ABSTRACT

EFFECTS OF NATIVE RUMEN MICROBES AS A DFM ON
COLOSTRUM IN HOLSTEIN DAIRY COWS

The use of direct-fed microbials (DFM) during the later stage of the transition period in the dairy industry is becoming more common. Although questions remain if there is an effect on colostrum quality and quantity. The purpose of this study is to determine if feeding a blend of native rumen microbes composed of *Clostridium beijerinckii, Pichia kudriavzevii, Butyrivibrio fibrisolvens, Ruminococcus bovis* influences the quality and quantity of colostrum. Holstein cows were brought into the close-up pen 3 weeks prior to calving and were separated into pens, two containing multiparous and two containing primiparous. Cows were fed identical total mixed ration (TMR) diets with the addition of the DFM Galaxis Frontier to the treatment pens. Galaxis Frontier was blended into the TMR at a rate of 5g/head/day. Upon freshening, colostrum was harvested from cows within a 12-hr period. Cows on treatment produced a larger amount of colostrum (8.17 kg) than those on the control diet (7.07 kg). Colostrum from cows on treatment also had a higher fat percentage (6.13) and kg of fat (0.51) than colostrum harvested from control cows (5.08, 0.36 respectively). No significant difference was found in analysis of protein percent (C=14.34, T=14.68) and kg of protein (C=1.12, T=1.27), refractometer (C=22.49, T=23.21), or misco readings (C=22.93, T=23.49) brix readings (C=24.41, T=24.04), IgG levels (C=14.34, T=14.68), or grams of IgG (C=610.63, T=665.10). However, a trend was found for moisture content (C=76.16, T=74.98), dry matter (C=23.84, T=25.02).

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December 2022

EFFECTS OF NATIVE RUMEN MICROBES AS A DFM ON COLOSTRUM IN HOLSTEIN DAIRY COWS

by

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A thesis

submitted in partial

fulfillment of the requirements for the degree of

Master of Science in Agricultural Science

in the Jordan College of Agricultural Sciences and Technology

California State University, Fresno

December 2022

APPROVED

For the Department of Animal Sciences and Agricultural Education:

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ACKNOWLEDGMENTS

I would also like to thank Fresno State and the Jordan College for introducing me to some of the best advisors, and friends. Throughout my six years at State, I have formed lifelong friendships that I will forever be grateful for. There is somethings special to be said about the experience JCAST provides for their students.

To my advisor Dr. Kyle Thompson- who knew six years ago, both our first year at Fresno State would lead us to where we are today. Because of your trust, support and guidance you have provided me with countless opportunities for me to grow and develop as an individual, both in the classroom and on the dairy. Though at sometimes it was rocky, we always found common ground and were able to build something better from it. I will forever be thankful for our time together.

To Dr. Gayle O’bannon, whose knowledge has no end- you may never know the impact you have on your students’ life. I for one can attest to the fact that I would not have half of the knowledge I do today if it were not for you. You are essential to the success of any animal science student, and you embody everything I hope to be. I will forever be thankful for your guidance, support, selflessness, and words of encouragement (W.W.G.O.D). I feel privileged to call you a friend. Thank you for everything.

To I would also like to thank my other committee members, Dr. Amanda McKeith and Lisa Marotz. Thank you for your constant support and help along the way. Finally, I owe a big thanks to Dr. Avery Culbertson for being a sounding board and breath of fresh air. I will forever be thankful for our friendship.

To my family and friends- thank you will never be enough. No matter what mountain I choose to climb I know I will have the strongest group of soldiers climbing with me. Thank you for believing in me even when I didn’t believe in myself.

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INTRODUCTION

With each passing year, it is becoming more challenging to be a dairy farmer. Every dairyman's top priority is to provide all that is necessary for their cows. They achieve this by finding ways to improve milk production, longevity, and overall health and performance. One approach dairymen use to meet their goals is by feeding direct-fed microbials (DFM). Direct-fed microbials (DFM) are continuously being researched to determine what type of effect they have on dairy cattle. Direct-fed microbials is a term that includes specific and nonspecific yeast, fungi, cell fragments, filtrates and bacteria (Beharka and Nagaraja, 1993; Sullivan and Martin 1999; Knowlton et al., 2002). Direct-fed microbials must also be natural occurring flora in the gastrointestinal tract (Fuller, 1997). It is important to associate the cow's productivity with the complex microbiome of the rumen because it plays a major role in the cow's ability to obtain nutrients and utilize them towards production (Dickerson et al., 2022). Research on dairy cows is mostly done during the transition period (AlZahal et al., 2014), pre- and post-partum. Studies are focused mostly on milk production, milk components, dry matter intake and ruminal digestion or feed efficiency. Research done on prepartum Holstein primiparous, and multiparous cows fed a *Propionibacterium* strain P169 DFM showed a 4% increase in fat-corrected milk (Stein et al., 2006). Some research shows no effect on milk production but a positive effect on ruminal digestion of forages (Nocek et al., 2006). There is a lack of research on the effects of supplementing a DFM during the close-up stage on colostrum quantity and quality. Research conducted on colostrum quality and quantity is commonly focused on the management of dry cows, time of year at parturition, and length on close-up diets (Borchardt et al., 2021). A study on feeding a silage plus concentrate concluded that there was no change in colostrum synthesis, specifically no change in colostrum IgG content (Dunn et al., 2017). More research is needed to determine if feeding a DFM during the close-up stage of the transition period has any effect on colostrum quality or quantity.

The current study tested Galaxis Frontier, a novel DFM containing *Clostridium beijerinckii, Pichia kudriavzevii, Ruminococcus bovis,* and *Butyrivibrio fibrisolvens.* These microorganisms were isolated from healthy, high producing dairy cows and were identified through next-generations sequencing experiments to be strongly associated with the most efficient dairy cows (Valldecabres 2022). The researchers hypothesized that feeding Galaxis Frontier to Holstein cows during the close-up stage would result in an increase in colostrum quality and quantity.

LITERATURE REVIEW

Feed Additives

Ionophores

Ionophores' mode of action is to interrupt transmembrane movement and intracellular equilibrium of ions in specific classes of protozoa and bacteria that inhibit the gastrointestinal tract (McGuffey et al., 2001). To increase the production efficiency, the rumen environment must be altered to better enhance ruminal fermentation. McGuffey et al. (2001) stated that ionophores provide an advantage for certain microbes. However, this comes with a cost of other microbes. There are three main areas that ionophores are known to affect. They provide an increase in energy metabolism of the rumen and animal, improved nitrogen metabolism of the rumen bacteria, and a decline of digestive improvement from abnormal rumen fermentation (Bergen and Bates, 1984; McGuffey et al., 2001). The animal is able to conform to these effects and use them to increase production. Monensin, or a form of monensin, is an extremely common ionophore fed to lactating dairy cows because it has proven to improve feed efficiency. Ionophore research spreads across the board hitting all the main targets, which include rumen fermentation, milk yield, metabolic disorders, and milk composition.

A commonly studied ionophore is lasalocid. McDougall et al. (2004) conducted a study on pasture-fed dairy cows and fed them 300 to 350 mg of lasalocid/day 3 weeks before and 18 weeks after the start of the calving period. McDougall et al. (2004) found that the lasalocid treatment increased milk volume, milk protein, and milk fat production by approximately 2%. The study also revealed that cows treated with lasalocid had fewer incidences of mastitis as well as no change in body condition or negative effect on metabolic processes postpartum. The authors also suggested that although monensin is the most predominantly researched ionophore related to lasalocid, they do not have the same effects overall. Monensin has a strong preference for sodium whereas lasalocid forms complexes with a mixed number of cations (Martineau et al., 2007). A study conducted by Martineau et al. (2007) attempted to find the bridge between lasalocid and monensin. The study blocked cows by milk production, and they were fed a TMR. The cows were either assigned to the control diet or a diet mixed with monensin or lasalocid. Among all three treatments, dry matter intake (23.5 kg), milk production (36.6 kg), milk fat (3.36%) and protein (3.38%) concentrations were all similar. Therefore, it is declared in this study that the addition of both DFM monensin and lasalocid have no effect on dry matter intake, milk production, or milk components.

