010). However, (Hill et al., 2016c) did report that high MR-fed calves weaned gradually (stepped down over 3 weeks), had increased post-weaning digestion and growth compared to high MR-fed calves weaned via step down over 1 week.

In this study, we further investigate the effects that MR feeding program and weaning strategy have on growth performance and diet digestibility in calves post-weaning. Prior to this post-weaning experiment, we performed a pre-weaning study (d 0-56), using the same 50 Holstein calves that incorporated two milk replacer amounts (moderate, MOD and high, HI) as well as two weaning methods (gradual, GR and abrupt, AB) in a 2x2 factorial design. Growth parameters and nutrient digestibility were recorded
to compare treatments. During the pre-weaning phase, we concluded greater ADG and BW as well as FE at weaning in HI calves and greater starter consumption in MOD calves. We also reported MOD calves were more efficient at digesting fibrous feed fractions. Calves assigned to AB weaning had lower starter consumption compared to GR prior to weaning. We hypothesized that calves on HI MR treatments (HI-AB and HI-GR) would experience a stagnation in growth after weaning due to the lower starter intake, allowing calves receiving MOD MR (MOD-AB and MOD-GR) to reduce the difference in BW and possibly surpass them. The increased starter consumption seen in GR vs. AB calves pre-weaning should have better prepared calves GIT for weaning, causing an enhanced growth and greater nutrient digestibility due to their more developed rumen. Gradual weaning could help solve the problems post-weaning seen in high MR fed calves by better preparing them for weaning.

**Materials and Methods**

*Animals, Facilities, and Treatments*

All animals were cared for as described in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 2010). Fifty Holstein bull calves, 2-3 days of age, were obtained from Fair Oaks Dairy (Fair Oaks, IN) and traveled 3.5 hours to Provimi’s Nurture Research Center (New Paris, OH) on April 11th, 2017. Before arriving at the Nurture Research Center, calves were housed in individual hutches with wire pens. They were fed 3 L of fresh colostrum within one hour of birth followed by another 3 L 12 hours later. Calves then received 2 L of pasteurized
whole milk twice daily until they left Fair Oaks Dairy. Upon arrival at the Nurture Research Center, calves were sorted in individual pens (1.2 m x 2.4 m) with a rock tile-drained base, bedded with wheat straw inside the facility with no added heat, natural ventilation, and curtain sides. Calves were fed 0.66 kg MR (DM) the first PM and the next AM. Then, calves were randomly assigned to one of four treatments: moderate milk replacer with an abrupt weaning period (MOD-AB), moderate milk replacer with a gradual weaning period (MOD-GR), high milk replacer with an abrupt weaning period (HI-AB), and high milk replacer with a gradual weaning period (HI-GR; Figure 3.1). The MOD treatments had 13 calves each and the HI treatments had 12 calves each.

During the pre-weaning phase, all calves received the same common MR (25% CP, 17% fat DM basis; 14% solids; Table 3.1), which was formulated with added synthetic amino acids (Hill et al., 2008a) and fatty acids (Hill et al., 2011). Milk replacer was fed in equal amounts twice daily at 0630 and 1530 hours. Calves were given ad libitum access to a texturized calf starter (20% CP, 42% starch; DM basis; Table 3.1) and water during the pre-weaning phase (first 56 d).

On 6/7/17 (day 56), after calves spent 7 d housed individually indoors, only consuming starter (weaned on d 49), they were moved to twelve group pens outside with 4-5 calves per pen, 3 pens per treatment. The pens consisted of 6.5 m² of outside space with concrete pad and rock, tile drained yard and 1.35 m² of inside space bedded with wheat straw. During the post-weaning phase, all calves were fed the same calf starter used during the pre-weaning phase blended with 5% chopped grass hay ad libitum as well as free choice water. Body weight (BW) and hip widths (HW) were measured on days 56,
70, 91, and 112, as well as body condition was scored. They remained in group pens until they left the Nurture Research Center on 8/2/17 (d 112).

The post-weaning phase of the trial took place from June 7\textsuperscript{th} to August 2\textsuperscript{nd}, 2017. The average temperature during the 56-day post-weaning phase was 22°C, with a range of 9-33°C. The average humidity during this period was 80\%, with a range of 25-100\%.

