UF IFAS Extension
UNIVERSITY of FLORIDA

UF/IFAS PEER REVIEWED

AN381

https://doi.org/10.32473/edis-AN381-2021

Inclusion of Direct-Fed Microbials in Lactating Dairy Cow Diets[1]

S. L. Bennett and A. P. Faciola[2]

Introduction

Direct-fed microbials (DFM), often referred to interchangeably as prebiotics and probiotics, are a widely used class of feed additives for dairy cattle to improve animal productivity and health. The FDA broadly defines them as "products that are purported to contain live microorganisms." Within DFM, there are two major groups that are currently fed to livestock: bacteria and fungi. Fungal DFM can be yeast based, with a common species being *Saccharomyces cerevisiae*. Bacterial DFM vary but tend to be from genera such as *Lactobacillus*, *Propionibacterium*, *Enterococcus*, and *Megasphaera*. The feeding of live microorganisms to livestock stems from the idea that they can be used to modify the ruminal environment to one that is more beneficial to the health and productivity of the animal (Yoon et al. 1996). Additionally, they can be an alternative to antibiotics, which are used as a preventative measure for farm health issues but are increasingly disapproved of by consumers (Krehbiel et al. 2003).

There is a widespread usage of DFM within the dairy industry. According to the USDA in the 2014 NAHMS Dairy Management report, around 30% of all farms were using DFM. This value is expected to increase over time; only 17% of operations were using them in 1996 and 26% in 2007, according to a NAHMS 2007 report. Their usage is more prevalent (43%) among large producers (farms with more than 500 cows), potentially because they are an inexpensive supplement which is easy to store and feed while still providing some production benefits (USDA 2016). The purpose of this report is to summarize the information relating to the usage of DFM, such as their modes of action and effectiveness in lactating dairy cows. This publication is for Extension agents, nutritionists, and dairy producers.

Mode of Action

The modes of action vary greatly depending on the type of DFM; however, all DFM share the general effect of modifying the ruminal environment. This may be mediated by controlling ruminal pH, scavenging O_2, using lactate, serving as nutrients to ruminal microorganisms, or altering microbial composition.

Yeast

There are multiple hypotheses regarding the way yeast alters ruminal fermentation. The most widely accepted is that yeast creates a more anaerobic ruminal environment. While the rumen is predominantly anaerobic, oxygen is still present. Yeast uses this oxygen, reducing oxygen in the ruminal environment (Newbold et al. 1996). Certain bacteria need these anaerobic conditions to function properly, specifically fibrolytic (fiber-degrading) and lactate-utilizing species, which are key groups of bacteria for adequate ruminal fermentation. Fibrolytic bacteria

1. This document is AN381, one of a series of the Department of Animal Sciences, UF/IFAS Extension. Original publication date December 2021. Visit the EDIS website at https://edis.ifas.ufl.edu for the currently supported version of this publication.

2. S. L. Bennett, animal scientist, MS, Ph.D. student, Department of Animal Science, The Pennsylvania State University; and A. P. Faciola, associate professor, livestock nutrition, Department of Animal Sciences; UF/IFAS Extension, Gainesville, FL 32611.

adhere to fiber, a complex nutritional component that can limit overall diet digestibility. The environment created by yeast enhances the growth of these bacteria and therefore increases fiber degradation within the rumen. Overall, this leads to more substrate in the rumen which can be used as energy sources for other bacterial species as well, creating a further enhanced environment for fermentation. Fibrolytic bacteria are very sensitive to pH changes, so the increase of lactate-utilizing bacteria is also a benefit to their growth (Yoon and Stern 1995; Callaway and Martin 1997).

Lactate-utilizing bacteria, such as *Megasphaera elsdenii*, also have enhanced growth with the inclusion of yeast in a dairy cow diet. These bacteria are responsible for preventing the buildup of lactate within the rumen, which is critical for prevention of acidosis. Through this mechanism, yeast can help regulate pH and lower lactate concentrations (Callaway and Martin 1997; Chaucheyras-Durand et al. 2008). There is research showing that yeast may also be utilizing lactate as a substrate (Desnoyes et al. 2009). Another method whereby yeast may be preventing lactate accumulation is through competing with bacteria for the highly fermentable carbohydrates and reducing the acid load that is produced during fermentation; however, this mechanism could also be considered antagonistic toward native rumen bacteria (Hatoum et al. 2012).

Yeast supplementation may foster the growth of ruminal microbes, which leads to increases in the amount of microbial protein produced. Because it can supply the animal with amino acids that are essential for growth and milk production, microbial protein is considered a high-quality protein. Increasing its availability would increase maximum performance. The additional nutrient flow of protein increases the amount and quality of protein available in the small intestine, where amino acids are absorbed for use by the animal (Yoon and Stern 1995). Recently, we tested yeast (*Saccharomyces cerevisiae*) and observed no changes in ruminal fermentation and digestibility (Bennett et al. 2021).