Monensin in the dairy industry gained popularity very fast. As results were starting to show through on its positive effects on milk yield and dry matter intake dairymen were quick to enact the addition of feeding monensin in lactating rations. One of the effects of monensin is that it increases ruminal propionate production (McCarthy et al., 2015). A study done by Akins et al., (2014) tested the effects of monensin on lactation performance. There was a reported increase in milk yield by 1.5 kg/d per cow along with an increase in milk protein percentage and lactose yield. There was no difference observed in dry matter intake (27.0 kg/d). Other findings included an increase in efficiency in those cows fed monensin compared to those who were not. Akins et al. (2014) concluded that monensin did increase feed efficiency and lactation performance across both dietary starch concentrations. Similar findings were revealed in a study done by McCarthy et al. (2015). Close-up diets for both primiparous and multiparous cows were top-dressed with monensin varying from 0-400 mg/d. Cows fed monensin regardless of quantity had a higher DMI as well as a higher milk yield during the first 9 weeks of lactation. This study also found that there were no significant effects of MON on milky yield during the first 3 weeks postpartum, however when data were evaluated from week 1 to week 9 postpartum, cows fed MON had produced 2.2 kg/d more milk than control cows. There was a trend found between the interaction of MON and parity in that primiparous cows fed MON had a lower feed efficiency than control cows. The authors suggested that the difference of DMI and feed efficiency between parities are present because of the differences in the ecosystem of the rumen itself (McCarthy et al., 2015).

Types of Direct-Fed Microbials

Direct-fed microbials (DFM) are commonly used in ruminant nutrition to stabilize ruminal fermentation in high-producing cattle (Raeth-Knight et al., 2007). One of the main purposes for feeding a DFM product is to improve overall health and performance by creating a more efficient cow. This starts with the health of the rumen. The term DFM is broad and includes specific and nonspecific yeast, fungi, bacteria, cell fragments, and filtrates (Beharka and Nagaraja, 1993; Sullivan and Martin, 1999). Currently, there are various types of DFM products already being utilized in animal production and are gradually growing in popularity as research continues to be conducted. The three different types of DFM that have been researched the most are bacteria, fungi, and yeasts (live or culture). It is not uncommon that these three types are fed as a blend of each other or on an individual basis. Each DFM serves a different purpose depending on lifecycle stage and age.

Lifecycle stages can be divided into neonates (pre-ruminants), young animals (weaned to pre-breeding), adult (mature bred or open), lactation stage (fresh, peak, mid, late, dry), and transition (nonlactating to lactating). During the pre-ruminant stage, the rumen is not functional and the majority of the diet is milk. The purpose of a DFM is to aid in the maintenance and production of the intestinal flora (Krehbiel et al., 2003; Fuller 1989). However, research does show that enterococci, lactobacilli, and yeast have a positive effect on weight gain and the prevention of diarrhea (Newbold, 1995). Whether feeding a DFM or not, the goal for young ruminants is to heighten the development of muscle and papillae growth in the rumen to encourage an earlier weaning age. Feeding *Enterococci*, *Lactobacilli,* and yeast, in addition to *Aspergillus oryzae* extract, together can achieve an earlier weaning age and advance rumen development (Newbold, 1995). Once an adult ruminant, the rumen flora and fauna are no longer developing, and the objective now becomes maintenance and increasing the ability of digestion and breakdown of feedstuffs.

The most common DFMs studied are two strains of *Enterococcus faecium* bacteria (Oetzel et al., 2007), either fed on its own or as a blend with *Saccharomyces cerevisiae* yeast or *Lactobacillus plantarum* (Nocek et al., 2003). A combination of *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* bacteria has been fed and measured to determine if there are any effects on milk yield, and nutrient digestibility (Boyd et al., 2011). One common yeast DFM fed on an individual basis is *Trichosporon sericeum* (Mwenya et al., 2005). Publications done on yeast based DFM are more common due to the lack of natural yeast growth ability in the rumen (Amin & Mao, 2021). One purpose behind feeding a DFM is to increase intake and the digestion of feedstuffs in addition to creating a healthier and habitual environment for microbes (Clemmons et al., 2018). More importantly, concern towards antibiotic resistance is becoming more prevalent in the industry as time goes on. One way to try and combat the usage of antibiotics is to find an alternative or to create a healthier cow from the start (Krehbiel et al., 2003). Given the results of the addition of DFM to cows throughout lifecycle stages helps prevent health incidences, thus reducing the utilization of antibiotics. Publications on all types of DFM show both significant and insignificant differences on overall effects on a dairy cow’s health and performance from feeding a DFM.

Transition Period and Cow Health

The transition period is defined as 3 weeks before and 3 weeks after parturition and is known to be the most difficult time in a cow's life (Oetzel et al., 2007). During the transition period, either prior or post calving, the cow is most susceptible to illnesses, specifically metabolic disorders due to having a suppressed immune system. In order for a cow to reach her genetic potential and peak for milk production, she needs to go through the full transition period with as little to no health issues and added stress. At this point the cow is going through major physiological and environmental changes, making it difficult for her to keep up with normal maintenance requirements. The cow also undergoes a significant ruminal microbiome change. A study done on the health of the rumen during the transition stage was able to isolate 34 different bacterial genera. The large variety of bacterial genres is the result of how quickly the cow goes through a diet change (Zhu et al., 2018). Zhu et al. (2018) stated that the main driver for such a dramatic change in the rumen microbiome during the transition period is because of dietary changes. However, the number of physiological changes the cow goes through during that time frame must also be considered. During the prepartum period, feed intake will drop significantly the closer the cow gets to parturition, but the cow is still consuming enough to meet maintenance and energy requirements, which is achievable by proper diet formulation. In the postpartum stage, feed consumption begins to increase significantly given the cow is feeling well and has had a successful calving period (Oetzel et al., 2007; Zhu et al., 2018). DFM has been shown to enhance the health of the rumen by manipulating the fermentation characteristics and microbial environment (Morrison et al., 2017). DFM studies are conducted during the transition stage because that is when the cow could use the most amount of support. One way to support the transition of the cow is to ensure overall health.

Managing the health of a cow is important during all stages; to achieve good health, each stage needs to be managed individually. All lifecycle stages have a different level of dietary requirements, thus nutrition balancing is a major part to success in dairy farming. Transitioning a cow off a lactating diet to a non-lactating diet while still providing necessary requirements, but not in excess, can be challenging. When the cow goes from the dry pen to the close-up pen there is another diet change in efforts to try and combat the cow from going into a severe negative energy balance postpartum. Cows naturally undergo a period of negative energy (Thompson, 2006). The reason for such high incidences of metabolic disorders is because milk production increases faster than energy intake during the first few weeks postpartum, resulting in the cow having a larger demand for nutrients and maintenance requirements (Luan et al., 2015). Once in the close-up pen, cows are fed a dietary cation-anion difference (DCAD) diet that contains a negative charge (Zhang et al., 2022). A study by Zhang et al. (2022) determined the effects on blood calcium and metabolic adaptation to lactation. Cows were fed a negative DCAD diet with low Ca, positive DCAD diet with low Ca, or negative DCAD diet with a high Ca supplementation. Results showed that cows fed a negative DCAD diet altered blood acid-base balance, induced metabolic acidosis at calving, and improved protein and lipid metabolism. In addition, those cows fed a negative DCAD diet with a high supplementation of Ca had a better metabolic adaptation to lactating than those cows fed a negative DCAD with low Ca supplementation. Luan et al. (2015) stated that the rumen fermentation pattern is physiologically altered after parturition. Other health issues that cows may face during the transition period include hypocalcemia or milk fever, displaced abomasum, ketosis (metabolic disorders), retained placenta, metritis, and mastitis (Thompson, 2006). By supplementing a DFM starting 2 weeks prepartum, Nocek and Kautz (2006) found that cows on treatment had a higher (13.6%) case of retained placenta than control cows (9.1%). They also found that metritis and ketosis was similar between treatments but occurrences of displaced abomasusms were lower in treatment cows (4.5%, 9.1%). Any type of transitional illness results in a decrease in overall 305-d mature equivalent milk yield (Thompson, 2006; Wallace et al., 1996). Dairymen focus on different management tools available to them in efforts to obtain a healthy, productive cow.

Milk production can be easily manipulated both positive and negatively. Factors that can negatively affect production can be as simple as the environment. One tool dairymen use to avoid negative factors is by feeding a DFM. Research was conducted on microbial supplements on pre- and postpartum cows and measured the effects on milk yield. Cows were fed two strains of *Enterococcus faecium* as well as *Saccharomyces cerevisiae* yeast products. They were fed 2 g/cow/d of the DFM mixed into the ration and were fed 10 d prepartum and 23 d postpartum. Supplementation of the DFM showed an increase in milk fat percentage for multiparous cows (Oetzel et al., 2007). Cows supplemented with the DFM product earlier in lactation did not show a statistical difference in milk yield compared to those cows fed the product at a later day postpartum. Overall, there was no statistical difference in milk yield between control and treatment cows but there was a numerical increase in yield from cows supplemented with a DFM. Nocek et al. (2003) fed *E. faecium* during pre and postpartum phases and came to the same conclusions as Oetzel et al. (2007. Results showed an increase in milk yield and milk protein content from cows fed the DFM pre and postpartum compared to those on control. In addition, there was no statistical difference but there was a numerical increase in production from cows fed only during the postpartum stage. The increase in milk fat is most likely caused by an increase in rumen butyrate concentrations as milk fat production has a direct correlation with butyrate (Huhtanen et al., 1993).

