Invited review: “Probiotic” approaches to improving dairy production: Reassessing “magic foo-foo dust”

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ABSTRACT

The gastrointestinal microbial consortium in dairy cattle is critical to determining the energetic status of the dairy cow from birth through her final lactation. The ruminant’s microbial community can degrade a wide variety of feedstuffs, which can affect growth, as well as production rate and efficiency on the farm, but can also affect food safety, animal health, and environmental impacts of dairy production. Gut microbial diversity and density are powerful tools that can be harnessed to benefit both producers and consumers. The incentives in the United States to develop Alternatives to Antibiotics for use in food-animal production have been largely driven by the Veterinary Feed Directive and have led to an increased use of probiotic approaches to alter the gastrointestinal microbial community composition, resulting in improved heifer growth, milk production and efficiency, and animal health. However, the efficacy of direct-fed microbials or probiotics in dairy cattle has been highly variable due to specific microbial ecological factors within the host gut and its native microflora. Interactions (both synergistic and antagonistic) between the microbial ecosystem and the host animal physiology (including epithelial cells, immune system, hormones, enzyme activities, and epigenetics) are critical to understanding why some probiotics work but others do not. Increasing availability of next-generation sequencing approaches provides novel insights into how probiotic approaches change the microbial community composition in the gut that can potentially affect animal health (e.g., diarrhea or scours, gut integrity, foodborne pathogens), as well as animal performance (e.g., growth, reproduction, productivity) and fermentation parameters (e.g., pH, short-chain fatty acids, methane production, and microbial profiles) of cattle. However, it remains clear that all direct-fed microbials are not created equal and their efficacy remains highly variable and dependent on stage of production and farm environment. Collectively, data have demonstrated that probiotic effects are not limited to the simple mechanisms that have been traditionally hypothesized, but instead are part of a complex cascade of microbial ecological and host animal physiological effects that ultimately impact dairy production and profitability. Key words: direct-fed microbial, eubiotic, postbiotic, prebiotic, microbiome

INTRODUCTION

In the United States, the dairy industry includes approximately 8.94 million cattle that produced 212 billion pounds of milk in 2020 (USDA-NASS, 2023). Over the past 4 decades the number of dairy farms has decreased steadily, while simultaneously the average dairy herd size has increased (USDA-NASS, 2023). Increasing the concentration of cattle on fewer, but larger farms has improved the efficiency of milk production, which has led to the development of dietary or nutritional strategies that improve this critical driver of dairy farm profitability (Losinger and Heinrichs, 1996; Capper and Cady, 2020). Dairy cattle are fed a variety of rations in an attempt to achieve this goal, ranging from very-high-grain diets to TMR, and even include solely grass-based grazing systems (Rotz et al., 2020; Wilkinson et al., 2020). Unfortunately, the inclusion of the energy-dense grains often leads to disruption in ruminal function, as well as severe metabolic disorders and reduced production (Dineen et al., 2020).

Ruminants are unique because they possess a pre-gastric fermentation chamber (the rumen), which is colonized by a dense, complex, and highly diverse microbial consortium (Hungate, 1966; Weimer, 1992; Al-Zahal et al., 2017; Wang et al., 2019b). The symbiotic relationship between the host animal and its ecologically and metabolically diverse gastrointestinal microbial ecosystem is critical to ensuring animal health and
maximizing production efficiency and allows the animal to utilize a broad spectrum of available feedstuffs (Russell and Hespell, 1981). A truly symbiotic relationship between the ruminal microbial ecosystem and the host animal allows ruminant animals to use the most abundant carbohydrate on earth, cellulose. End products of this ruminal microbial fermentation provide the animal with short-chain fatty acids (SCFA), microbial cell mass, and vitamins that the animal utilizes to meet its energy and protein requirements (Fahey and Berger, 1988; Ferrell, 1988). Microbial degradative enzymes (including polysaccharide utilization loci) are responsible for catabolism of nearly all the feedstuffs consumed by the animal before degradation in the acidic abomasum (Seshadri et al., 2018; Stewart et al., 2019; Wang et al., 2019b). In addition to ruminal fermentation, the cecal and colonic secondary fermentation in ruminants has become increasingly viewed as being highly impactful on ruminant efficiency and body composition (Li et al., 2016; Shabat et al., 2016; Krause et al., 2020; Welch et al., 2020), as well as animal health (Gressley et al., 2011; Li et al., 2011).

Despite the ruminal and gastrointestinal fermentation of feedstuffs being inherently inefficient energetically speaking (relative to monogastric animals), it provides the ruminant with the ability to utilize a wide variety of substrates that are not available to other animals (Fahey and Berger, 1988; Ferrell, 1988). Compared with monogastric animals, cattle are inefficient on a feed:gain or feed:milk production basis, which has led to research to understand the relationship between the gastrointestinal microbial community composition and milk production (Shabat et al., 2016; Sasson et al., 2017; Ort et al., 2018; Wallace et al., 2019). Manipulation of the gastrointestinal microbial community has been sought through antibiotic as well as probiotic approaches to improve milk production efficiency and alter milk composition (Weimer et al., 2010; Bernard, 2015; Souza et al., 2017) and often has demonstrated a positive effect on dairy profitability (Desnoyers et al., 2009; Heinrichs et al., 2020).

Although antimicrobial inclusion in animal rations has improved milk production efficiency, the emergence of antibiotic resistance and the risk of therapeutic failures to treat infections in humans and animals has led to the implementation of the Veterinary Feed Directive in the United States (Hoelzer et al., 2017; Tagliabue and Rappuoli, 2018; Lees et al., 2020). This effort to reduce antibiotic usage in agriculture has increased scientific emphasis on developing alternatives to antimicrobials (Seal et al., 2013; Lhermie et al., 2017; Kurt et al., 2019; Callaway et al., 2021). One of the best understood alternative strategies to alter the microbial population of the gut is the use of probiotics, which are included in the category of direct-fed microbials (DFM; McAllister et al., 2011; Callaway et al., 2012). Many rations fed to dairy cattle in recent years have included probiotics or DFM to improve efficiency by changing the microbial community composition (Wisener et al., 2015; Maldonado et al., 2018; Tesfaye and Haihu, 2019; Nalla et al., 2022). Furthermore, some DFM are also aimed at reducing the deleterious effects of grain feeding to cattle and can reduce negative associative feed effects (e.g., acidosis; Olayinka, 2021). The objective of the current review is to provide an update on the use of DFM as probiotic-type feed additives with a known or demonstrated effect on the microbial ecosystem, the host immune system, and on the production and development of dairy cattle and calves.

**DIRECT-FED MICROBIALS: EMERGING CATEGORIES OF PROBIOTIC APPROACHES**

The broad category of “probiotic approaches” or DFM in the animal industry encompasses a large number (and many categories) of products that have been used to improve performance, enhance animal health, and ensure food safety (Bunty et al., 2016; Khan et al., 2016). Direct-fed microbials roughly correspond to the term “probiotics” in humans, but in animals DFM includes “traditional probiotics,” which are now often referred to as probiotics, eubiotics, prebiotics, postbiotics, and symbiotics are shown in Table 1 (Kareem et al., 2017; Miniello et al., 2017; Yaşar et al., 2017). Different products have very distinct modes of action and efficacies, which underlies the variability in effectiveness of DFM products that is encountered when taking research results onto real farms (Figure 1). It should be noted that many categorizations of DFM or probiotic approaches are not firm delineations, but are evolving, fluid categories. Furthermore, some commercial products can potentially be classified under several categories depending on their composition. Direct-fed microbial-type preparations that are used in dairy cattle are typically individual species or mixtures of lactic acid bacteria (LAB), yeasts, or their end products and are not animal species-specific (not limited to use in cattle), can be comprised of one or more strains or species, or even necessarily originally isolated from animals (Oliveira et al., 2017; Kaewpila et al., 2021; Lambo et al., 2021).