**Digestibility Estimates**

During the post-weaning phase of the study, fecal samples were collected to estimate diet apparent digestibility coefficients (dC). Sampling was performed on d 66-70, 87-91, and 108-112 (indicated as d 66, 87, and 108) and they were composited by pen for each five-day sampling period to estimate total tract diet digestibility as described by (Hill et al., 2016b). Fecal grab samples were taken from each pen on d 66 to 70, d 87 to 91 and d 108 to 112, frozen daily, combined on an equal wet weight basis, and subsampled for analysis. Fecal samples were carefully taken from the pen floor to minimize bedding and ground material contamination. Acid-insoluble ash was used as an internal marker for feed and fecal samples taken during both periods (Van Keulen and Young, 1977).

**Feed and Digestibility Analyses**

Every bale of grass hay and every other bag of MR and starter was sampled and composited for nutrient analysis. Feeds and feces were analyzed according to AOAC International (2000) for DM (oven method 930.15), ash (oven method 942.05), CP (Kjeldahl method 988.05), fat (alkaline treatment with Roese-Gottlieb method 932.06 for MR; diethyl ether extraction method 2003.05 for starters and hay), NDF with ash (Van
Soest et al., 1991) without sodium sulfite or alpha-amylase, ADF with ash (Robertson and Van Soest, 1981), starch (α-amylase method; (Hall, 2009), sugar (colorimetric method; (DuBois et al., 1956), and acid insoluble ash (Van Keulen and Young, 1977).

**Statistical Analyses**

Data for the 56-d post-weaning phase were analyzed as a completely randomized design in a 2x2 factorial arrangement with repeated measures when applicable to identify changes over time by PROC MIXED in SAS (version 9.4; SAS Institute., Cary, NC). A statistical model was developed for the analysis that included terms for the fixed factor of milk replacer rate (M; i = 1, 2), weaning strategy (S; g = 1, 2), and period (P; j = 1 to 8); MS is the interaction of M and S, PM is the interaction of P and M, PS is the interaction of P and S, and PMS is the interaction of P, M, and S; C is the random effect of calf within treatment (M and S), and e is the residual error. The model was represented as follows:

\[ Y_{igjk} = \mu + M_i + S_g + P_j + MS_{ig} + PM_{ij} + PS_{jg} + PMS_{jig} + C_{kig} + e_{igjk}. \]

where \( Y_{igjk} \) is a continuous, dependent response variable and \( \mu \) is the overall mean. Calf was the experimental unit (n=50) for the growth measurements and pen was the experimental unit for the digestibility measurements (n=12). BW and intake data were grouped by week, and HW and BCS data were grouped by 14-day periods. The digestibility data were analyzed by individual 5-d collection periods as well as for the entire 56-d pre-weaning phase. Analysis of Variance followed by Fisher’s Protected Least Significant Difference Test were used to evaluate the terms in the model. P-values,
least squares means, and standard errors of least squares means are presented in tables. Statistical significance was declared at $P \leq 0.05$ and trends discussed at $0.10 \geq P \geq 0.05$.

**Results and Discussion**

Post-weaning concentrate and hay chemical composition are presented in Table 3.1. Treatments were planned to differ during the pre-weaning phase and a common diet was offered during the post-weaning phase. Thus, nutrient composition was the same among experimental treatments. Daily fecal scores were not reported due to calves being in group pens. There were no medical treatments given during the post-weaning phase.

**Milk Replacer Feeding Rate Effect**

**Growth Performance**

Initial body weight (BW), hip width (HW), and hip height (HH) were greater for HI compared to MOD calves (Table 3.2). These differences are due to the treatments implemented during the pre-weaning phase of this trial. Calves that received HI MR had greater BW, HW, and HH at the end of the pre-weaning phase. This is believed to be due to increased nutrient availability seen in calves fed high MR rates (Khan et al., 2007b). Starter consumption prior to weaning was greater for MOD calves compared to HI calves ($P<0.01$). Decreased pre-weaning starter intake, has been observed in many studies using intensive MR feeding regimens (Terré et al., 2007; Hill et al., 2016c; Dennis et al., 2018). This pattern is believed to be due to HI calves satisfying a high amount of their nutrient requirements from MR, therefore reducing starter consumption. However, this decreased starter intake caused by greater MR consumption has led to an underdeveloped rumen, a
reduction in growth post-weaning, as well as reduced structural growth (Hill et al., 2016b; Hill et al., 2016c) and starter digestion (Terré et al., 2007; Hill et al., 2010).