Bacteria

The mode of action used by a bacterial-based, direct-fed microbial is dependent on the species of bacteria present within it. Lactate-utilizing bacteria are one of the most common groups of fed bacteria in dairy cattle. As previously discussed, they are critical for balancing lactate concentrations and preventing metabolic disorders. The predominant lactate-utilizer of interest in current research is *M. elsdenii* due to its ability to convert large proportions (up to 95%) of lactate into acetate and propionate and to mitigate lactic acidosis. While these are still acids, they do not have as strong of an effect on ruminal pH as lactate; thus, they do not cause as drastic of a decrease. Additionally, they can be absorbed through the rumen wall, which limits accumulation (Counotte et al. 1981).

Lactate-producing bacteria are often used in DFM mixtures as well. While it may seem counterintuitive to feed a product that will lead to an increase in lactate concentrations, these bacteria are critical for the control of lactate within the rumen. Lactate-producing bacteria increase the "resting" concentration of lactate slightly without raising basal lactate concentrations to a level that would cause metabolic stress on the animal, which stimulates the growth of native lactate-utilizing bacteria (Philippeau et al. 2017). When an animal encounters a challenging situation later, such as acidosis, it will have an elevated level of lactate-utilizing bacteria that prevents the excessive accumulation of lactate within the rumen and reduces the lag time in these microbes' growth.

Other bacteria used as a DFM are those within the genus *Propionibacterium*, which produce propionate from lactate. By shifting the rumen volatile fatty acid (VFA) production towards propionate, it creates more energetically favorable conditions within the rumen. Propionate is a 3 C molecule and is the VFA most easily converted to glucose in the liver after being absorbed through the rumen wall. Since it serves as a glucose precursor, this provides more energy to the animal and reduces the energetic waste that occurs from the production of other VFA (Krehbiel et al. 2003). Additionally, increases in the proportion of propionate reduce the amount of methane produced, which is both an energetic loss to the animal and an environmental health concern (Jeyanathan et al. 2014). Recently, we tested *Lactobacillus animalis, Propionibacterium freudenreichii, Bacillus lichenformis, Bacillus subtillis*, and *Enterococcus faecium* and observed no changes in ruminal fermentation and digestibility (Bennett et al. 2021). We have also evaluated *Lactobacillus plantarum* and observed a reduction in ruminal ammonia, which may represent an improvement in ruminal nitrogen utilization (Monteiro et al. 2020).

Bacterial DFM also function as a competitive microorganism (i.e., competitors) within the rumen, aiding in the prevention of pathogen-induced disease. This is a long-held hypothesis to describe the function of bacterial DFM beyond their role in fermentation. This competition occurs along the rumen and intestinal wall and prevents the colonization of pathogens such as *Escherichia coli* which causes numerous gastrointestinal tract diseases. While this is an indirect method of controlling pathogens, some species of bacterial DFM can be used in a more direct way due to their production of antibacterial compounds. *Lactobacilli*, in addition to their role as lactate-utilizers, produce hydrogen peroxide, which has an inhibitory effect on pathogens (Krehbiel et al. 2003). Overall, all these modes of action may lead to the improved health status and productivity of dairy cows.

Production Responses

There are many potential methods for DFM to influence the ruminal environment and animal productivity; however, there is also variation among studies, making it hard to come to a clear consensus on the methods' efficacy. The variation in results does not indicate negative impacts, as much it shows that there is no effect of inclusion in the diet. Nevertheless, there have been positive associated outcomes for all major production parameters such as nutrient digestibility and milk production (Martin and Nisbet 1992; Desnoyes et al. 2009).

Challenging Periods

Perhaps the most important way that DFM can be used on a dairy operation is during times of metabolic challenge for the animal. There may be times when an animal is at an increased risk for a ruminal pH challenge or heat stress. Stress can trigger dysbiosis within the rumen and large intestine, which is a shift towards imbalance in the microbial community. This imbalance allows pathogenic bacteria to be favored and may lead to further health incidents (Chaucheyras-Durand et al. 2008).

Ruminal Acidosis

Subacute ruminal acidosis is a serious metabolic concern and is prevalent on most farms. It occurs due to high levels of rapidly fermentable carbohydrates in the diet that produce elevated levels of organic acids and cause a drop in pH to below 5.6 for more than 3 hours. If there is an accumulation of lactate, acidosis becomes more severe, dropping the pH to below 5.2 (Owens et al. 1998). Since many DFM influence lactate in some capacity, they seem to be more effective when animals are undergoing or at risk of an acidosis challenge. Some strains, such as *Lactobacillus* species, have been specifically identified due to their ability to increase rumen pH when the animal consumes a high starch (38% DM) diet (Philippeau et al. 2017). In a study where subacute ruminal acidosis was induced, pH was increased (5.84 vs. 5.41) and the severity of acidosis, which is measured as the amount of time ruminal pH is below 5.6, was also reduced when cows were fed a yeast (*Saccharomyces cerevisiae*) and bacteria (*Enterococcus faecium*) combination (Chiquette 2009).