Supplementing rations with any class of DFM has the ability to improve productive efficiency by an increase ruminal digestion and dry matter intake (Luan et al., 2015; Nocek et al., 2003). The addition of *Bacillus pumilus* 8G-134 to pre and postpartum cows had mixed results. Prepartum DMI results for supplemented cows was down 0.15 kg/d and control was down 2.16 kg/d. This can be because *Bacillus* might target the intestinal and microbiota or even modulate the immune system (Luan et al., 2015). DMI did increase faster in control cows as compared to cows on treatment; this could be pivotal for the cow to go through a healthier transition, potentially resulting in an increase in 305-d lactation (Luan et al., 2015). A different approach to rumen digestibility is looking at propionate in the rumen. Compared to glucose, propionate is 108% a source of energy for ATP; therefore feeding Propionibacterium may naturally help influence metabolism (Stein et al., 2006). As a result of the addition of *Propionibacterium* strain P169, ruminal propionate levels were increased by 18.5% and 17.0%. Cows on both levels of treatment had an increase in DMI.

Yeast and Fungus

Compared to bacteria, yeast products are a more prominent DFM utilized in research. Most research has focused less on live yeast (**LY**) and more on yeast culture (Moallem et al., 2009) The most popular yeast culture research is done on the strain of *Saccharomyces cerevisiae* (Mwenya et al., 2005). *S. cerevisiae* is essential in the fermentation process (Mahmoud et al., 2020). Similar to other DFM products, the overall results on dairy cows' performance from a yeast supplementation remains inconsistent. Some research found an increase in milk production and dry matter intake (**DMI**), but others have found there to be no differences. Moallem et al. (2009) supplemented dairy cows with 1g of LY (*Saccharomyces cerevisiae*) and found an increase in feed intake and milk yield during the hot season. They were also able to determine a 2 kg/d increase in fat corrected milk. Dry Matter Intake was 3.7% greater in cows supplemented with LY than those on the control diet. It was concluded by Sniffen et al. (2004) that the diet balance between degradable and nondegradable protein is a key factor in the response to yeast supplementation. Yeast culture provides growth factors for intestinal flora and other stimulants for bacteria to grow in the rumen (Miller-Webster et al., 2002; Moallem et al., 2009). Moallem et al. (2009) stated that enhancing the environment within the rumen provides a more livable habitat for the rumen microbes allowing, the cow to overall become more efficient. A reliable strategy used to achieve this is by enhancing the host's microbiota by feeding live microorganisms (Michalak et al., 2021; Pinloche et al., 2013; Valldecabres et al., 2022). Another study done on *Saccharomyces cerevisiae* by Grigoletto et al. (2021) analyzed the effects of the LY on intake, digestibility, and milk yield. Cows were fed a diet containing MON or MON and milk sacc+ (MS+; a blend of live yeast and organic materials). The study found that cows fed the MS+ showed an increase in dry matter intake (P=0.033), milk yield (P=0.036), and fat-corrected milk (P < 0.028), where the cows fed MON had an increase in milk urea nitrogen (P< 0.080). The authors stated that DFM, especially those that contain *Saccharomyces cerevisiae*, increase the cellulolytic bacteria activity and population of lactic acid utilizing bacteria in the rumen, thus contributing to the increase in milk components and dry matter intake.

Further research done on yeast DFM products tends to be driven towards the effects of ruminal pH and ruminal fermentation. Mwenya et al. (2005) fed a yeast culture (*Trichosporon sericeum*) at four different rates some mixed with galacto-oligosaccharides (**GOS**). Overall, there were no major effects of utilizing the supplements GOS and YC in this particular study (Mwenya et al., 2005). A similar study using *Saccharomyces cervisiae* in combination with a fungal culture *Aspergillus oryzae* looked at the effects on ruminal fermentation, microbial populations, and nutrient supply to the small intestine. Four cows were fitted with ruminal and duodenal cannulas and fed the same basal diet except the treatment groups added either 57 g/d of yeast, 3 g/d of fungal culture, or 57 g/d of yeast and 3 g/d of fungal culture. Results across all diets for ruminal pH, ammonia concentration, and overall VFA concentration were similar. Cows fed a yeast or fungal culture had a higher molar percentage of ruminal isoacids than the cows on both yeast and fungal cultures. Fiber digestion in the rumen was also similar among all treatments (Yoon and Stern 1996).

The most common fungal (AO) product researched is *Aspergillus oryzae*. Significant growth of AO or *S. cervisiae* does not naturally occur within the rumen. Yoon and Stern (1996) reported that the results found in this study determined that yeast and fungal DFM either together or standing alone can influence ruminal fermentation and microbial populations. Though this study declared that yeast and fungal cultures have an effect on ruminal fermentation and microbial populations, there is still clarification needed on the mode of action. Martin and Nisbet (1992) broke down the different quantities of the natural inhabitants of the rumen. They stated that anaerobic fungi are less concentrated than aerobic and that as a result, fungal feed additives have little effect on the natural population of anaerobic fungi. As a result, there are few studies done just on the effects of feeding a fungal DFM, as they are usually paired with yeast DFM.

Bacteria

Bacteria is a type of DFM that is utilized to alter the health of the rumen in efforts to achieve a healthier, more efficient cow. When comparing bacteria to yeast DFM, bacteria have gotten less attention even though most bacterial DFM products are naturally present within the rumen. This can also be why ruminal fermentation is not impacted as dramatically by other DFM products. Philippeau et al. (2017) reported that because of the ability to modulate ruminal fermentation, bacterial DFM have been proposed as potential enteric methane mitigation additive for ruminants. Bacteria organisms most widely known and fed to dairy cows are *Lactobacillus acidophalus* and *Propionibacterium freudenreichii*. It is not uncommon that studies are done with a combination of the two because both bacteria products feed off each other (Boyd et al., 2011; Lawrence et al., 2021). *Lactobacillus acidophalus* is a lactate-producing bacteria and *Propionibacterium freudenreichii* are bacteria that produce propionate by utilizing lactate (Boyd et al., 2011). Previous research utilizing a combination of both *L. acidophalus* and *P. freudenreichii* focused on milk yield, efficiency of yield, and nutrient digestibility (Boyd et al., 2011). Cows were separated into groups based on assigned treatments. Treatment cows were fed the same diet as control, with the addition of 4 x 10^9 cfu/head/day. Results showed that with the inclusion of both bacteria based DFM in the diet, it increased milk and protein yields along with energy corrected milk. Monteiro et al. (2021) and Nocek et al. (2011) suggested that one-way bacteria-based DFM are able to achieve an increase in milk production, components, and feed efficiency is by using the lactate in the rumen. Originally, the idea behind feeding bacteria was to potentially improve the health of the gastrointestinal tract, increase the quantity of desirable microflora, and decrease the risk of pathogenic organisms (Krehbiel et al., 2003). It has since then been utilized more for its ability to increase milk production or because of its positive effects on cows' overall health and performance during the transition stage. Bacterial based DFM have shown to affect the innate, humoral and cellular aspects of the immune system. This provides evidence that bacterial DFM are able to aid in the protection against pathogenic organisms, by adhering to and colonizing the gastrointestinal tract. Lactobacilli are known to mostly target the innate immune response (Krehbiel et al., 2003). Lactobacilli are also unique in the fact that they also help aid in the reduction of ruminal acidosis. Ruminal acidosis occurs when the pH of the rumen gets below a 5.6. The presence of Lactobacilli (*Lactobacillus* and *Enterococcus)* in the rumen helps the microorganisms to adapt to the concentration of lactic acid, thus reducing acidosis (Krehbiel et al., 2003).

Philippeau et al. (2017) conducted a study on the effects of bacterial DFM on ruminal fermentation, microbial characteristics, milk fatty acid, diet digestibility, and methane production while fed a diet focused on increasing VFA profiles. Cows were separated into groups based on parity, days in milk, milk production and body weight. They were either assigned to a high starch or low starch diet. The cows were fed one of the four diets with the addition of *Propionibacterium* P63; P63 and *Lactobacillus plantarum* 115; or P63 and *Lactobacillus rhamnosus* 32. Direct Fed Microbials were fed at a rate of 10^{10} cfu/d. Results show that the two diets (high and low starch) induced different ruminal VFA profiles, having a larger proportion of it being propionate at the expense of the other two prominent VFA, acetate and butyrate. For the high starch diet, there were greater concentrations of total bacteria, while protozoa concentration decreased. Ruminal pH was increased across both low and high starch diets when compared to control. Overall, bacterial DFM does have an effect on VFA production but is dependent on the strain of bacteria and nutritional value of the diet.