Calves receiving HI MR maintained an increased BW until d 91 of the study (Figure 3.3). However, final BW, HW, and HH (d 112) did not differ among treatments. Change in HH ($P<0.01$) was greater for MOD vs. HI calves. This is due to MOD calves having a lower HH at the start of the post-weaning phase but then the same HH as HI calves at the end of the post-weaning phase, accounting for the greater overall change during this period. These results indicate that during the post-weaning phase, HI calves lost their growth advantage over MOD calves, as a result of an inadequate amount of starter consumption prior to weaning causing an underdeveloped rumen that is not fully capable of digesting the nutrients in the starter (Khan et al., 2007a; 2007b). This allowed the MOD calves that were more accustomed to starter intake and possessed a more developed rumen (microbial establishment, papillae formation, initiation of rumination, and epithelial muscularization and vascularization; (Khan et al., 2016), the opportunity to make up the difference in BW. Dennis et al. (2018) compared the effects of MOD and HI MR and also reported no differences in final BW and HW. After the 56-d pre-weaning phase, there was a trend for increased BW in HI compared to MOD calves but after the post-weaning phase, HI calves no longer had a trend for increased BW. There was no difference between MOD and HI calves based on BW by the end of the post-weaning phase.

There were no differences seen in DMI based on MR amount or weaning strategy at any time during the grower phase (Figure 3.2). However, when comparing each
treatment, there was an effect at d 70 where MOD-GR had significantly greater DMI compared to MOD-AB, meaning when calves receive MOD MR and are weaned GR they are better adapted to solid feed intake compared to AB weaning. There was also an effect at d 98 where both HI-AB and MOD-GR consumed significantly more DM compared to HI-GR calves. An interaction ($P=0.03$) was seen for FE, it was reduced with GR weaning in MOD calves whereas it was increased with GR weaning the HI calves (Figure 3.5). This could be because MOD-GR received the lowest overall amount of MR pre-weaning and increased MR is linked to increased nutrient availability and digestion (Khan et al., 2007b), so this low amount of MR is not being efficiently digested, absorbed, and utilized by the body. During the pre-weaning phase of this experiment, HI MR had greater FE compared to MOD and AB was greater than GR. This advantage in HI MR and AB weaning could be due to increased availability of nutrients with large amounts of MR and AB calves receiving more MR for a longer period than GR (Khan et al., 2007b). Calves receiving HI MR maintained this increased FE until d 70 of the grower phase (Figure 3.4), but by d 91 this advantage was lost. Average FE (Figure 3.5) during the grower phase shows that MOD-AB calves are more accustom to digesting greater quantities of nutrients compared to MOD-GR causing them to have greater FE while HI-AB calves are not adapted to solid feed intake allowing HI-GR calves, who are more adapted to solid feed intake, to have greater FE post-weaning. This could indicate that MOD MR regimens might not need to be gradually weaned whereas there is a major advantage for HI MR regimens when weaned gradually.
Diet Digestibility

Table 3.3 shows the apparent dC for d 66, 87, and 108 as well as them combined, to identify digestibility differences over the entire 56 d post-weaning phase and by sampling period. When reporting combined data, apparent dC of DM, NDF, and ADF were greater (P<0.05) for MOD vs. HI. Hill et al. (2016c) reported the same results when comparing MOD vs. HI MR feeding programs over a 56 d post-weaning phase. This reflects increased development in the digestive tract of MOD calves, promoting nutrient digestion including fiber (Table 5). There were no significant differences between OM, CP, fat, starch, or sugar apparent dC based on treatment and no interactions were observed in the combined data set. These results suggest that calves receiving MOD amounts of MR pre-weaning are more adapted to consuming more complex nutrients such as fiber (Hill et al., 2016b; Dennis et al., 2018). This increased nutrient digestion allowed MOD calves to converge the BW and structural growth gap within HI calves.