As a primary lactate-utilizer in the rumen, *M. elsdenii* has been utilized in a number of trials with acidosis challenges and does seem to have effects. For example, high-producing cows spent 73 fewer minutes with a ruminal pH below 5.6 and less pH fluctuation throughout the days after the challenge (Aikman et al. 2011). *M. elsdenii* may be more beneficial for high-producing cows that are fed high concentrate levels (60%–70% DM as concentrate) as shown through production responses because they are undergoing a higher metabolic challenge. Under these conditions, milk yield (39.3 kg/d vs. 35.9 kg/d) and milk fat (3.21% vs. 3.56%) both tended to be greater for the cows that were supplemented than for those that were not (Henning et al. 2011). While the role in mitigating lactate-related acidosis is beneficial, one potential reservation regarding the inclusion of *M. elsdenii* in the diets of lactating dairy cows is its association with milk fat depression (MFD), which would be important for producers in component pricing systems. The main source of concern stems from its elevated levels during MFD, which is often caused by diets with high fermentable carbohydrate (high grain) loads similar to those which cause the pH drops seen in acidosis (Weimer et al. 2015). Beyond this, there is its potential to produce *trans*-10, *cis*-12 conjugated linoleic acid, which is closely associated with MFD, although this effect seemed to be strain dependent when tested under *in vitro* conditions (Kim et al. 2002). This relationship has not been fully studied and the exact effects of *M. elsdenii* on milk fat production are still unanswered questions because there has been variation in the literature with studies showing negative effects (0.5% decrease) (Aikman et al. 2011) or no difference (Weimer et al. 2015), in addition to the positive effects discussed previously.

Heat Stress

While acidosis-based challenges are diet based, there can be environmental stresses that impair rumen function. In Florida, heat stress is a prime example of an environmental factor that causes metabolic stress. This is attributed in part to the decrease in DMI many cows exhibit as well as the increased energy demands to maintain body temperature. However, yeast supplementation can limit these negative impacts. When supplemented in heat stress conditions, cows had an increased DMI (2.5% greater over control) and subsequent milk yield (Moallem et al. 2009), likely from the stabilizing effect yeast had in the rumen. Cows supplemented with yeast also exhibited less respiration, indicating improved efficiency at dissipating heat. The cows in this study also had increased milk yield (26.7 kg/d vs. 25.4 kg/d), potentially due to more energy going into production and less into heat management mechanisms (Salvati et al.

2015). Both of these cases show there is a time when DFM can be beneficial in managing challenging situations.

Conclusions

Table 1 includes a summary of the results from experiments investigating the effects of various DFM on lactating dairy cow performance. Overall, there is conflicting data regarding the effectiveness of DFM inclusion in lactating dairy cow rations. Part of this stems from the many different modes of action DFM can utilize, making it important to understand how specific microorganisms function and serve different purposes in the diet. With this knowledge, it is critical to identify the needs of the farm and match those with the appropriate DFM in order to maximize chances of improvements in production and health. Finally, producers should keep in mind that the literature shows that these types of feed additives are more effective during times of stress, both metabolic and environmental, so these are the situations where animals are more likely to benefit from the feeding of DFM.

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Table 1. Summary of the results from experiments investigating the effects of various direct-fed microbials on lactating dairy cow performance.

Direct-Fed Microbial	Feeding Rate	Conditions	Effects	Citation
Megasphaera elsdenii	10^{10} cfu/day	Acidosis	Decreased milk fat percentage, less time with pH below 5.6	Aikman et al. (2011)
Enterococcus faecium and *Saccharomyces cerevisiae*	2 g/d (5 x 10^{9} cfu)	Acidosis	Increased minimum ruminal pH	Chiquette (2009)
Saccharomyces cerevisiae	56 g/d	High-starch diet	Increased DM and OM digestibility	Ferraretto et al. (2012)
Megasphaera elsdenii	10^{11} cfu/day	High concentrate, transition period	Increased milk production, increased milk fat yield, increased dry matter intake	Henning et al. (2011)
Saccharomyces cerevisiae	10^{10} cfu/day	Heat stress	Increased milk production, increased milk fat yield, increased dry matter intake	Moallem et al. (2009)
Propionibacterium P63, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*	10^{10} cfu/day	Acidosis	Increased ruminal pH, decreased lactate concentrations	Philippeau et al. (2017)
Saccharomyces cerevisiae	25 x 10^{10} cfu/day	Heat stress	Increased milk yield	Salvati et al. (2015)
Megasphaera elsdenii	2 x 10^{12} cfu/day	Normal	Decreased DMI, decreased milk fat production	Weimer et al. (2015)