Dairy producers have used various DFM for years primarily to increase growth rate, milk production, or production efficiency (Vieco-Saiz et al., 2019). Historically the efficacy of DFM were variable, with little understanding of why or how each DFM worked or, perhaps more importantly, why they did not work. In fact, there are many references to DFM being described
as “magic foo-foo dust” because of this lack of consistency of action (LeJeune et al., 2006; Kim et al., 2007). However, as we have elucidated their modes of action, we understand why some DFM work better in specific production situations than others; furthermore, newer effects of DFM treatment on cattle performance have been uncovered (Buntyn et al., 2016; Ghazanfar et al., 2017; Adjei-Fremah et al., 2018), providing new perspectives about the modes of DFM action (Sherman, 2009; Sherman et al., 2009).

**HOW DO WE THINK DFM WORK?**

Historically eubiotics, probiotics, prebiotics, postbiotics, and synbiotics (Table 1) have been used to benefit animal health; as well as dairy cattle performance (Krishnan et al., 2020; Nalla et al., 2022), via direct and indirect effects on the microbial community composition through several mechanisms (Figures 2, 3, and 4). Although any or all of these mechanisms may play a role in a specific DFM product action, the relative weight of each individual mode of action will vary considerably among products. Generally, it is thought that many benefits to the animal of DFM occur by (1) a shift in the microbial environment via acid production (including the resultant pH decrease from lactate production); (2) a shift in molar proportion of SCFA (especially butyrate), which increases gut epithelial integrity—particularly related to tight junction proteins such as occludins and zonula occludens (ZO-1)—ensuring physical separation of self from nonself due to reduced gut permeability (Resta-Lenert and Barrett, 2003; Luyer et al., 2005a,b; Resta-Lenert et al., 2005; Resta-Lenert and Barrett, 2006); (3) increasing competition for nutrients, substrates, and cofactors among the microbial consortium members (Oelschlaeger, 2010); (4) increasing competition for physical binding sites along the gastrointestinal epithelium, possibly by development of exclusionary microbial biofilms along the gut epithelial surface or by stimulation of mucin production (Cocomnier et al., 1993; Hudault et al., 1997); (5) increased production of antimicrobial compounds (e.g., colicins, bacteriocins, antimicrobials) by the resident microbial consortium (Gillor et al., 2008; Oelschlaeger, 2010); (6) reduced systemic inflammation via immunostimulation; (7) stimulation of growth hormone and inflammatory pathways of the host (Adjei-Fremah et al., 2018; Sanders et al., 2021); or (8) a combination of any or all of these mechanisms. The mode of action is specific for each DFM product and can vary based on host species and production status (e.g., early lactation vs. dry cow). Furthermore, pre-, post- and eubiotics can exhibit more than one mode of action.

**Table 1. Direct-fed microbial categories and definitions used in the review**

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
<th>Product type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eubiotics</td>
<td>Feed additives that play an essential role in supporting animal performance and animal welfare by supporting gut health</td>
<td>Organic acids, essential oils, probiotics, prebiotics, phytobiotics</td>
<td>Miniello et al., 2017; Santovito et al., 2018</td>
</tr>
<tr>
<td>Probiotics</td>
<td>“Living microorganisms, which, when administered in adequate quantities, are beneficial to the health of the host” (FAO, 2001)</td>
<td>Live bacterial, yeast, or fungal cultures</td>
<td>Fuller, 1989; FAO, 2001</td>
</tr>
<tr>
<td>Prebiotics</td>
<td>Fermentable substrate not used by host animal</td>
<td>Oligosaccharides (e.g., fructooligosaccharides), inulin</td>
<td>Gibson and Roberfroid, 1995</td>
</tr>
<tr>
<td>Postbiotics</td>
<td>Yeast or fungal products or products of their fermentation</td>
<td>Yeast or fungal fermentation end products; includes cell-wall products and heat-treated cultures (non-living)</td>
<td>Tomasik and Tomasik, 2020; Salminen et al., 2021</td>
</tr>
<tr>
<td>Synbiotics</td>
<td>Feed additives that work synergistically through multiple modes of action (e.g., probiotic along with a prebiotic)</td>
<td>Probiotic coupled with a prebiotic, or a yeast product that contains prebiotics; some products exhibit synbiotic activity naturally</td>
<td>Collins and Gibson, 1999; Schrezenmeir and De Vrese, 2001</td>
</tr>
</tbody>
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Several specific live cultures of bacteria, fungi, and yeasts have been used widely to alter the dynamics and composition of the gastrointestinal microbial community (Jayne-Williams and Fuller, 1971; Fuller, 1989; Nurmi et al., 1992; Waters et al., 2005) and can colonize different sections of the gastrointestinal tract. In vitro studies have shown that the presence of *Lactobacillus* in the culture medium reduces the attachment and invasion of intestinal epithelial cells by enteropathogenic bacteria, such as *Salmonella* (Coconnier et al., 1993; Hudault et al., 1997). The mechanisms by which pathogen colonization is reduced are still not clearly established but appear to be related to probiotic adhesion to epithelial cells, resulting in physical hindrance of pathogen adhesion to the epithelial cell receptors (Coconnier et al., 1993), particularly those that are entero-invasive (Oyofo et al., 1989b, a;c; Shokryazdan et al., 2014). Changes in the microbial community composition can also affect SCFA production and luminal concentrations, which

![Figure 2](image_url)

**Figure 2.** Benefits of the probiotic, eubiotic, or prebiotic categories of direct-fed microbials in dairy cattle. The mechanisms listed in the figure are further described in the “How Do We Think DFM Work?” section. A:P = acetate:propionate ratio.
**Figure 3.** Mode of action of direct-fed microbials in the rumen of cattle that supplement the activities of the resident native ruminal microbial consortium. (Created with BioRender.com.)

**Figure 4.** Mode of action of probiotic (direct-fed microbials) approaches in the hindgut of cattle. (Created with BioRender.com.)
are responsible for (1) acidification of the environment, which is unfavorable to the development of pathogenic bacteria (Hollowell and Wolin, 1965; Wolin, 1969), and (2) maintenance of the integrity of the intestinal epithelium, in particular by induction of genes encoding the proteins that make up the tight junctions (Parada Venegas et al., 2019).

Interestingly, some eubiotics and probiotics can stimulate host cells to increase mucin production; mucin is a glycoprotein secreted by intestinal epithelial cells and a principal component of the mucus present in the surface of the intestinal epithelium (Mack et al., 1999; Murugesan et al., 2014). Lactobacilli populations increase the expression of the gene coding for mucin by intestinal epithelial cells (Mack et al., 1999; Mattar et al., 2002), which is also associated with a decrease in the adhesion of enteropathogenic bacteria to epithelial cells (Mack et al., 1999). Increased mucin production is thought to be involved in the maintenance of intestinal epithelial stability (Resta-Lenert and Barrett, 2003; Luyer et al., 2005a), possibly related to expression or maintenance of the phosphorylation activity of the tight junction proteins occludins and zonula occludens (Resta-Lenert and Barrett, 2003; Song et al., 2014). Furthermore, increased butyrate production associated with some DFM treatments increased epithelial stability (e.g., gut health), as well as villus and papillae length in the gut (Sordillo, 2016; Van Niekerk et al., 2021). Improving gut health and epithelial stability helps ensure the physical separation between self and nonself, which helps exclude potential toxins (e.g., lipopolysaccharide) and fermentation end products and microbes from reaching the blood stream and catalyzing an uncontrolled (and a significant nongrowth energy expenditure) immune response (Malmuthuge and Guan, 2017; Kim and Lilleshoj, 2019; Lourenco et al., 2019).