At d 66 and d 87, apparent dC of DM, NDF, and ADF were greater (P<0.05) for MOD vs. HI calves (Table 3.3; Figure 3.6). Hill et al. (2016c) measured digestibility on d 77 and reported greater NDF and ADF dC in MOD compared to HI and a trend for greater DM dC in MOD calves. Dennis et al. (2018) also reported increased dC of DM, NDF, and ADF for MOD vs. HI calves at d 84. Calves fed HI amounts of MR pre-weaning do not have an adequately developed rumen (establishment of the microbiome, papillae formation, and muscularization and vascularization of the rumen wall) to effectively digest the nutrients found in starter, causing them to have lower DM, NDF, and ADF dC up to 6 wks. post-weaning (weaned on d 49). There were no other
differences in digestibility at d 66. At d 87, apparent dC of sugar ($P=0.02$) were greater for MOD vs. HI. There was also an interaction seen between MR feeding rate and weaning strategy for apparent dC of DM, CP, and sugar ($P<0.05$). The combination of HI MR with GR weaning seems to drastically improve nutrient digestion compared to HI MR and AB weaning. In fact, HI-GR dC for DM, CP, and sugar are almost as high, if not greater than the MOD treatments. This indicates that the combination of feeding calves increased amounts of MR and then gradually reducing it until weaning, both promotes the growth of the animal pre-weaning and adequately prepares the rumen for solid feed consumption (Khan et al., 2007b). Apparent dC of all reported nutrients for HI-AB appears to be less than the other three treatments. Feeding calves HI MR and weaning them abruptly does not adequately prepare them for solid feed consumption post-weaning leading to low nutrient digestibility. At d 108, apparent dC for starch was greater for HI vs. MOD ($P=0.04$). Dennis et al. (2018) also observed increased starch digestion in high MR regimens compared to moderate at d 112 of the post-weaning phase. No other differences or interactions were reported. Calves receiving HI MR were initially reported to have lower dC for DM, NDF, and ADF compared to MOD calves up until d 87 (Figure 3.6). This is believed to be due to an inadequate amount of starter consumption prior to weaning and an underdeveloped rumen caused by HI MR programs (Terré et al., 2007). This is logical, calves fed MOD MR have reduced BW up through d 91 but by d 112 there was no different in BW compared to the HI treatments (Figure 3.3). The reduced dC for DM, NDF, and ADF seen in HI calves early in the post-weaning phase, allowed the MOD MR calves to converge and reduce the differences in BW. Chapman et al. (2016)
also reported lower dC post-weaning in calves fed increased amount of MR, which resulted in reduced ADG and structural growth. However, by the end of the 56 d post-weaning phase, there were no differences seen for nutrient apparent dC except for starch, which was greater for HI vs. MOD MR.

During the 56-day post-weaning phase of this study, DM, NDF, and ADF digestibility overall were greater for MOD vs HI calves. Moreover, looking at individual sampling times, DM, NDF, and ADF dC were greater for MOD vs. HI calves for d 66 and 87 but there were no significant differences seen in the last sampling period (d 108; Table 3.3; Figure 3.6), indicating that all calves were adapted to solid feed consumption (Hill et al., 2016c; Dennis et al., 2018). DM apparent dC was greater for MOD vs. HI calves over the entire 56 d post-weaning period. There was not a significant difference seen in starch digestibility based on MR feeding rate overall, at d 66 or 87, but at d 108 starch apparent dC was significantly greater for HI vs. MOD calves. By the end of the post-weaning phase, HI calves were able to adapt to solid feed consumption, allowing their rumens to develop, and increasing nutrient digestibility. This is evident by MOD calves no longer having greater dC for any nutrient at d 108 and HI calves having greater starch dC compared to MOD, indicating that MR effect on diet digestibility is lost by the end of the post-weaning phase of this experiment.