Native Rumen Microbes

A more recent addition to the world of DFM is the use of microbes native to the rumen. Using a DFM not native to the rumen works more towards enhancing the environment of the rumen and creating a larger source of nutrition for the microbes, whereas feeding a DFM composed of microbes native to the rumen has the potential to influence the population and efficiency of microbes naturally inhabiting the rumen. Both work towards the same goal that creates a more efficient and healthier cow while having a different area of target. Although there is not a large number of publications done on the utilization of natural rumen microbes and its effects on the cow, the research published has shown positive results. Stein et al. (2006) conducted a field study on the effects of feeding *Propionibacterium* strain P169 that was isolated from rumen fluid collected from fistulated cows. There were three treatment groups, one control diet, one on a low dose, and the other a high dose of DFM. Cows were grouped by primiparous and multiparous and were fed the control diet with the addition of the *Propionibacterium* at a rate of 6×10^{10} cfu/cow and 6×10^{11} cfu/cow. The study found an increase in fat corrected milk across all treatment diets, both primiparous and multiparous. A difference was found in milk fat from multiparous cows over all groups and primiparous cows, although not significant, had a higher fat concentration than control cows (Stein et al., 2006). Given the maturity of the cows, it is not uncommon that multiparous cows have a higher fat percentage regardless of treatment. With that, the authors suggested that it is much more difficult to alter the efficiency of primiparous cows than multiparous cows (Stein et al., 2006). Similar to other suggestions, the authors predicted that the reason for the increase in milk components is related to the increase in *Propionibacterium*.

Research on native rumen microbes is being conducted, however there is no research done comparing a native rumen microbial based DFM against a foreign DFM. Commercial DFM products are not native to the rumen, which could lessen their ability to interact with the native microbiome (Goldsmith et al., 2022). One of the recent studies evaluated the effects of feeding a mix of two native microbes and a mix of four native microbes to determine possible positive changes in cow profitability and efficiency. The first DFM (MFS1) comprised of a minimum of *Clostridium beijerinckii* at 2×10^{6} CFU/g and *Pichia kudriavzevii* at 2×10^{7} CFU/g and the second (MFS2) was made up of *C. beijerinckii* at 2×10^{6} CFU/g, *P. kudriavzevii* at 2×10^{7} CFU/g, *Ruminococcus bovis* at 2×10^{7} CFU/g, and *Butyrivibrio fibrisolvens* at 2×10^{7} CFU/g). This study supports that there is an increase in cow efficiency and profitability by determining that the supplementation of MFS2 in the diet did effectively improve milk yield by 3.0 kg/d, energy corrected milk by 3.7 kg/d, fat by 0.12 kg/d, and protein by 0.12 kg/d overall feed efficiency (Valldecabres et al., 2022).

The dairy industry continues to be one of the most advanced industries in the way that dairymen put forth their most effort into increasing productivity and cow efficiency while trying to minimize outputs and environmental impact (Dickerson et al., 2022). How they achieve this is by enhancing or altering the complex microbiome of the rumen since it plays a major role in cow productivity and efficiency. One other study has been conducted on these specific MSF1 and MFS2 during the lactating stage and found different results on milk production, and BCS. The same DFM (MSF1 and MSF2) were fed to Holstein cows that were blocked by parity, days in milk and energy-corrected milk (ECM). From there cows were randomly assigned to treatments and the diets were top-dressed daily with the respective DFM for 112 d. Although results were not significant, there was a trending increase in milk yield, fat, and protein (Dickerson et al., 2022). A intriguing finding from this study was that the cows who were started on the DFM at later days in milk have a smaller effect on yield than those who were supplemented the DFM earlier on in lactation (Dickerson et al., 2022). In order for a cow to be efficient, she must have a healthy rumen ecosystem for the microbes to thrive. Volatile fatty acids (VFA) are vital to the cow so that they can be used for milk production. The microorganisms in the rumen digest the different feedstuffs consumed by the cow and convert them into VFA for the cow (Brown et al., 1960). With results being consistent across all three studies on the blend of the four native rumen microbes, there is potential that this is the direction that the industry may begin to go after.

Native rumen microbes that make up Galaxis Frontier focus on specific feedstuffs that result in the production of certain VFA. The two bacteria microbes *Ruminococcus bovis* and *Butyrivibrio fibrisolvens* affect the process of fermentation (Dickerson et al., 2022). *B. fibrisolvens* produces stearic acid, resulting in an effect on milk fat synthesis by fermenting structural carbohydrates. *R. bovis* produces acetate by fermenting starch and other carbohydrates within the rumen. *Clostridium beijerinckii* creates hydrogen, acetate, and butyrate by creating a relationship with complex carbohydrate digesters (Dickerson et al., 2022). The yeast microbe, also found naturally in the rumen, is *Pichia kudriavzevii*, which breaks down cellulose and hemicellulose into monosaccharides because of the high cellulase activity, benefiting the rumen by providing a source of energy for the other microbes (Dickerson et al., 2022).

Colostrum

Before the cow starts lactogenesis, she first goes through colostrogenesis, or the production of colostrum. Colostrogenesis occurs during the last few weeks of gestation otherwise known as the prepartum transition stage (Borchardt et al., 2021). The first few milkings postpartum is true colostrum; the next five milkings after initial harvest of colostrum is referred to as transitional milk (Yang et al., 2015). Not only does colostrum contain five classes of immunoglobulins (IgG, IgG1, IgG2, IgA, and IgM), but it also is made up of nutrients like fat-soluble vitamins, vitamin B12 and iron (Godden et al., 2009; Yang et al., 2015). There are few known factors that affect colostrum production such as length of dry period, parity, time of year, and nutrition (Borchardt et al., 2021; Dunn et al., 2017). Most research focuses on dry cow management and nutrition during the dry cow and close-up stages and the effects those factors have on colostrum production. Borchardt et al. (2021) conducted a study on the effects of management-related factors in dry cows and colostrum quality. Data collected for both primiparous and multiparous cows included days in far off pen, days in close-up pen, calving ease (0-5), calf sex, quantity (kg) and quality (brix) of colostrum. They found that there was a seasonal pattern for colostrum quantity and quality. For primiparous cows, the quality was affected by month of calving having the highest colostrum harvested in the month of December and lowest in the month of August. Quality for multiparous cows was affected by parity, having the greatest colostrum given by third and above lactation and the lower quality from second lactation cows. The biggest finding was that shortening the dry period from 60 d to 40 d did not negatively affect overall colostrum quality determined by IgG content. The authors suggested that nutritional management during both dry off and the close-up stage has a larger effect on colostrum quality versus length of dry period.

Because of the maturity difference between multiparous and primiparous cows, it is not uncommon for multiparous cows to produce greater quality and quantity of colostrum than primiparous cows. Primiparous cows are still developing initial mammary tissue, whereas multiparous cows are not. As a result, altering the quantity and quality of colostrum is extremely difficult to do for both multi and primiparous cows. During the prepartum dry or far-off stage, the cow should be focusing on maintaining the pregnancy, apoptosis if a mature cow, and development of the mammary tissue (Borchardt et al., 2021). Balancing and managing nutrition during the prepartum stage is important because prepartum mothers have an increased need for energy and protein to support the production of mammary tissue, colostrum synthesis, as well as uterine and fetal development (Borchardt et al., 2021). The most common way that dairymen attempt to alter or improve the quality and quantity of colostrum is by vaccinating the mothers at dry off and when they are moved up to the close-up pen. Aside from vaccination, one important tool used to achieve high quality colostrum is by shortening the time from calving to harvesting the colostrum (Borchardt et al., 2021). The longer the time frame between calving and collection provides more opportunity for the dam to begin reabsorbing the nutrients in colostrum, degrading the quality that could have been fed to the newborn calf.

Calves are born with a limited functioning immune system; therefore, it is vital to calf health that it is fed the proper quantity and quality of colostrum at birth to initiate maternal passive immunity. Calves rely solely on the consumption of colostrum for protection against disease exposure for the first few weeks of their life (Arthington et al., 2000; Quigley et al., 2002). Without the proper intake of colostrum, there is a higher occurrence of mortality, pneumonia, diarrhea, and overall morbidity in neonate calves

(Borchardt et al., 2021). Passive immunity is achieved from colostrum through the intestinal epithelium, which only remains permeable for 24 h after birth. (Arthington et al., 2000). After the 24-h period the intestinal wall becomes impermeable to larger proteins leaving it no longer possible to absorb colostrum thus meaning leaving no room for colostrum to be effective. Lombard et al. (2020) fed calves at different timings after birth and found that the faster the calf consumes colostrum will result in higher IgG levels. They indicate that the highest IgG levels were found in calves that were fed within 2 h of birth. Not only is it important the calf receives colostrum, but it is important to ensure it is of the proper quality and quantity. Quality of colostrum is measured on a brix scale by determining the number of solids within. Two most common methods to measure total solids is by using a refractometer or a digital refractometer. Acceptable brix percentage indicating good quality is 22% (Bielmann et al., 2010). To provide the proper amount of colostrum it is recommended that newborn calves be fed 10–12% of their birth weight (Arthington et al., 2000). Borchardt et al. (2021) suggested that when a calf doesn't receive the right amount of colostrum there is potential for permanent effect on overall lifetime performance and production. After calves have received colostrum, a blood sample is obtained and tested for total protein levels to determine if the calf had successfully gone through passive transfer or if they did not. If IgG levels do not exceed 10 g/L within the first 2 days of life, they are considered a failure for passive transfer (Silva-del-Río et al., 2017).