Eubiotic approaches can act differentially on immune cells that constitute the complex defensive system, as has been reviewed in detail previously in several food-animal species; however, the mode of probiotic action remains unclear (Palamidi et al., 2016; Parada Venegas et al., 2019; Wang et al., 2019a). Dead or inactive DFM products (e.g., yeast cell-wall products or heat-treated or lysed bacteria or fungi) have been shown to stimulate a broad swath of the immune system (Cummings et al., 2004; Kogut and Swaggerty, 2012; Swaggerty et al., 2019), including in dairy calves and cows (Broadway et al., 2017; Broadway et al., 2020). Direct-fed microbial products designed to strengthen the immune system are indeed available on the market (Yang et al., 2017; Bergman et al., 2020) and may enhance the phagocytic capacity (Delcenserie et al., 2008). By acting on the maturation, gene expression for cytokines, and surface expression of the major histocompatibility complex surface molecules of dendritic cells DFM may modulate their function (Delcenserie et al., 2008) and can also modulate T lymphocyte activity (Delcenserie et al., 2008). Some DFM products increased natural killer cell activity (Delcenserie et al., 2008), which could be related to the production of cytokines such as interleukins (IL-12 and IL-15; Delcenserie et al., 2008). Direct-fed microbial products stimulate the production of primarily IgA (Delcenserie et al., 2008), and increase production of IgA by B lymphocytes after mitogen stimulation in mice or children suffering from acute rotavirus diarrhea (Isolauri, 2001; Isolauri et al., 2001; Collado et al., 2009). Some eubiotic products are associated with decreased lymphocyte proliferation (Delcenserie et al., 2008; Penha Filho et al., 2015), but this is highly strain dependent. Recent research has demonstrated that DFM treatment could change the expression of many thousands of genes of lactating dairy cows, especially genes involved in systemic inflammation and growth hormone regulation (Adjei-Fremah et al., 2018). It is exciting to see these associations between DFM and probiotics and immune system activity, and it is important to ensure that ruminant-specific data is explored, especially with respect to diet–microbial interactions.

We typically view the modes of action of commercial DFM and probiotic products through a simple dichotomous lens (e.g., it is either a prebiotic or a probiotic), but in real-world conditions this is often not reflective of reality. Although prebiotics have been widely used in monogastrics (Máteová et al., 2008; Janardhana et al., 2009; Kim et al., 2011), in ruminants the intentional use of prebiotics is complicated (and limited) by the catabolic activity of the native ruminal microbial community, which can degrade most oligosaccharides (Accetto and Avgustin, 2015; Seshadri et al., 2018; Accetto and Avgustin, 2019; Tomazetto et al., 2020). The large and very biochemically active rumen microbial population means that many of the oligosaccharides used commonly as prebiotics can be readily degraded, requiring these relatively expensive compounds to be encapsulated for use in ruminants (Accetto and Avgustin, 2019; Tomazetto et al., 2020). However, many commercial DFM also naturally contain prebiotic-type compounds (Callaway and Martin, 1997; Druart et al., 2014). Prebiotic usage can be coupled with probiotics, and the combination is known as symbiotics, which enhance the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract (Collins and Gibson, 1999).

GUT INTEGRITY AND HEALTH

The phrase “gut health” has become more widely used in animal agriculture (Gorbach, 2000; Bischoff,
2011; Plaizier et al., 2018). The epithelial barrier is composed of the apical cell membrane and the tight intercellular junctions, such as ZO-1, which acts as a link between the cytoskeleton and other tight junction proteins (Johnson-Henry et al., 2008). Disruption of the epithelial barrier leads to an increase in permeability and allows penetration of antigens and pathogens that induce and drive inflammation (Pizarro-Cerda and Cossart, 2006). DFM protect the epithelial barrier function from damage, but the mechanisms involved are not well understood. For example, the pretreatment of epithelial cells with \textit{Lactobacillus} strains prevents pathogen-induced loss of barrier integrity, notably by increasing the expression of the ZO-1 protein (Parassol et al., 2005; Sherman et al., 2005; Johnson-Henry et al., 2008). The use of LAB as probiotics has broadly demonstrated improved epithelial integrity, intestinal protein transport, and intestinal glucose absorption (Kengang et al., 2014; Abdollahi-Roodsaz et al., 2016; Michael et al., 2021; Sanders et al., 2021).

Prebiotic approaches have been used to improve intestinal health attributes, such as intestinal mucosal integrity in dairy calves (Dar et al., 2017; Singh et al., 2017a,b). Prebiotic and synbiotic supplementation improved gut conditioning and enhanced immunity outcomes from a 90-d feeding trial examining the effect of probiotic, prebiotic, and synbiotic feed additives on fecal counts of coliform and \textit{Escherichia coli}, along with cell-mediated immune response in crossbred calves (Dar et al., 2017). Calves were assigned to different dietary treatments using a \textit{Lactobacillus acidophilus} culture, or a prebiotic treatment (mannanoligosaccharide; MOS), or both treatments (synbiotics; Dar et al., 2017). There was a significant reduction in fecal shedding of coliform and \textit{E. coli} in treated groups, indicating reduced pathogenic bacteria proliferation in the gut, and this was coupled with an increased lymphocyte count in probiotic treated groups, as well as increased skin fold thickness at 48 h of phytohaemagglutinin injection (Dar et al., 2017).

**DIRECT EFFECTS OF PROBIOTICS ON THE HOST**

For many years, the primary effects of probiotic or DFM were assumed to be mediated directly or indirectly by the microbial community of the gastrointestinal tract. However, recent studies that examined the effects of probiotic supplementation on growth and global gene expression in dairy cows found some exciting results that have changed how we view the interactions between the animal and its resident microbial community (Adjei-Fremah et al., 2018). Direct-fed microbial-induced gene expression was observed previously with DFM composed of \textit{Lactobacillus plan-}

**WHAT DO WE STILL NEED TO KNOW IN TERMS OF MODE OF ACTION?**

Although we have increased our understanding of DFM and probiotic modes of action, our knowledge remains incomplete; however, recent developments have rapidly deepened our understanding of the resident microbial ecosystem in ruminants. The emergence of next-generation sequencing techniques and the rapid reduction of costs for sequencing have allowed the widespread adoption of these techniques to more precisely characterize each specific probiotic, as well as the effects of DFM treatment on the gastrointestinal microbial ecosystem (Neves et al., 2017; Weinroth et al., 2018; Henderson et al., 2019). Advancements that next-generation sequencing can lead to in the future include the development (or refinement) of specialized probiotic products (or modes of actions) for use in specific production situations (e.g., lactation and the dry period) to produce next-generation probiotics, eubiotics, and postbiotics (Firkins and Yu, 2015; Denman et al., 2018; Islam and Lee, 2018). Future studies will change how we feed DFM and include the potential use of modified microorganisms that produce specific molecules that affect the host (Le et al., 2017;
CALVES: NEEDS FOR AND USE OF PROBIOTIC APPROACHES

Alarming high rates of mortality and morbidity persist in the calf industry, with the majority of calf illnesses attributed to digestive diseases and disorders (Urie et al., 2018; Winder et al., 2018). In commercial production, the high incidence of diarrhea is typically followed by a bout of respiratory disease (Klein-Jobst et al., 2014; Clymer et al., 2015). Given the myriad health challenges that afflict both female (replacement heifer) and male (veal or beef) calf industries, our production systems have become reliant on antimicrobial use, both prophylactic and therapeutic (Urie et al., 2018). The high risk of the dissemination of antimicrobial resistance through these practices, as well as consumer concerns (Smith, 2015), have catalyzed the investigation of alternatives to antibiotics (Callaway et al., 2021), such as probiotics from bacterial and yeast origin, to mitigate societal pressures and alleviate gut health challenges.