**Weaning Strategy Effect**

**Growth Performance**

Only initial HH was greater for AB vs. GR calves ($P=0.02$; Table 3.2). These differences are due to the treatments implemented during the pre-weaning phase of this
trial. Calves that were weaned abruptly had greater HH at the start of the post-weaning phase. This is logical because AB weaned calves received MR at higher amounts compared to GR, leading to increased structural growth. However, final HH based on weaning strategy was not different. Even though calves weaned abruptly consumed more MR and had increased structural growth, they may not have been adequately adapted to solid feed consumption causing their structural growth advantage to be lost post-weaning. Khan et al. (2007b) compared how conventional (reduced milk and abrupt weaning) and step-down (increased milk and gradual weaning) feeding method affected structural growth. They reported that both at weaning and after weaning step-down had increased HH compared to conventional. This differed from our results and could be due to our study having a HI and MOD MR amount offered with AB and GR weaning. Whereas they only had a moderate milk with abrupt weaning (10% of BW for 44 d) and high milk with gradual weaning (20% of BW for 23 d, then 10% of BW for 16 d). There were no other differences between weaning method. Final BW, HW, and HH did not differ among treatments. Weaning strategy alone might not be vital to optimize BW and structural growth in calves. Instead, it might be the combination with HI MR that we see advantages because it leads to increased ADG as well as promotes the consumption of solid feed prior to weaning, initiating rumen development and better preparing the calf for a completely solid diet post-weaning. This advantage, however, is lost and all calves are the same in BW and structural growth by d 112 of the experiment (Table 3.2; Figure 3.3; Dennis et al. (2018)).
**Diet Digestibility**

Table 3.3 shows the apparent dC for d 66, 87, and 108 as well as them combined, to identify digestibility differences over the entire 56 d post-weaning phase and by sampling period. At d 66, there were no dC differences between AB and GR weaning for NDF or ADF. Hill et al. (2016c) also reported no differences in NDF or ADF based on a more abruptly weaned and a more gradually weaned treatment at d 77 indicating that early in the post-weaning phase weaning strategy did not help stimulate rumen development enough to increase fiber digestion. At d 87, apparent dC of DM and ADF were greater for GR vs. AB. The same increase in ADF dC for gradual weaning vs. abrupt was reported by Dennis et al. (2018) at d 84. This shift from no differences seen in ADF dC at d 66 to greater ADF dC in GR vs. AB at d 84 could be the result of cellulolytic bacteria within the rumen taking a longer time to establish (Anderson et al., 1987), causing GR weaning to have a delayed increase in ADF dC. At d 108, apparent dC for starch was greater for GR vs. AB weaning ($P=0.01$). Again, this transition from no differences seen initially (d 66 and 87) in starch dC to greater starch dC in GR vs. AB weaning at d 108, indicates that GR weaning is having a positive carry over effect on dC for both structural and nonstructural carbohydrates. The only differences seen over the entire 56 d post-weaning period were greater DM and ADF dC for GR vs. AB. This indicates that GR weaning can have a positive impact on nutrient digestion by promoting starter consumption and stimulating rumen development prior to weaning, better preparing the animal to transition to digesting solid feed after weaning.
Conclusion

During the pre-weaning phase of this study, we reported that increased MR promotes growth early in life but a more conventional amount of MR promotes starter intake, rumen development, and the digestion of fibrous feed fractions. During the post-weaning phase of this study, we observed that the increased growth parameters seen prior to weaning lasted through d 91, but all treatments converged by d 112 of the study. There were no significant differences seen for ADG, final BW, final HH, final HW, or final BCS based on MR amount or weaning strategy by day 112. This confirms our hypothesis that HI MR calves would experience a stagnation in growth post-weaning, allowing the MOD MR calves to reduce the growth gap by d 112. There were also no dC differences at d 108 between treatments except for starch. In conclusion, when feeding calves a MOD MR, GR weaning them is not necessary to ensure growth and development. However, offering calves a HI MR should be paired with a GR weaning process to ensure successful growth and development.
Table 3.1 Chemical composition of grower phase experimental feeds

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<td>87.9 ± 2.10</td>
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<tr>
<td>DM basis, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>6.3 ± 0.49</td>
<td>9.8 ± 1.05</td>
</tr>
<tr>
<td>CP</td>
<td>20.7 ± 2.25</td>
<td>5.8 ± 0.62</td>
</tr>
<tr>
<td>Fat</td>
<td>3.5 ± 0.34</td>
<td>1.9 ± 0.24</td>
</tr>
<tr>
<td>ADF</td>
<td>7.5 ± 0.97</td>
<td>40.8 ± 0.39</td>
</tr>
<tr>
<td>NDF</td>
<td>14.2 ± 1.96</td>
<td>62.4 ± 0.69</td>
</tr>
<tr>
<td>Starch</td>
<td>42.4 ± 0.54</td>
<td>0.7 ± 0.13</td>
</tr>
<tr>
<td>Sugar</td>
<td>6.7 ± 0.82</td>
<td>11.1 ± 1.28</td>
</tr>
</tbody>
</table>