Colostrum management is important because calves are the future generation of a herd. Raising healthy calves into replacement heifers is critical for sustainability and overall economic performance of a dairy operation (Borchardt et al., 2021). The more research done on the different areas of raising healthy calves and heifers, the more dairymen have begun to shift focus from milk production to replacement rearing. A good calf makes a good cow; in order to allow a calf to reach her genetic potential the calf must be set up to do so at birth and in the hutch. If calfhood scours, pneumonia, and other illnesses are high in the hutch, causing lack of or extended time for growth, overall lactational production will be decreased (Svensson and Hultgren, 2008). Research published on the effects of colostrum quality on the development in calves compares feeding colostrum and transitional milk at the first feeding. There were similar effects on the calves between feeding colostrum and transitional milk; however, calves fed colostrum had an accepted rate of IgG (>10 mg/mL of serum) (van Keulen et al., 2020) an increase in villus width and length, crypt depth and mucosal thickness compared to those calves fed only transitional milk (Yang et al., 2015). Yang et al. (2015) stated that the higher the colostrum quality and quantity, the more likely the calf will establish their own immune defense mechanism and antioxidant system quickly after birth. assisting in a decrease in morbidity and mortality. Van Keulen et al. (2021) indicated that large numbers of calves had failed passive transfer because they were left with their dam longer than others, which suggests the quality of colostrum is far more important than leaving the calf with their dam. Calves that failed passive transfer suffered from scours during weeks 2 and 3 of age and it was found via fecal sample that calves were infected with *Rotavirus* (Van Keulen et al., 2021). It was suggested by van Keulen et al. (2021) that due to the lack of colostrum to the calves, they were more susceptible to *Rotavirus*. Calves fed a high-quality colostrum had significantly fewer cases of scours during the first 3 weeks of age suggesting again that quality of colostrum is essential to calf health.

Since calves are born with limited immune system function (Arthington et al., 2000), in order to initiate the startup, dairymen focus on feeding the calf good quality (22% brix; Bielmann et al., 2010) and quantity (10-12% of birth weight) (Arthington et al., 2000). As a result, it is important to improve colostrum quality. One possible way to achieve this is by feeding a DFM to preparturition cattle. DFM products are beneficial in decreasing metabolic disorders, mastitis cases, and body condition scores (AlZahal et al., 2014; Nocek et al., 2003; Oetzel et al., 2007). They also increase milk yield, milk components and dry matter intake. There is lack of research found on the effects of DFM fed during the close-up stage on colostrum quality and quantity; in addition, there is a lack of research on the effects in primiparous cows. No previous research was found on native rumen microbes fed as a DFM and tested to see the effects on colostrum quantity and quality. Therefore, the following study was completed to determine if feeding a blend of native rumen microbes during the close-up stage influences colostrum quality and quantity. This study is the first research conducted using a blend of four native rumen microbes, *Clostridium beijerinckii, Pichia kudriavzevii, Butyrivibrio fibrisolvens,* and *Ruminococcus bovis* to establish effects on colostrum.

MATERIALS AND METHODS

This study was conducted at a 6,500 Holstein dairy located in the Central Valley of California. Eight groups of prepartum cows were moved to the close-up pen 21 d prior to their expected calving date. Of the four groups of primiparous cows and four groups of multiparous cows, odd ear tags were assigned to the treatment (Galaxis Frontier, Native Microbials San Diego, CA) and even ear tags were assigned to control. There were two groups per treatment for both primiparous and multiparous. Primiparous cows had 130 hd in both treatment and control pens. The remaining two pens were designated for overflow. The multiparous pens housed 220 hd; cows over that went into the overflow pens designated for multiparous cows. All groups, control and treatment, were fed once a day. Multiparous treatment and control cows were fed identical rations (**Table 1**) except for Galaxis Frontier (Native Microbials, San Diego, CA), a blend of native rumen microbes added to the treatment groups. Primiparous treatment and control cows were fed identical rations (**Table 2**) except for the addition of Galaxis Frontier to the treatment group. Galaxis Frontier was fed at 5 g/hd/d, blended adequately into the ration. Galaxis Frontier contains a minimum of 2,000,000 CFU/g of *Clostridium beijerinckii,* and a minimum of 20,000,000 CFU/g each of *Pichia kudriavzevii, Butyrivibrio fibrisolvens, Ruminococcus bovis*.

Harvest of Colostrum

Upon calving, both primiparous and multiparous cows' colostrum was collected within 12 h post calving. Samples were collected at 0300 or 1400, depending on when the cow freshened. Sample collection started the last week of January 2022 and was taken once every morning on Tuesdays, Wednesdays, and Thursdays. On Mondays and Fridays, samples were collected during the afternoon shift. Samples were collected during both the morning and afternoon shifts on Saturdays and Sundays. Once 200 multiparous cows were sampled, collection was completed. Weights and samples were done on an individual basis by freshly calibrated IACAR approved meters from Fresno Dairy Herd Improvement Association (DHIA, Fresno, CA). Weights were recorded on site at the dairy once cows completed milking, followed by collecting a sample. A minimum of 50 mL of colostrum was collected from each fresh cow and stored in two separate sterile tubes. One tube contained 45 mL of colostrum, the other contained the remaining 5mL. Tubes were labeled with the cow identification number and collection date. Samples were then stored on ice until they arrived at the Fresno State Dairy (Fresno, CA). Once at the Fresno State Dairy, samples were inverted and tested with a digital Misco PA200-008 Palm Abbe Digital Refractometer (Solon, OH) and an AGTEC Portable Refractometer with Copper ATC 0-32 Brix (Hampden, ME). One mL of each sample was transferred with disposable pipettes and placed on the refractometer and Misco. Before placing the sample in the Misco, it was calibrated using sterile water. Once calibration was set, the sample was placed in the Misco and was not recorded until it read the same number three times. The sample was also placed on the refractometer, held up to the light, and read. The refractometer glass was cleaned with sterile water between each sample. After both readings were collected, the remaining samples were stored in a 20°C freezer. Only colostrum samples from multiparous cows were sent off for further analysis. The 45 mL samples were sent in batches of 100 to SDK Laboratories (Hutchison, KS) to be tested for percentage of dry matter by vacuum oven, fat percentage by acid hydrolysis and crude protein totals by Kjedahl method. The 5 mL samples were sent in one batch of 200 samples to Saskatoon Colostrum Company (Saskatchewan, Canada) to be tested for radial immunodiffusion assay to determine IgG levels and grams of IgG.

Statistical Analysis

The data were analyzed using the GLM procedure in SAS (Version 9.4) to compare treatment cows against control using LS means. Values were considered significant at ($P \leq 0.05$); if the value was less than 0.1 and above 0.05 it was considered to be a trend towards significance. Categorical Brix was analyzed using the freq procedure in SAS (Version 9.4) to compare control cows against treatment cows using a chi-squared and Fisher test.

RESULTS AND DISCUSSION

Multiparous data showed no difference among treatments when comparing total protein percent (C=14.34%, T=14.68%; *P*=0.56) and protein kg (C=1.12, T=1.27; *P*=0.28), (**Table 3**). In addition, there was no statistical difference in refractometer (C=22.49, T=23.21; *P*=0.23) or misco readings (C=22.93, T=23.49; *P*=0.38) performed at Fresno State (**Table 3**). Misco readings (C=22.33, T=23.16; *P*=0.23) and refractometer readings (C=22.24, T=22.82; *P*=0.39) from SDK Laboratories (Hutchison, KS) were also not statistically significant (**Table 3**). Cows fed Galaxis Frontier had a higher fat percentage than those on the control diet (C=5.08%, T=6.13%; *P*=0.01; (**Table 3**). Similarly, there was a statistical difference found in kg of fat (C=0.36, T=0.51) from cows fed Galaxis Frontier versus those who were not (*P*<0.05; **Table 3**). Cows fed Galaxis Frontier had a higher quantity of colostrum (8.17 kg) than the control (7.07 kg) cows (*P*=0.03). Colostrum was tested on a brix scale and either tested good or bad (**Table 4**), and from the good readings, there was no difference calculated (*P*=0.34) between treatment groups. No difference was found in moisture content; however there was a trend towards significance (C=76.16%, T=74.98%; *P*=0.08) among treatments (**Table 3**). Identically, there was no difference found but instead a trend was found in dry matter content (C=23.84%, T=25.02%; *P*=0.08) (**Table 3**). There was no difference in grams of IgG levels (C=610.63, T=665.10; *P*=0.09) between treatment groups. Galaxis Frontier had no effect (C=85.88, T=84.80; *P*=0.89) on IgG percentage content of colostrum (**Table 3**). Furthermore, there was no difference found among treatments for brix readings (*P*=0.25) from SCCL laboratories (Saskatchewan, Canada) (**Table 3**).