Bacterial- and yeast-based DFM efficacy in dairy calves seems to be largely dependent on mode of delivery and calf age. For example, during the preweaning period, supplementation is focused on bacterial-based probiotics to decrease the incidence of diarrhea, increase the speed of resolution, and improve growth. The most common bacterial probiotics used during the preweaning period are *Lactobacillus* spp., *Bifidobacterium* spp., *Bacillus* spp., and *Enterococcus* spp., which are used either independently or in combination (Cangiano et al., 2020; Ban and Guan, 2021). Based on health-scoring data, several strains of bacteria have proven efficacious in improving calf gut health (Timmerman et al., 2005; Frizzo et al., 2010; Signorini et al., 2012) and are commonly fed prophylactically. Lactic acid–producing bacteria improve barrier function in relation to mucin production and the release of luminal antimicrobial peptides (Ban and Guan, 2021). Bacterial-based probiotics can improve calf health responses, and, in some cases, can improve average daily gain during the preweaning period (Cangiano et al., 2020). Health responses are typically assessed by health-scoring procedures (Olson et al., 2019); however, there is a paucity of information about the specific mechanisms by which probiotics alter microflora and interact with the host when bacterial-based probiotics are used in calves.

Live yeast (LY) and yeast cultures (YC) have been used in calves during the preweaning and weaning periods. Live yeast products are dried fermenting living yeasts that typically contain a minimum of $10 \times 10^9$ LY cells per gram. In comparison, YC are products of yeast fermentation and include the media they are grown in and can include bioactive components such as cell-wall constituents that may have immunomodulatory effects (Delecenserie et al., 2008). Most calf research has investigated adding yeast to dry feed (starters) to improve intake, growth, and health. However, their application as a supplement in milk during the preweaning period has been recently investigated (He et al., 2017). *Saccharomyces cerevisiae boulardii* in milk decreased the incidence of diarrhea in a commercial male calf operation (Villot et al., 2019). No overall treatment differences were detected in growth; yet, of all calves diagnosed with diarrhea, those supplemented with probiotics in milk achieved greater growth than the control group (Villot et al., 2019). Further investigation into the potential mechanisms suggest that *S. cerevisiae boulardii* did not alter the gastrointestinal tract microbial community but did alter host defenses, such as the production of IgA in the intestine (Villot et al., 2020), highlighting the importance of investigating a variety of host-response metrics in probiotic research.

To date, the majority of yeast-supplementation research in calves has focused on supplementation in dry feed, and primarily has targeted time periods after solid-feed intake is substantial in an effort to improve the weaning transition. Yeast products have been shown to improve starter intake, likely by stabilizing ruminal pH and improving fiber digestion by stimulating the growth (directly or indirectly) of fibrolytic ruminal species, potentially via vitamin or intermediate production (Fonty and Chaucheeyras-Durand, 2006). Some studies investigating yeast-product inclusion in solid feed have reported conflicting results regarding starter intake and growth, but some have shown advantages during the weaning period (Lesmeister et al., 2004; Galvão et al., 2005). In addition to the positive response in intake and growth,YC fed with dry feed has also been shown to reduce fecal scores, diarrhea incidence (Hill et al., 2009; Brewer et al., 2014), the humoral response to vaccine (Kim et al., 2011), and neutrophil function (Magallães et al., 2008). The impact on the host immune response is an interesting field that requires more attention because published studies often lack replication and robustness.

EFFECT OF DFM ON CALF GROWTH

The benefits of DFM on calf growth are largely mediated by changes in the intestinal and ruminal micro-
bacterial community compositions, which improve gut and overall health and animal welfare or well-being (Isik et al., 2004; López-Valencia et al., 2017; Broadway et al., 2018). Feeding DFM often improves weight gain and feed conversion ratios, likely by increasing the assortment of end products that are utilized by the host animal (e.g., VFA vs. CH₄ or CO₂; Shabat et al., 2016; Deng et al., 2012; Song et al., 2018). In terms of productivity, there is significant variability in animal response to DFM treatment in terms of growth and feed efficiency. Beneficial responses from DFM treatment are more marked in calves grown under poor nutritional or health conditions or undergoing production or environmental stresses (Krehbiel et al., 2003; Reuter et al., 2008). Such variability is not unexpected because modification of the intestinal ecosystem is widely variable among trials due to microorganism communities native in the environment, the gut, and host animal genetics, as well as calf age and nutritional and health status. Collectively, results suggest that the use of DFM can benefit calves undergoing stresses, whether heat, transition, transportation, or dietary changes (Deng et al., 2012; Song et al., 2014; Roul et al., 2015).

USE IN MATURE AND LACTATING DAIRY COWS

Direct-fed microbial products can enhance dairy production efficiency and thus improve dairy farm profitability (Desnoyers et al., 2007; Palmer, 2018; Moreira et al., 2019). However, the efficacy can vary greatly based on the DFM or probiotic product type (i.e., fungal vs. bacterial; live culture vs. fermentation extract, prebiotic, or eubiotic), organism selected, the diet fed to cattle, and stage of lactation (Windschitl, 1992). Generally, probiotic-type approaches improve average daily gain from 2.5 to 5% and feed efficiency (amount of gain per amount of feed ingested) by approximately 2% (Krehbiel et al., 2003; Desnoyers et al., 2007). The introduction of DFM or probiotic bacteria and postbiotics to the ration of dairy cows reduced subacute and acute acidosis, lowered the risk of liver abscesses, and eased dietary and lactational transitions (Jouany, 2006a; Oetzel et al., 2007; Ferraretto and Shaver, 2015). Other studies found that probiotic approaches directly affected animal health, often mediated through improved gut health and integrity, reduced metritis, and reduced incidence of mastitis (Crispie et al., 2008; Beecher et al., 2009; Tanbayeva et al., 2016; Pellegrino et al., 2019).