Table 3.2 Growth performance of calves during the grower phase of the trial as influenced by the four pre-weaning treatments

<table>
<thead>
<tr>
<th>Item</th>
<th>Pre-weaning Treatments1</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MOD-AB</td>
<td>MOD-GR</td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td>75.00</td>
<td>73.53</td>
</tr>
<tr>
<td>Final BW, kg</td>
<td>133.42</td>
<td>129.84</td>
</tr>
<tr>
<td>Initial hip width, cm</td>
<td>20.87</td>
<td>20.77</td>
</tr>
<tr>
<td>Final hip width, cm</td>
<td>25.87</td>
<td>25.70</td>
</tr>
<tr>
<td>Initial hip height, cm</td>
<td>82.97</td>
<td>81.10</td>
</tr>
<tr>
<td>Final hip height, cm</td>
<td>95.30</td>
<td>94.07</td>
</tr>
<tr>
<td>Initial BCS</td>
<td>2.20</td>
<td>2.30</td>
</tr>
<tr>
<td>Final BCS</td>
<td>2.70</td>
<td>2.73</td>
</tr>
<tr>
<td>DM intake, kg/d</td>
<td>3.13</td>
<td>3.25</td>
</tr>
<tr>
<td>DM intake, % BW</td>
<td>3.01</td>
<td>3.20</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>1.04</td>
<td>1.01</td>
</tr>
<tr>
<td>Feed efficiency2</td>
<td>0.33</td>
<td>0.31</td>
</tr>
<tr>
<td>Hip width change, cm</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Hip height change, cm</td>
<td>12.33</td>
<td>13.00</td>
</tr>
<tr>
<td>BCS change</td>
<td>0.50</td>
<td>0.47</td>
</tr>
</tbody>
</table>

1 Calves assigned to MOD-AB were fed 0.66 kg (DM basis) MR for first 42 d then 0.33 kg for last 7 d, MOD-GR were fed 0.66 kg MR for 28 d, 0.33 kg for 14 d, and 0.17 kg for the last 7 d, HI-AB were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 28 d, and 0.66 kg for the last 7 d, HI-GR were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 14 d, 0.66 kg for 14 d, and 0.33 kg for the last 7 d
2 BW gain divided by DMI
Table 3.3 Apparent digestibility coefficients (dC) of nutrients in calves during the grower phase of the trial as influenced by the four pre-weaning treatments