When analyzing primiparous data, there was no difference observed between treatments on any of the three data points. Refractometer readings were not different between treatments (C=20.76, T=21.45, *P*= 0.40; **Table 5**). Misco readings (C=20.71, T=21.63; *P*=0.27) were not different between treatment and control groups (**Table 5**). In addition, weights collected from primiparous cows on control and treatment groups were not statistically different (C=5.61 kg, T=6.10 kg, *P*=0.42; **Table 5**).

Discussion

The goal of this trial was to determine the effects of feeding a blend of native rumen microbes *Clostridium beijerinckii, Pichia kudriavzevii, Butyrivibrio fibrisolvens,* and *Ruminococcus bovis* on colostrum quality and quantity.

Quality

There were similar findings discovered in a trial based on mannan oligosaccharide (MOS) fed to prepartum Holstein dairy cows. Westland et al. (2017) reported that cows fed MOS during the close-up stage of transition, produced significantly more colostrum than those who did not receive MOS during the close-up stage. Cows treated with MOS had produced an average of 1.96 kg more milk per cow, which is similar to the 1.10 kg increase found in the current study. There is reason to believe that with an enhancement of the immune system due to the health of the rumen, it could correlate to the increased production of colostrum levels as a result of a more efficient metabolism (Westland et al., 2017). With the addition of a healthy rumen, there is a larger VFA concentration. An increase in acetate along with butyrate directly correlates with an increase in milk fat (Dickerson et al., 2002). A study conducted by Aragona (2020) on the addition of nicotinic acid (NA) to the close-up diet and its effects on colostrum quality and quantity showed that there was no difference in colostrum quantity from those cows on treatment. The findings of the Ort et al. (2017) study showed no difference in colostrum yield from cows fed the DFM, and it was suggested by the authors that because there was no increase in dry matter intake, that was why there was no increase in colostrum yield.

Nutritional Value and Quality

In the present study there was no significant difference found in the levels of IgG, protein, or quality readings. Cows fed Galaxis Frontier produced 1.05% and 0.15 kg more fat than the control group. It is likely that there was an increase in fat production from those cows on treatment because of an increase in butyrate production in the rumen. Increased rates of butyrates are known to have a positive effect on fat production (Huhtanen et al., 1993). The study by Westland et al. (2017) showed no difference in IgG levels between cows supplemented with MOS and cows who were not. Westland et al. (2017) suggested that no difference was seen in IgG concentration potentially because of lactation number and the time difference varying from cow to cow between calving and harvest of colostrum. However, Aragona (2020) found a positive effect on IgG levels from supplementing the close-up diet with NA. By supplementing prepartum cows with cellulase and amylase enzymes Ort et al. (2018) found that there was no positive effect on colostrum composition, but there was a decrease in IgA, fat yield, ash concentration and total solids. Brix good data for control cows showed that 57.97% of the brix readings were good and 42.03% was bad. Treatment cows had 67.74% of good readings and 32.36% bad readings with the probability of 0.25 that there is no difference between the treatments. Although there was no statistical difference in brix, refractometer or Misco readings, multiparous cows on treatment did show a numerical increase of 0.46% increase across all quality measures.

Limitations and Future Research

For the present study, there are potential limitations, one of which being the sample size. Since there was a trend towards significance in the analysis of dry matter and moisture, it can suggest that a larger sample size is needed to determine if there are either not enough cows to declare the analysis significant or not enough cows to prove insignificance. In addition, the collection times can manipulate the quality of colostrum.

Colostrum was harvested within 12 h of calving, which allows for a potential 12 h window to allow the cow to begin reabsorption of colostrum, thus hindering the true quality of the colostrum. Lastly, when looking at previous published data, there are very few studies that have looked at the effects on colostrum quality and quantity from feeding a DFM. Previous literature published on DFM research is done on the transition period, but the findings do not focus on its effects on colostrum instead studies are focused on DMI, feed efficiency, milk yield and transitional cow health. This is the first study to test the effects of four native rumen microbes on colostrum quantity and quality. More research needs to be conducted to further the understanding of DFM effects on colostrum quality and quantity in close-up cows. A suggestion for future research would be to increase sample size per treatment to provide more data specifically for the analysis that came back insignificant, but leaning towards a trend. Moreover, harvesting colostrum within a smaller window postpartum to better ensure that the reading taken from colostrum is more accurate.

Table 1. Ingredient and nutrient composition of the multiparous

Ingredient	
Alfalfa Hay DCAD, %	5.61
Almond Hulls, %	2.81
Canola, %	1.70
Rolled Corn, %	5.58
Mineral-CU Supplement, %	1.50
Corn, Silage %	12.36
Water, %	0.45
Components	
Dry Matter, %	60
Moisture, %	40.1
CP, %	16.5
ADF, %	21
ADF NDF, %	67.9
NDF, %	30.9
Lignin, %	3.87
Lignin NDF, %	12.52
ESC, %	1.9
ESC NFC, %	4.4
Starch, %	26.1
Starch NDC, %	61.4
Fat EE, %	3.54
TDN, %	69.6
Nel Mcal, lb	0.72
NEm Mcal, lb	0.79
Neg Mcal, kg	0.51
NFC, %	42.6
NSC, %	28
DCAD, %	-16.80
ME, %	1.2
Ash, %	8.85
Ca, %	1.34
P, %	0.38
Mg, %	0.32
K, %	1.25
S, %	0.34
Na, %	0.26
Cl, %	1.36
Fe	283
Mn, %	62
Zn	120
Cu, %	24

Table 2. Ingredient and nutrient composition of the primiparous diet

Ingredient	
Alfalfa Hay DCAD, kg	5.61
Almond Hulls, kg	2.81
Canola, kg	1.70
Rolled Corn, kg	5.58
Mineral-CU Supplement, kg	1.50
Corn Silage, kg	12.36
Water, kg	0.45
Components	
Dry Matter, %	59.6
Moisture, %	40.4
CP, %	16
ADF, %	21.4
ADF NDF, %	68
NDF, %	31.6
Lignin, %	3.94
Lignin NDF, %	12.49
ESC, %	1.3
ESC NFC, %	3.1
Starch, %	26.2
Starch NDC, %	62.2
Fat EE, %	3.47
TDN, %	69
Nel Mcal, lb	0.71
NEm Mcal, lb	0.78
Neg Mcal, kg	0.50
NFC, %	42.2
NSC, %	27.5
DCAD, %	-18.90
ME, %	1.19
Ash, %	9.13
Ca, %	1.33
P, %	0.39
Mg, %	0.34
K, %	1.18
S, %	0.36
Na, %	0.26
Cl, %	1.33
Fe	369
Mn, %	66
Zn	123
Cu, %	25

Table 3. Effects of native DFM on colostrum quality and quantity in multiparous cows.[1]

Item	Con	TRT	CSE	TSE	*P*-Value
Nutritional Value					
Fat, %	5.08	6.13	6.15	4.51	0.0070
Protein, %	14.34	14.68	0.28	0.30	0.56
Fat, kg	0.36	0.51	0.04	0.06	0.0013
Protein, kg	1.12	1.27	0.18	0.97	0.28
Dry Matter %	23.84	25.02	0.32	0.36	0.08
Colostrum Quality					
Refractometer, Brix [2]	22.49	23.21	0.29	0.30	0.23
Misco, Brix[3]	22.93	23.49	0.32	0.32	0.38
SDK Refractometer, Brix	22.24	22.82	0.32	0.34	0.39
SDK Misco, Brix	22.33	23.16	0.33	0.37	0.23
SCCL Brix[4]	24.41	24.04	1.03	0.40	0.90
Colostrum Quantity					
Weight, kg	7.07	8.17	0.54	0.61	0.03
IgG content					
IgG[5]	85.88	84.80	3.82	3.08	0.88
gIgG[6]	610.63	665.10	39.09	43.55	0.35

[1] Data presented are Least Square Means, treatment, n=94 control, n=111
[2] IgG quantity measured on a brix scale
[3] Digital refractometer measurement of IgG quantity on a brix scale
[4] Measurement of IgG
[5] Immunoglobulin G, antibody quantity