Broadly, eubiotics, probiotics, and prebiotics (DFM) improve fiber digestion by the ruminal microbiota by modifying the ruminal environment, primarily through pH stabilization and alteration of the native microbiome (Oelschlaeger, 2010; Elghandour et al., 2015; Ghazanfar et al., 2017). An increase in the ruminal bacterial population density (cfu/mL) was observed in the presence of some (live and dead) yeast products, for example, in conjunction with an increase in the degradability of feedstuffs, especially fiber, which can lead to improvements in animal health and productivity (Chiquette et al., 2007; Chiquette et al., 2008; Souza et al., 2017; Shakira et al., 2018). Some of the improvement in fiber degradability conferred by some of these yeast products has been hypothesized to be related to the ability of the added yeast to use oxygen carried into the rumen during feed and water consumption, thereby reducing the ruminal redox potential (Mathieu et al., 1996), creating a biochemically favorable environment for the growth of more strictly anaerobic fungi, bacteria, and archaea (Cord-Ruwisch et al., 1988; Mathieu et al., 1996; Zheng et al., 2014). In addition, some probiotic yeasts, such as *Saccharomyces cerevisiae*, provide micronutrients and metabolic intermediates, including B-vitamins; phenylacetic acid or phenylpropionic acid, or both; trace minerals; AA; and organic acids used by bacteria for their growth through a cross-feeding process that contributes to increasing the ruminal microbial environmental stability (Russell, 2002; Vandana et al., 2013; Mayorgas et al., 2021). The high variability in DFM efficacy has long been noted and is related to the nutrient and environmental limitations facing the ruminal and gastrointestinal microbial communities; such variability is likely due to the presence or absence in the product of these micronutrients or metabolic intermediates of the probiotic or DFM.

EFFECTS ON MILK PRODUCTION

Whether used in cows, ewes, or goats, LY products (probiotics) generally had a positive effect on milk production volume (Chiquette et al., 2012; Lettat et al., 2012; Bernard, 2015; Shakira et al., 2018). The use of probiotic bacteria (especially LAB) in adult dairy cows improved the lactation performance and feed efficiency of those cattle (Aleman et al., 2007; Raeth-Knight et al., 2007; Lehloenya et al., 2008). *Lactobacillus acidophilus* DFM cultures improved feed efficiency in dairy cows (Boga and Gorgulu, 2007; Ferraretto and Shaver, 2015); however, the addition of *L. acidophilus* and *Propionibacterium freudenreichii* to the diet of midlactation cattle had no effect on DMI, feed efficiency, or milk production (Raeth-Knight et al., 2007; Jami et al., 2014). Other researchers found that feeding *Propionibacterium* decreased plasma glucose and insulin concentrations in cattle (Francisco et al., 2002; Aleman et al., 2007; Kim et al., 2007; Azad et al., 2017). Propionibacteria-based DFM increased daily milk yield and fat-corrected milk yield across a 30-wk lactation interval, but no changes in reproductive parameters were noted (Stein et al., 2018).
2006; Narvaez et al., 2014). When early-lactation cows were treated with the same _Propionibacterium_ strain, total ruminal SCFA concentrations were increased, but milk yield remained unchanged. Feeding a mixture of yeast and _Propionibacterium_ increased plasma glucose and increased milk production by midlactation, but not during early lactation (Lehloeny et al., 2008; Philippeau et al., 2017). Two different species of _Bacillus_ were used in dairy cows, and increased milk production was observed with _Bacillus licheniformis_, but no milk yield differences were detected from treatment with _Bacillus subtilis_ (Qiao et al., 2010). Using _B. subtilis_ spores (bacteria) in dairy cows resulted in an increase of 1.7 kg/day in milk yield, 0.05 kg/d in milk protein, 0.15 kg/d of total solids, and 4 MJ/d of energy, but milk urea-N was reduced (1.5 mg/dL), when the probiotic was fed for 16 weeks (Le et al., 2017).

Results of LY DFM supplementation in dairy cows often demonstrate large variability in the responses related to the quantity of milk produced. Similarly to studies using live bacterial populations, live fungal DFM must be continuously fed to maintain detectable ruminal populations of these products (Kung Jr. et al., 1997; Finck et al., 2014). Similarly, the fermentation extracts, end products, or cell-wall products must be fed daily to maintain their benefits. Early-lactating cows had a greater response to a LY supplementation than did later lactation cows (Wohlt et al., 1998). This improvement in peak milk production increased average milk production by 1.5 kg/day over 126 d of lactation (Wohlt et al., 1998). Including _S. cerevisiae_ cultures in high-producing dairy cow rations increased DMI and milk yield (Muñoz et al., 2016). Milk production increases of 0.7 to 2.4 kg per day have been reported in a variety of studies, but the reasons underlying the wide variance remains unclear.

In an outstanding meta-analysis of yeast DFM feeding studies that examined more than 150 experiments and over 375 treatments or doses, it was concluded that yeast supplementation increased rumen pH and SCFA concentration and also decreased the ruminal lactic acid concentrations, yet it had no effect on the ruminal acetate:propionate ratio (Desnoyers et al., 2009). Dry matter intake, milk yield, and fat-corrected milk were also increased by yeast supplementation (Desnoyers et al., 2009). Lower serum levels of lipomobilization markers and liver enzyme activities, as well as higher glucose levels, may suggest that LY slightly mitigated negative energy balance and had a liver-protective effect (Kumprechtová et al., 2019). In in vitro studies, the addition of _S. cerevisiae_ to fermentations decreased lactate accumulation and methane production, indicating that these DFM shifted the flow of carbon and energy in the ruminal fermentation, resulting in more available energy (Hu et al., 2017). Some authors have demonstrated a decrease in methanogenesis in dairy cows treated with _Aspergillus oryzae_ (Frumholz et al., 1989) or _S. cerevisiae_; others have not observed an effect (Mathieu et al., 1996) or have noted an increase in methane production (Takahashi et al., 1997). Variability of effects by individual DFM cultures on methanogenesis have been demonstrated in vitro as well (Mwenya et al., 2004; Jeyanathan et al., 2016; Latham et al., 2018, 2019).

Feeding live cultures and fermentation extracts (which can include enzymes produced by the postbiotic) of the fungal fermentations from _A. oryzae_ increased ruminal pH and SCFA concentration (Wiedmeier et al., 1987; Oellermann et al., 1990). Other _A. oryzae_ cultures (and possibly the end products of their fermentation) had a greater impact, in terms of increased ruminal pH and SCFA production, in animals on low-forage diets than in animals on high-forage diets (Gomez-Alarcon et al., 1990). In other studies, milk yield and production efficiency were improved in early-lactation cows fed a high-grain diet supplemented with an _A. oryzae_ culture, but the effects were less pronounced in later lactation cows (Gomez-Alarcon et al., 1990). When fungal and yeast cultures were directly compared, ruminal pH, ammonia nitrogen concentration, and total SCFA concentration were similar (Yoon and Stern, 1996). Overall, YC feeding increased the organic matter and crude protein digestibility, and the use of fungal postbiotic products increased cellulolytic bacterial counts, possibly through increased availability of vitamins, phenylpropionic acid, or other cofactors needed by the microbes that occupy the fibrolytic or cellulolytic ecological niche (Yoon and Stern, 1996).

Collectively, the evidence supports that DFM (bacterial or fungal) can improve milk production and production efficiency in dairy cows (Desnoyers et al., 2009; Jewell et al., 2015; Dill-McFarland et al., 2019). However, DFM effects have not always been consistent in magnitude, although the reasons behind this variability are still unknown. The advent of molecular community population estimates of the rumen and intestinal tract of cattle may help fill in this gap. This is an area that should be addressed in future research, because studies of the effects on quantification of microbial populations (both native and exogenous) have rarely been performed. Many of the greatest benefits of DFM feeding appear to occur in animals undergoing stresses or transitions (e.g., parturition, negative energy balance); DFM appears to make their greatest contribution to improving production in situations where animals are exposed to hot weather (Yu et al., 1997), low-quality diets, or other physiological stressors (Al-Shawi et al., 2020).
HEALTH BENEFITS OF DFM IN COWS

Dairy cows face numerous challenges during their lactation cycle, many of which are rooted (at least in part) in a nutritional problem, ranging from negative energy balance, to ketosis, to grass tetany, to displaced abomasums (Mulligan and Doherty, 2008; Zebedi et al., 2015; Khiaosa-Ard and Zebedi, 2018). Many of these challenges include a component caused (or exacerbated) by the resident (or a transient) microbial population (Schären et al., 2018; Shakira et al., 2018; Zhang et al., 2022). Challenges with a microbial component or driver include laminitis, liver abscesses, and hemorrhagic bowel disease and can include other production problems such as metritis, respiratory disease, and mastitis (Nader-Mácasías et al., 2008; Amachawadi and Nagaraja, 2015, 2016; Jones et al., 2022). The use of exogenous probiotics or DFM can alter or stabilize the microbial communities of the gut of adult cows when fed continuously, thereby affecting the incidences of these challenging conditions (Nader-Mácasías et al., 2008).