<table>
<thead>
<tr>
<th>Digestibility, %</th>
<th>Pre-weaning Treatments</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MOD-AB</td>
<td>MOD-GR</td>
</tr>
<tr>
<td>d 66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>83.6</td>
<td>83.3</td>
</tr>
<tr>
<td>OM</td>
<td>85.3</td>
<td>85.2</td>
</tr>
<tr>
<td>NDF</td>
<td>57.7</td>
<td>56.5</td>
</tr>
<tr>
<td>ADF</td>
<td>56.1</td>
<td>55.1</td>
</tr>
<tr>
<td>CP</td>
<td>84.1</td>
<td>84.0</td>
</tr>
<tr>
<td>Fat</td>
<td>80.2</td>
<td>81.9</td>
</tr>
<tr>
<td>Starch</td>
<td>97.7</td>
<td>97.7</td>
</tr>
<tr>
<td>Sugar</td>
<td>97.8</td>
<td>97.3</td>
</tr>
<tr>
<td>d 87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>83.0</td>
<td>84.0</td>
</tr>
<tr>
<td>OM</td>
<td>85.3</td>
<td>85.5</td>
</tr>
<tr>
<td>NDF</td>
<td>58.1</td>
<td>61.3</td>
</tr>
<tr>
<td>ADF</td>
<td>56.9</td>
<td>61.3</td>
</tr>
<tr>
<td>CP</td>
<td>84.6</td>
<td>83.4</td>
</tr>
<tr>
<td>Fat</td>
<td>87.7</td>
<td>87.9</td>
</tr>
<tr>
<td>Starch</td>
<td>96.7</td>
<td>97.3</td>
</tr>
<tr>
<td>Sugar</td>
<td>97.2</td>
<td>96.9</td>
</tr>
<tr>
<td>d 108</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>80.3</td>
<td>81.0</td>
</tr>
<tr>
<td>OM</td>
<td>81.4</td>
<td>82.3</td>
</tr>
<tr>
<td>NDF</td>
<td>51.6</td>
<td>53.0</td>
</tr>
<tr>
<td>ADF</td>
<td>50.6</td>
<td>51.8</td>
</tr>
<tr>
<td>CP</td>
<td>82.5</td>
<td>81.8</td>
</tr>
<tr>
<td>Fat</td>
<td>82.2</td>
<td>82.8</td>
</tr>
<tr>
<td>Starch</td>
<td>93.8</td>
<td>96.1</td>
</tr>
<tr>
<td>Sugar</td>
<td>94.7</td>
<td>94.6</td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>82.3</td>
<td>82.8</td>
</tr>
<tr>
<td>OM</td>
<td>84.0</td>
<td>84.3</td>
</tr>
<tr>
<td>NDF</td>
<td>55.8</td>
<td>56.9</td>
</tr>
<tr>
<td>ADF</td>
<td>54.5</td>
<td>56.1</td>
</tr>
<tr>
<td>CP</td>
<td>83.8</td>
<td>83.1</td>
</tr>
<tr>
<td>Fat</td>
<td>83.4</td>
<td>84.2</td>
</tr>
<tr>
<td>Starch</td>
<td>96.0</td>
<td>97.0</td>
</tr>
<tr>
<td>Sugar</td>
<td>96.6</td>
<td>96.3</td>
</tr>
</tbody>
</table>

1 Calves assigned to MOD-AB were fed 0.66 kg (DM basis) MR for first 42 d then 0.33 kg for last 7 d, MOD-GR were fed 0.66 kg MR for 28 d, 0.33 kg for 14 d, and 0.17 kg for the last 7 d, HI-AB were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 28 d, and 0.66 kg for the last 7 d, HI-GR were fed 0.66 kg MR for 7 d, 0.82 kg for 7 d, 1.1 kg for 14 d, 0.66 kg for 14 d, and 0.33 kg for the last 7 d.
**Figure 3.1** Milk replacer amounts for the four pre-weaning experimental treatments

![Graph showing milk replacer amounts for four treatments over time](image)

**Figure 3.2** Dry matter intake (DMI) change over the 56 d (d 56-112) post-weaning phase as influenced by the four pre-weaning treatments

![Graph showing DMI change for four treatments over time](image)

- a=MOD-GR > MOD-AB, \( P < 0.05 \)
- b=HI-AB > HI-GR, \( P < 0.05 \)
- c=MOD-GR > HI-GR, \( P < 0.05 \)
Figure 3.3 Body weight (BW) change over the 56 d (d 56-112) post-weaning phase as influenced by the four pre-weaning treatments.

Weaning strategy effect, $P < 0.05$
Milk replacer rate effect, $* = P < 0.05$

Figure 3.4 Feed efficiency (FE) change over the 56 d (d 56-112) post-weaning phase as influenced by the four pre-weaning treatments.

Weaning strategy effect, $P < 0.05$
Milk replacer rate effect, $* = P < 0.05$
**Figure 3.5** Average feed efficiency (FE) over the 56 d (d 56-112) post-weaning phase as influenced by the four pre-weaning treatments.
Figure 3.6 Apparent nutrient digestibility coefficients (dC) during the post-weaning phase as influenced by the four pre-weaning treatments.