Table 4. Effects of native DFM on colostrum quality in multiparous cows.[1]

Item	Con Good	Con Bad	TRT Good	TRT Bad	P-Value
Colostrum quality					
Categorical Brix	57.97%	42.03%	67.74%	32.26%	0.25

[1] Data presented in chi square

Table 5. Effects of native DFM on colostrum quality and quantity in primiparous cows. [1]

Item	Con	TRT	CSE	TSE	P-Value
Colostrum Quality					
Refractometer, Brix[2]	20.76	21.45	0.39	0.43	0.40
Misco, Brix[3]	20.71	21.63	0.44	0.39	0.27
Colostrum Quantity					
Weight, kg	5.61	6.10	0.71	0.64	0.43

[1] Data presented are Least Square Means, treatment, n= 44, control, n= 51
[2] IgG quantity measured on a brix scale
[3] Digital refractometer measurement of IgG quantity on a brix scale

CONCLUSION

Feeding a combination of native rumen microbes prepartum (*Clostridium beijerinckii, Pichia kudriavzevii, Butyrivibrio fibrisolvens,* and *Ruminococcus bovis*) increased the total weight and percentage fat in multiparous animal colostrum. No differences were found for protein pounds, protein percentage, refractometer and misco readings from both Fresno State and SDK laboratories (Hutchinson KS), brix good, IgG levels, and brix readings from SCCL (Saskatchewan, Canada). A trend leading towards significance was found in dry matter content, moisture, and gIgG. No differences were found in weight, refractometer and misco readings for prepartum primiparous cows. This data suggest that supplementation with rumen native microbes during the last 3 weeks of the transition period (prepartum) can positively influence colostrum quantity and some aspects of colostrum quality.

REFERENCES

AlZahal, O., H. McGill, A. Kleinberg, J.I. Holliday, I.K . Hindrichsen, T.F. Duffield, and B.W. McBride. 2014. Use of a direct-fed microbial product as a supplement during the transition period in dairy cattle. *J. Dairy Sci*. 97:7102–7114.

Amin, A.B., and S. Mao. 2021. Influence of yeast on rumen fermentation, growth performance and quality of products in ruminants: A Review. *Ani. Nut*. 7:31–41.

Aragona, K.M., E.M. Rice, M. Engstrom, and P.S. Erickson. 2020. Supplementation of nicotinic acid to prepartum Holstein cows increases colostral immunoglobulin G, excretion of urinary purine derivatives, and feed efficiency in calves. *J. Dairy Sci*. 103:2287–2302.

Arthington, J.D., M.B. Cattell, J.D. Quigley, G.C. McCoy, and W.L. Hurley. 2000. Passive immunoglobin transfer in newborn calves fed colostrum or spray-dried serum protein alone or as a supplement to colostrum of varying quality. *J. Dairy Sci*. 83:2834–2838.

Akins, M.S., K.L. Perfield, H.B. Green, S.J. Bertics, and R.D. Shaver. 2014. Effect of monensin in lactating dairy cow diets at 2 starch concentrations. *J. Dairy Sci*. 97:917–929.

Beharka, A. A., and T. G. Nagaraja. 1993. Effects of *Aspergillus oryzae* extract (Amaferm) on in vitro fiber digestion. *J. Dairy Sci.* 76:812–18.

Bergen, W.G., and D.B. Bates. 1984. Ionophores: Their effect on production efficiency and mode of action. *J. An Sci*. 58:1465–1483.

Bielmann, V., J. Gillan, N.R. Perkins, A.L. Skidmore, S. Godden, and K.E. Leslie. 2010. An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle. *J. Dairy Sci*. 93:3713–3721.

Borchardt, S., F. Sutter, W. Heuwieser, and P. Venjakob. 2021. Management-related factors in dry cows and their associations with colostrum quantity and quality on a large commercial dairy farm. *J. Dairy Sci*. 105:1589–1602.

Boyd, J., J.W. West, and J.K. Bernard. 2011. Effects of the addition of direct-fed microbials and glycerol to the Diet of lactating dairy cows on milk yield and apparent efficiency of yield. *J. Dairy Sci*. 94:4616–4622.

Brown, R.E., C.L. Davis, J.R. Staubus, and W.O. Nelson. 1960. Production and absorption of volatile fatty acids in the perfused rumen. *J. Dairy Sci.* 43:1788–1795.

Clemmons B.A., Voy B.H., Myer P.R. Altering the gut microbiome of cattle: Considerations of host-microbiome interactions for persistent microbiome manipulation. *Microb Ecol*. 2018 Feb;77(2):523-536. doi: 10.1007/s00248-018-1234-9. Epub 2018 Jul 22. PMID: 30033500.

Dickerson, A.M., F. Yang, H.B. Green, M.M. Embree, and J.K. Drackley. 2022. Feeding native rumen microbial supplements increases energy-corrected milk production and feed efficiency by Holstein Cows. *JDS Communications*. 3:239–244.

Dunn, A., A. Ashfield, B. Earley, M. Welsh, A. Gordon, M. McGee, and S.J. Morrison. 2017. Effect of concentrate supplementation during the dry period on colostrum quality and effect of colostrum feeding regimen on passive transfer of immunity, Calf Health, and performance. *J. Dairy Sci*. 100:357–370.

Fuller, R. 1997. PROBIOTICS 2 (Applications and Practical Aspects). Chapman & Hall, London.

Fuller, R. 1989. A review: Probiotics in man and animals. J. Appl. Bacteriol. 66:365–378

Godden, S.M., D.M. Haines, and D. Hagman. 2009. Improving passive transfer of immunoglobulins in calves. I: Dose effect of feeding a commercial colostrum replacer. *J. Dairy Sci*. 92:1750–1757.

Goldsmith, K., J. Lefler, M. Embree, and M.J. VandeHaar. 2022. The effect of supplementing native rumen microbes on milk production of dairy cows. *JDS Communications*.

Grigoletto, N.T.S., L.G. Ghizzi, L.S. Gheller, M.S. da S. Dias, A.T. Nunes, T.B.P. Silva, G.G. da Silva, L.F. Costa e Silva, D.N. Lobato, and F.P. Rennó. 2021. Effects of a blend of live yeast and organic minerals or monensin on performance of dairy cows during the hot season. *J. Dairy Sci*. 104:11634–11645.

Huhtanen, P., H. Miettinen, and M. Ylinen. 1993. Effect of increasing ruminal butyrate on milk yield and blood constituents in dairy cows fed a grass silage-based diet. *J. Dairy Sci*. 76:1114–1124.

Knowlton, K. F., J. M. McKinney, and C. Cobb. 2002. Effect of a direct- fed fibrolytic enzyme formulation on nutrient intake, partitioning, and excretion in early and late lactation Holstein cows. *J. Dairy Sci.* 85:3328–3335.

Krehbiel, C. R., S. R. Rust, G. Zhang, and S. E. Gilliland. 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. *J. Dairy Sci.* 81 (E. Suppl. 2): E120-E132

Lawrence, M., S. Polukis, A.M. Barnard, M.A. Miller, L. Kung, and T.F. Gressley. 2021. Evaluating the effects of lactobacillus animalis and Propionibacterium Freudenreichii on performance and rumen and fecal measures in lactating dairy cows. *J. Dairy Sci*. 104:4119–4133.

Lombard, J., N. Urie, F. Garry, S. Godden, J. Quigley, T. Earleywine, S. McGuirk, D. Moore, M. Branan, M. Chamorro, G. Smith, C. Shivley, D. Catherman, D. Haines, A.J. Heinrichs, R. James, J. Maas, and K. Sterner. 2020. Consensus recommendations on calf- and herd-level passive immunity in dairy calves in the United States. *J. Dairy Sci*. 103:7611–7624.

Luan, S., M. Duersteler, E.A. Galbraith, and F.C. Cardoso. 2015. Effects of direct-fed bacillus pumilus 8G-134 on feed intake, milk yield, milk composition, feed conversion, and health condition of pre- and postpartum Holstein cows. *J. Dairy Sci*. 98:6423–6432.

Martin, S.A., and D.J. Nisbet. 1992. Effect of direct-fed microbials on rumen microbial fermentation. *J. Dairy Sci*. 75:1736–1744. doi:10.3168/jds.s0022-0302(92)77932-6.

Martineau, R., C. Benchaar, H.V. Petit, H. Lapierre, D.R. Ouellet, D. Pellerin, and R. Berthiaume. 2007. Effects of lasalocid or monensin supplementation on digestion, ruminal fermentation, blood metabolites, and milk production of lactating dairy cows. *J. Dairy Sci*. 90:5714–5725.

McCarthy, M.M., T. Yasui, C.M. Ryan, G.D. Mechor, and T.R. Overton. 2015. Performance of early-lactation dairy cows as affected by dietary starch and monensin supplementation. *J. Dairy Sci*. 98:3335–3350.