IMPROVING FIBER UTILIZATION

The rumen contains several thousand polysaccharide utilization loci that enable the microbial consortium to degrade a wide variety of dietary feedstuffs, such as NDF (Flint and Bayer, 2008; Accetto and Avgustin, 2015, 2019; Tomazetto et al., 2020). Thus, the introduction of exogenous organisms (probiotics or postbiotics) to supplement the catabolic capacity of the rumen faces numerous practical challenges. Inclusion of the ruminal fibrolytic bacterium Ruminococcus flavefaciens increased the total-tract digestibility of hay, but had to be fed daily to maintain this effect (Chiquette et al., 2007). Feeding a Prevotella bryantii (a common ruminal bacterium that is not fibrolytic) probiotic to early-lactation cattle increased milk-fat concentration and ruminal fermentation parameters, including digestibility of fiber, potentially by providing intermediates required by fibrolytic organisms (Chiquette et al., 2008; Fraga et al., 2018).

In addition, yeast products have been suggested to promote the growth of cellulolytic (i.e., Fibrobacter succinogenes, Ruminococcus albus), and amylolytic bacteria (i.e., Ruminobacter, Bifidobacterium), which were correlated with improved performance, but the linkage to the yeast treatment being the sole cause was tenuous (Jiang et al., 2017). But it should be noted that a higher relative abundance of ruminococccaceae did not translate into improved fiber digestion (Jiang et al., 2017). In vitro studies reported that a fungal species, A. oryzae stimulated growth of ruminal fungi (e.g., Neocallimastix frontalis EB 188) and cellulolytic microbes (Sun et al., 2014). In addition, the abundance of genes encoding for specific microbial fibrolytic enzymes has been increased in cow supplemented with S. cerevisiae Y1242 (AlZahal et al., 2014a,b). Yeast can use transient oxygen that enters with feed or water, thus improving the redox status of the rumen (Newbold et al., 1996). Fibrolytic and lactate-utilizing bacteria (LUB) require anaerobic and reduced conditions to function properly, and these are critical keystone bacterial guilds that support an adequate and healthy ruminal fermentation (Chaucheyras-Durand and Fonty, 2001). This promotion effect may be additionally explained by the fact that the S. cerevisiae product may provide growth factors, such as peptides, amino acids, or vitamins, to stimulate ruminal bacterial communities (Fonty and Chaucheyras-Durand, 2006).

REDUCING RUMINAL AND HINDGUT ACIDOSIS AND SARA

To meet the vast energy demands faced during lactation, dairy cows are commonly fed high-grain diets. Feeding a starch-rich diet may negatively affect rumen function due to high acid production (particularly of the strong acid lactate) and reduced buffering capacity, resulting in dramatically reduced pH (Owens et al., 1998). Acute ruminal acidosis has been widely understood as a profoundly low ruminal pH (often <5.5), driven by large amounts of lactate production, which affects the host animal physiology and performance (e.g., acidosis, laminitis, DMI reduction) and can in severe cases lead to death (Slyter, 1976). Many have viewed SARA as simply a milder case of ruminal acidosis (subacute) that self-corrects over time; however, it is a chronic condition with long-term and broad effects on both the animal and the resident microbial population and is linked to altered rumen microbial communities and functions (Petri et al., 2013a,b), such as a reduction in fibrolytic bacteria growth and fermentation (thus reducing fiber degradation; Plaizier et al., 2018). It appears that many cases of SARA were more correlated with a low pH (<6.0), which was driven by SCFA concentrations rather than lactate (Krause and Oetzel, 2005). When cattle are mildly acidic or are undergoing SARA, they are subject to cyclic feeding (and associated production disruption), as well as peritonitis, liver abscesses, and laminitis (Nagaraja and Tiggesmeyer, 2007). Although we typically understand and discuss acidosis in terms of ruminal effects, it is not just a “rumen disorder,” because it also affects the total digestive tract (Plaizier et al., 2018). Many of our acidosis-intervention strategies have focused on
Subacutal ruminal acidosis has been associated with the overgrowth of the lactate-producing ruminal bacterium *Streptococcus bovis*; however, severe SARA or acute acidosis was associated with a high abundance of *E. coli* (Khapipour et al., 2009a,b). Therefore, it was speculated that competitive lactic acid producing bacteria such as *Lactobacillus* and *Enterococcus* might prevent ruminal acidosis (Nocek et al., 2002, 2003) by facilitating the growth of ruminal microbes adapted to the presence of lactic acid in the rumen or by stimulating LUB populations or activity (Martin and Nisbet, 1990; Nisbet and Martin, 1991; Martin, 1992; Yoon and Stern, 1996). Based on in vitro data, it was recently speculated that LAB could also compete with *Strep. bovis* and their produced bacteriocins could inhibit ruminal *E. coli* involved in severe SARA development (Ban and Guan, 2021). *Saccharomyces cerevisiae* can metabolize lactate, and its effect on altering ruminal lactate concentration has been confirmed through in vivo studies (Reis et al., 2018a,b; Kumprechtová et al., 2019). Additionally, it appears that yeast products can stimulate the growth of native ruminal microbes that compete with LAB for available sugar(s), potentially contributing to the alleviation of ruminal pH decreases and SARA development (Amin and Mao, 2021). It must be noted that because the yeast products must be fed daily, it is not likely a direct competition of the yeasts for the sugars in the rumen. Butyrate-oriented SARA also appears to be at least partially alleviated by yeast supplementation (Brossard et al., 2004; Lettat et al., 2010). A single strain of *S. cerevisiae* fed to sheep with butyric ruminal acidosis had a pH stabilizing effect (Lettat et al., 2010). This DFM strain promoted ciliate Entodiniomorphid protozoa, which are known to engulf starch granules very rapidly and thus compete effectively with amylolytic bacteria (Owens et al., 1998). The fact that ciliate protozoa digest starch at a slower rate than amylolytic bacteria, and their main end products of fermentation are SCFA rather than lactate, might explain why they have a stabilizing effect in the rumen by delaying fermentation.