Weaning strategy effect, $Q = P < 0.05$, $QQ = P < 0.10$
Milk replacer rate effect, $* = P < 0.05$, $** = P < 0.10$
Interaction between weaning strategy and MR feeding rate, $I = P < 0.05$, $II = P < 0.10$
CHAPTER FOUR

SUMMARY

The objective of this study was to identify the effects that milk replacer (MR) feeding rate and weaning strategy, as well as the combined effects of the two, have on growth performance and nutrient digestibility in Holstein calves to 4 months of age. Calves were assigned to one of four experimental treatments, moderate (MOD) MR with an abrupt (AB) weaning strategy, MOD MR with a gradual (GR) weaning, high (HI) MR with an AB weaning, or HI MR with GR weaning. At d 56, one week after calves were weaned, HI MR fed calves and those weaned abruptly had increased growth, feed efficiency, and digestion of readily available nutrients compared to MOD MR calves and those weaned gradually who had increased starter intake and digestion of fibrous feed fractions. At d 112, there were no longer any differences in growth performance and nutrient digestibility based on MR amount and weaning strategy. However, HI MR calves had increased feed efficiency when paired with a gradual weaning strategy compared to an abrupt. In conclusion, when feeding calves a MOD amount of MR it is not necessary to wean them gradually to ensure successful growth and development. However, when feeding calves a HI amount of MR it is necessary to wean them gradually to allow them to adapt to solid feed consumption prior to weaning and start the development of the rumen.
APPENDICES

Appendix A

Technical Note: A simple rumen collection device for calves: An adaptation of a manual drenching system

Technical note: A simple rumen collection device for calves: An adaptation of a manual drenching system


ABSTRACT

A limited amount of research is available related to the rumen microbiota of calves, yet there has been a recent spike of interest in determining the diversity and development of calf rumen microbial populations. To study the microbial populations of a calf's rumen, a sample of the rumen fluid is needed. One way to take a rumen fluid sample from a calf is by fistulating the animal. This method requires surgery and can be very stressful on a young animal that is trying to adapt to a new environment and has a depressed immune system. Another method that can be used instead of fistulation surgery is a rumen pump. This method requires a tube to be inserted into the rumen through the calf's esophagus. Once inside the rumen, fluid can be pumped out and collected in a few minutes. This method is quick, inexpensive, and does not cause significant stress on the animal. This technical note presents the materials and methodology used to convert a drenching system into a rumen pump and its respective utilization in 2 experiments using dairy heifer calves.

Key words: calf, rumen fluid, simple technique, rumen pump

Technical Note

Rumen fluid collection in calf nutrition research is essential to study the function of the digestive system and the rumen microbial community. The rumen microbiome is composed of bacteria, archaea, protozoa, and fungi, and these communities, by their metabolic activities, transform diet components into VFA, microbial protein, and other metabolites that will serve as a source of energy, AA, and growth promoters to the animal (Ribeiro et al., 2017). Several techniques have been designed to study the rumen microbial population and its changes between the different stages of the calf. Fistulas are commonly used for these types of studies, as they simplify the collection of rumen fluid. But one of their main disadvantages is the invasiveness of the surgery during early stages of development in calves (Tirfuald et al., 1973). They can also be cost prohibitive compared to a rumen pump and put more stress on the animal when the calf is at the early stages of developing an active immune system.

A technique previously used in different studies for rumen fluid sample collection was the use of oral stomach tubes attached to a syringe (Abdelkadi et al., 1996). Geisheiser (1993) designed an alternative technique for ruminal fluid sampling, an oro-ruminal probe with a suction pump. Nevertheless, it presents a significant limitation when collecting samples from animals with limited fluid present in the ventral sac of the rumen, such as the case of young calves; the hole in the probe’s head and in the suction tube tend to get obstructed. Terru et al. (2012) compared samples obtained from both ruminal fistulas and oro-ruminal tubes, finding no significant difference between the pH or VFA profile; this implies that little sample integrity is sacrificed when using a stomach tube. Moreover, Paz et al. (2016) used a modified esophagostomy tube attached to a metal strainer (Barn and Firrou, 1962) using suction from a vacuum pump and observed no differences in the structure of rumen bacteria communities when compared with samples taken from the same animals through rumen cannulas. Another alternative is rumenocentesis (rumen puncture), an invasive technique that requires surgical creation of the rumen and suffers the risk of localized abscesses or peritonitis (Duthold et al., 2004) and might not be feasible in calves less than 90 d old.

Here, we describe a device to collect rumen samples from postweaned calves (Figure 1) created from a modification of the Castle Pump System designed for drenching fluids into the rumen of adult cattle (Springer Magnus, Glenrothes, MN). This device has been used in calves as early as 15 d old, but intensive...
REFERENCES