McDougall, S., L. Young, and F.M. Anniss. 2004. Production and health of pasture-fed dairy cattle following oral treatment with the ionophore lasalocid. *J. Dairy Sci*. 87:2967–2976.

McGuffey, R.K., L.F. Richardson, and J.I.D. Wilkinson. 2001. Ionophores for Dairy Cattle: Current status and Future Outlook. *J. Dairy Sci*. 84.

Moallem, U., H. Lehrer, L. Livshitz, M. Zachut, and S. Yakoby. 2009. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. *J. Dairy Sci*.

Mahmoud, A.H., J.R. Slate, S. Hong, I. Yoon, and J.L. McGill. 2020. Supplementing a saccharomyces cerevisiae fermentation product modulates innate immune function and ameliorates bovine respiratory syncytial virus infection in neonatal calves. *J. Ani Sci*. 98.

Michalak, M., K. Wojnarowski, P. Cholewińska, N. Szeligowska, M. Bawej, and J. Pacoń. 2021. Selected alternative feed additives used to manipulate the rumen microbiome. *Animals*. 11:1542.

Miller-Webster, T., W.H. Hoover, M. Holt, and J.E. Nocek. 2002. Influence of yeast culture on ruminal microbial metabolism in continuous culture. *J. Dairy Sci*. 85:2009–2014.

Monteiro, H.F., A.L.J. Lelis, P. Fan, B. Calvo Agustinho, R.R. Lobo, J.A. Arce-Cordero, X. Dai, K.C. Jeong, and A.P. Faciola. 2022. Effects of lactic acid-producing bacteria as direct-fed microbials on the ruminal microbiome. *J. Dairy Sci.* 105:2242–2255.

Morrison, S.Y., J.M. Campbell, and J.K. Drackley. 2017. Amino acid supplementation of calf milk replacers containing plasma protein. *J. Dairy Sci*. 100:4637–4649.

Mwenya, B., B. Santoso, C. Sar, B. Pen, R. Morikawa, K. Takaura, K. Umetsu, K. Kimura, and J. Takahashi. 2005. Effects of yeast culture and galacto-oligosaccharides on ruminal fermentation in Holstein cows. *J. Dairy Sci*. 88:1404–1412.

Newbold, C.J. (1995). Microbial feed additives for ruminants. In *Biotechnology in Animal Feeds and Animal Feeding* (eds. R.J. Wallace and A. Chesson). doi.org/10.1002/9783527615353. ch13

Nocek, J. E., and W.P. Kautz. 2006. Direct-fed microbial supplementation on ruminal digestion, health, and performance of pre- and postpartum dairy cattle. *J. Dairy Sci*. 89:260–266.

Nocek, J.E., M.G. Holt, and J. Oppy. 2011. Effects of supplementation with yeast culture and enzymatically hydrolyzed yeast on performance of early lactation dairy cattle. *J. Dairy Sci*. 94:4046–4056.

Nocek, J.E., W.P. Kautz, J.A.Z. Leedle, and E. Block. 2003. Direct-fed microbial supplementation on the performance of dairy cattle during the transition period. *J. Dairy Sci*. 86:331–335.

Oetzel, G.R., K.M. Emery, W.P. Kautz, and J.E. Nocek. 2007. Direct-fed microbial supplementation and health and performance of pre- and postpartum dairy cattle: A field trial. *J. Dairy Sci*. 90:2058–2068.

Ort, S.B., K.M. Aragona, C.E. Chapman, E. Shangraw, A.F. Brito, D.J. Schauff, and P.S. Erickson. 2017. The impact of direct-fed microbials and enzymes on the health and performance of dairy cows with emphasis on colostrum quality and serum immunoglobulin concentrations in calves. *J. Ani. Phys. Ani. Nut*. 102. doi:10.1111/jpn.12806.

Philippeau, C., A. Lettat, C. Martin, M. Silberberg, D.P. Morgavi, A. Ferlay, C. Berger, and P. Nozière. 2017. Effects of bacterial direct-fed microbials on ruminal characteristics, methane emission, and milk fatty acid composition in cows fed high- or low-starch diets. *J. Dairy Sci*. 100:2637–2650.

Pinloche, E., McEwan N., Marden J. -P., Bayourthe C., Auclair E., and Newbold C. J. (2013). The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle. *PLoS One* 8:e67824. doi: 10.1371/ journal.pone.0067824

Quigley, J.D., C.J. Kost, and T.A. Wolfe. 2002. Effects of spray-dried animal plasma in milk replacers or additives containing serum and oligosaccharides on growth and health of Calves. *J. Dairy Sci*. 85:413–421.

Raeth-Knight, M. L., J.G. Linn, and H.G. Jung. 2007. Effect of direct-fed microbials on performance, diet digestibility, and Rumen Characteristics of Holstein dairy cows. *J. Dairy Sci*. 90:1802–1809.

Silva-del-Río, N.,D. Rolle, A. García-Muñoz, S. Rodríguez-Jiménez, A. Valldecabres, A. Lago, and P. Pandey. 2017. Colostrum immunoglobulin G concentration of multiparous jersey cows at first and second milking is associated with parity, colostrum yield, and time of first milking, and can be estimated with Brix refractometry. *J. Dairy Sci*. 100:5774–5781.

Sniffen, C. J., F. Chaucheyras-Durand, M. B. De Ondarza, and G. Donaldson. 2004. Predicting the impact of a live yeast strain on rumen kinetics and ration formulation. Pages 53–59 in Proc. Southwest Nutr. Manag. Conf., Tempe, AZ.

Stein, D. R., D.T. Allen, E.B. Perry, J.C. Bruner, K.W. Gates, T.G. Rehberger, K. Mertz, D. Jones, and L.J. Spicer. 2006. Effects of feeding propionibacteria to dairy cows on milk yield, milk components, and reproduction. *J. Dairy Sci*. 89:111–125.

Sullivan, H. M., and S. A. Martin. 1999. Effects of *Saccharomyces cerevisiae* culture on in vitro mixed ruminal microorganism fer- mentation. *J. Dairy Sci.* 82:2011–2016.

Svensson, C., and J. Hultgren. 2008. Associations between housing, management, and morbidity during rearing and subsequent first-lactation milk production of dairy cows in southwest Sweden. *J. Dairy Sci*. 91:1510–1518.

Thompson, K. S. (2006). Effect of Site of Infusion of *Lactobacillus Acidophilus* and *Propionibacterium Freudenreichii* on Production and Nutrient Digestibility In Lactating Dairy Cows [Unpublished Masters Thesis]. Oklahoma State University

Valldecabres, A., S.P. Gilmore, J.J. Embree, I.Z. Zhelev, J.R. Gaffney, C.A. Marotz, F. Yang, A.S. Izzo, M.M. Embree, and A. Lago. 2022. Effects of rumen-native microbial feed supplementation on milk yield, composition, and feed efficiency in lactating dairy cows. *J. An Sci.* 100.

van Keulen, P., S.A. McCoard, J. Dijkstra, H. Swansson, and M.A. Khan. 2021. Effect of postpartum collection time and colostrum quality on passive transfer of immunity, performance, and small intestinal development in preweaning calves. *J. Dairy Sci*. 104:11931–11944.

Wallace, R. L., G. C. McCoy, T. R. Overton, and J. H. Clack. 1996. Effect of adcerse health events on dry matter consumption, milk production, and body weight loss of dairy cows during early lactation. *J. Dairy Sci.* 79:205

Westland, A., R. Martin, R. White, and J.H. Martin. 2017. Mannan oligosaccharide prepartum supplementation: Effects on dairy cow colostrum quality and quantity. *Animal*. 11:1779–1782.

Yang, M., Y. Zou, Z.H. Wu, S.L. Li, and Z.J. Cao. 2015. Colostrum quality affects immune system establishment and intestinal development of neonatal calves. *J. Dairy Sci*. 98:7153–7163.

Yoon, I. K., and M.D. Stern. 1996. Effects of saccharomyces cerevisiae and aspergillus oryzae cultures on ruminal fermentation in dairy cows. *J. Dairy Sci*. 79:411–417.

Zhang, X., K.M. Glosson, S.S. Bascom, A.D. Rowson, Z. Wang, and J.K. Drackley. 2022. Metabolic and blood acid-base responses to prepartum dietary cation-anion difference and calcium content in transition dairy cows. *J. Dairy Sci*.105:1199–1210.

Zhu, Z., L. Kristensen, G.F. Difford, M. Poulsen, S.J. Noel, W. Abu Al-Soud, S.J. Sørensen, J. Lassen, P. Løvendahl, and O. Højberg. 2018. Changes in rumen bacterial and archaeal communities over the transition period in primiparous Holstein Dairy Cows. *J. Dairy Sci*. 101:9847–9862.