Lactate production and accumulation in the gut has been linked to reduced pH, ruminal acidosis, liver abscesses, and leaky gut syndrome; conditions that are linked with production issues such as reduced DMI and cyclic feeding, as well as other health effects (Hernández et al., 2014; Chen et al., 2019). Thus it has been important for researchers to understand how lactate-utilizing ruminal bacteria could affect pH and animal health. *Megasphaera elsdenii* is a ruminal bacteria that can ferment glucose rapidly to lactate, but can then ferment lactate, and the addition of this bacterium reduced ruminal lactate accumulation, at least in vitro (Kung Jr. and Hession, 1995; Weimer et al., 2015). Lactate-utilizing bacteria found naturally in the rumen, such as *Selenomonas ruminantium* and *Megasphaera elsdenii*, can use lactic acid to produce SCFA, primarily ending up as propionate and butyrate (Forsberg, 1978; Cournotte et al., 1981; Marounek et al., 1989; Nisbet and Martin, 1994). Thus, LUB-based DFM have been shown to aid in preventing the accumulation of lactate in the rumen and maintaining a higher ruminal pH as well as increasing SCFA production, especially propionate and butyrate (Cournotte et al., 1981; Krehiel et al., 2003; McAllister et al., 2011; Al Ibrahim et al., 2012). To date, there has been little evidence of the decrease in lactate concentrations in the rumen in vivo, but the in vitro results have been promising in terms of pH increase and stabilization and a shift in the ruminal SCFA profile.

Lactate-utilizing bacterial populations in the rumen were correlated to improved dairy production (Aikman et al., 2011; Shabat et al., 2016), and many have hypothesized that specific LUB (or others of this niche) could be used to capture dietary energy that would normally be lost (Krehiel et al., 2003; Fernando et al., 2010; McAllister et al., 2011). The rate and extent of lactate utilization by the ruminal-origin LUB *M. elsdenii* and *Sel. ruminantium* was significantly increased by some DFM, demonstrating a prebiotic-type effects (Nisbet and Martin, 1991; Martin and Nisbet, 1992; Nisbet and Martin, 1994). Some of the DFM-catalyzed increase in lactate utilization was hypothesized to be due to concentrations of malate or fumarate, or both, naturally occurring in yeast products, which increases the rate of lactate fermentation by these important ruminal LUB (Nisbet and Martin, 1993; Martin and Streeter, 1995; Callaway and Martin, 1996; Newbold et al., 1996). This suggests a potential future symbiotic approach combining a probiotic of LUB and a prebiotic including dicarboxylic acids (e.g., malate, fumarate) to reduce the risk of acidosis in cows fed high levels of starch, and further may explain the benefits of some existing DFM that successfully modify the ruminal fermentation (Martin et al., 1999; Tejido et al., 2005; Jouany, 2006). As a primary lactate utilizing in the rumen, *M. elsdenii* is a major component of commercial DFM products that have been used to prevent ruminal acidosis in dairy cows fed a high-concentrate diet, especially during the postpartum period (Aikman et al., 2011; Arik et al., 2019; Chen et al., 2019). The present utilization of *M. elsdenii* in commercial products offers an exciting opportunity to reduce acidosis and
improve performance and can be used as a coculture product with Propionibacterium, and can alter the microbial composition of the rumen and increase the molar proportion of propionate produced (Weimer et al., 2015; Luo et al., 2017; Arik et al., 2019; Chen et al., 2019). However, it must be noted that no studies have demonstrated the exogenously added \textit{M. elsdeni} populations to persist in the rumen, meaning they must be fed daily. This contrasts with the theory that the addition of a “rumen-native” bacterium would have a greater chance to persist in the ruminal microbial community, and this poor ruminal persistence merits further investigation.

For all bacterial DFM (e.g., eubiotics), it has been suggested that their efficacy in preventing SARA might depend on the dietary grain types and the endogenous rumen microbiota because several studies did not demonstrate a beneficial effect in mild- or low-challenge situations (Arik et al., 2019). Fungal DFM especially LY (i.e., \textit{S. cerevisiae} and \textit{S. boulardii}) have been more widely used to alleviate cases of ruminal acidosis in dairy cattle. Using 18S and internal transcribed spacer sequencing, Ishaq et al. (2017) showed that diet-induced SARA modified the diversity of rumen fungi and protozoa and selected against fiber-degrading species. There have been several yeast-based SARA-prevention studies because of its specific metabolism (Ishaq et al., 2017). Yeast promotes shifts in microbial communities involved in the release of lactate or those implicated in lactic acid removal (or both), leading to an optimized balance between lactate producers and lactate utilizers (Pinloche et al., 2013). Probiotic feeding increased the availability of vitamin B1 (thiamine), which promotes the colonization of plants by the ruminal microbes and enhances fiber digestibility (Chiquette et al., 2015). These factors explain how the use of \textit{S. cerevisiae} cultures promoted cellulolytic bacteria growth in the rumen (Newbold et al., 1995; Newbold et al., 1996).

Live yeast supplementation (\textit{S. cerevisiae} CNCM I-1077) was shown to improve dairy cow performance in 14 trials (de Ondarza et al., 2010) and also demonstrated an improvement in feed efficiency. The feed efficiency was the greatest, with an extra 80 g of milk per kilogram of DMI when animals were at a high risk of SARA (low-fiber diet, >25% starch). Great stability over a day and better rumen pH supports higher activity of the fiber-degrading niche, thus explaining the increase of small-meal frequency. Susceptibility of individual cattle to acidosis (Nagata et al., 2018) and the instability of microbial fermentations in the rumen characteristic of SARA or emerging acute acidosis (Schwartzkopf-Genswein et al., 2003; Gao, 2016) are thought to be responsible for the variable or even contradictory effects of probiotics used to prevent the development of acidosis.

Inflammation is one of the hallmarks and underlying effects of acidosis and SARA, including widespread systemic inflammation. Few studies have looked at the potential of probiotics to alleviate inflammation or to modulate the immune system during acidosis (Isolauri et al., 2001). In heifers fed diets with high concentrations of starch or fructose, a combination of the ionophore monensin and LY reduced histamine concentrations in ruminal fluid (Golder et al., 2014). A decrease in plasma LPS concentrations, as well as serum amyloid A (\textit{SAA}), an acute phase protein, was demonstrated after supplementing steers with LY products, but these products had no effect on ruminal or duodenal LPS concentrations (Garcia Diaz et al., 2018). Plasma SAA levels were reduced by using a LY product, which also had no effect on ruminal LPS concentrations (Silberg et al., 2013). The differences in LPS concentration effects may be because the large intestine, rather than the rumen, is the most probable site for LPS translocation in ruminants because the monolayer intestinal epithelium is more prone to damage by acidity compared with the reticulorumen epithelium (Khiaosa-Ard and Zebeli, 2018). These effects can lead to reduced epithelial integrity and subsequent LPS translocation to the bloodstream of cattle, which can cause severe systemic inflammation, can initiate laminitis (sore feet), and drives many early culling decisions in cattle (Nielsen and Dohoo, 2011; Hernández et al., 2014).

\textbf{Metritis}

Metritis is an inflammation of the uterine wall caused by a bacterial infection that typically occurs during the postpartum period and can lead to temporary or permanent impairment of fertility. Studies have demonstrated some DFM containing LAB modulated endometrial infection and inflammation in cattle by reducing in vitro and ex vivo infections caused by \textit{E. coli} (Geníš et al., 2016, 2017). The addition of bovine vaginal LAB (Gärnter et al., 2015) as a probiotic approach inhibited the growth of metritis-causing organisms in dairy cattle (Otero and Nader-Macías, 2006; Otero et al., 2006; Bayatkouhsar et al., 2013; Peter et al., 2018). Some vaginal \textit{Lactobacillus} strains have anti-	extit{Staphylococcus} activity due to their production of \textit{H}_2\textit{O}_2 (Otero and Nader-Macías, 2006). A comparative study of vaginal microbial community structures in 5 healthy and 5 endometritic dairy cows found that the disruption of the normal vaginal microflora (e.g., dysbiosis) may contribute to the onset of endometritis and that the use of DFM may provide stability to this microbial ecosystem to help resist development of metritis.
Mastitis

Mastitis is an inflammatory reaction of the mammary gland caused by infection, trauma, or toxicity and is one of the most detrimental diseases affecting the dairy industry (Bradley, 2002). Although mastitis can be a result of physical or chemical trauma, it is most commonly caused by pathogenic microorganisms. If untreated, mastitis can lead to the deterioration of cow welfare and health, milk production, and milk quality. Studies on probiotic preparations found that the bacteria living on the surface of a healthy udder could inhibit the in vitro growth of mastitis-causing organisms, including Aracanobacterium pyogenes (Al-Qumber and Tagg, 2006; Bouchard et al., 2015; Rainard and Foucras, 2018).

Probiotics based on LAB have been used as alternatives to chemical pre- and postmilking teat disinfectants to maintain udder health. When applied via intramammary infusion, the immunomodulatory activity of Lactococcus lactis DPC3147 strain was demonstrated in an improved mastitis cure rate equivalent to that of antibiotic treatment (Crispie et al., 2008). The Lactococcus strain used stimulated the mammary gland immune response (in particular IL-1 and IL-8 gene expression), favoring the elimination of the pathogen (Beecher et al., 2009; Kitching et al., 2019). The LAB disinfectant exhibited long-term effectiveness against Strep. agalactiae (Yu et al., 2017), and similar DFM have been equally effective against other mastitis-causing pathogens (Genis et al., 2018; Wu et al., 2019; Gao et al., 2020). It should be noted that applying these products as intramammary infusion may be impractical for use at the farm level, but instead offer a proof of concept and avenue of approach for future dips or surface treatments to reduce entry of mastitis-causing organisms (Crispie et al., 2008; Krishnan et al., 2020).

REPRODUCTIVE EFFECTS

Several strains of both gram-negative and gram-positive pathogenic bacteria have been identified as the main causative agents of uterine infections. Infectious diseases of the uterus directly affect the fertility and productivity of dairy cows, and, consequently, the financial bottom line of the dairy industry. Recent research suggests that lactobacilli present in the vaginal microflora of healthy cows constitute the primary microbiological barrier to infection (Otero et al., 2006; Loir and Even, 2017). Other recent studies indicated that small populations of bacteria can be present in the uterine horns of otherwise normal healthy cows (Walker et al., 2023); however, the role of this community remains unknown.

A LAB cocktail (Lactobacillus sakei and Pediococcus acidilactici) was intravaginally infused and lowered the incidence of metritis and total uterine infections (Deng et al., 2015). Moreover, the intravaginal infusion produced lower concentrations of systemic lipopolysaccharide-binding protein, SAA, and greater vaginal mucus concentration of secretory IgA. However treatment did not affect serum concentrations of haptoglobin, tumor necrosis factor, IL-1, IL-6 and total IgG among the treatment groups (Deng et al., 2015). Further studies have demonstrated the presence in the bovine vaginal tract of probiotic bacteria that have the ability to produce H₂O₂ (Pellegrino et al., 2017). Lactating cows that received 10¹⁰ cfu/g of S. cerevisiae Sc47-CNCM I-4407 over 13 mo required fewer inseminations (from 3.1 down to 2.7) to achieve pregnancy during the probiotic yeast-supplementation period (Julien et al., 2018). Simultaneously, the fat and protein yield in these cows improved by approximately 5%, demonstrating that it is possible to simultaneously improve milk performance and reproductive health (Julien et al., 2018). Additional studies found that the success rate of the first insemination is generally better in dairy cows receiving a high dose of probiotic yeast during the 6 wk around calving time. These cows also had better feed efficiency and lower negative energy balance when beginning lactation, and as such, supplementation may improve reproductive function in dairy cows (Julien et al., 2017).

HOLISTIC VIEWS OF EUBIOTIC APPROACHES: A CONCLUSION

Microbial feed additives, such as DFM or eubiotic approaches, seem to be an effective solution to improve the well-being of ruminants (Nocek and Kautz, 2006; Buntyn et al., 2016; Ort et al., 2018). For many years we have depended on antibiotics to improve animal performance and animal health, which in turn has improved the affordability of animal protein for millions of people, but there are increasing consumer and regulatory pressures to reduce the nontherapeutic use of antimicrobials and replace them with alternative strategies such as the use of probiotic approaches (Callaway et al., 2021; Low et al., 2021), which have none of the deleterious effects of antimicrobial resistance (Ioannou et al., 2018; Seal et al., 2018).

A wide variety of probiotic-type approaches exist, ranging from live cultures (bacteria, fungi, or yeast; probiotics) to killed (or inactivated) cultures, and this category includes end products of fermentation or cell products (postbiotics), or both, to specific compounds (prebiotics) to consider when deciding which approach is needed to improve gut health and animal performance. Generally, most of these products are thought
to modify the intestinal flora (directly or indirectly) and have been shown to have other effects on the host, especially in terms of gene expression and immune function (Novak et al., 2012; Ballou, 2014; Adjej-Fremah et al., 2018). One of the biggest proposed benefits of modifying the composition of the microbial community of the gut is through stabilization of the gastrointestinal community structure (Hernández et al., 2014; Krishnan et al., 2020). A more stable community is thought to be able to resist changes (reduced plasticity) and prevent exogenous challenges from pathogens that can affect host health and productivity. However, to date there are no specific metrics of what constitutes a “more stable” community, and whether this truly benefits the animal remains unknown.

Although we cannot describe all effects of the different probiotic approaches, we can draw some general conclusions regarding the beneficial effects. The benefits of probiotic approaches are not limited to the gut; they can reduce diseases that affect cattle after parturition (often around peak lactation) and improve fertility measures as well. When cattle undergo stress or transitions, it appears that eubiotics can be a valuable tool in the arsenal of dairy producers. In calves, DFM prevent pathogen penetration into the gastrointestinal consortium, reduce wasteful energy expenditures, and can stimulate the calf’s immune defenses through increased production of vitamins, cofactors, and trace elements. In mature cows, probiotics and prebiotics improve fiber digestion by providing limiting nutrients (e.g., branched-chain SCFA, vitamins, phenylacetic acid) to the cellulolytic microbiota. Further immunostimulatory effects have been demonstrated from a variety of pro- and prebiotic approaches and can be used to strengthen this important first line of defense for cattle against pathogens. However it must be noted that the use of DFM or probiotics in dairy cattle has suffered from a great deal of variability in efficacy, with no clear explanation for the differences between failures and successes. Therefore it is imperative that we develop integrated models of microbial communities of the gastrointestinal tract, along with microbial and host animal metabolites, animal performance, and health metrics. Furthermore, little research has examined if, and for how long, the added probiotic organisms persist in the gut when not fed daily or how long the changes in the microbial ecology of the gastrointestinal tract continue following treatment withdrawal.

We like to think that DFM operate by a defined set of rules or mechanisms of action that we can identify; however, the effects of DFM vary widely with the type of probiotic, animal status (calf vs. mature cow), and environmental conditions. As we broaden our understanding of the host-native microbial symbiotic relationship, specifically understanding how antibiotics affect animal health and productivity, we will shine a light on potential specific targets that we can address with probiotic or DFM approaches. Today there are no indicators of what the hallmarks of a “good” or “bad” microbiome is, or even if there is such a thing applicable at more than the individual-animal level. As we begin to understand the native microbial composition of the gastrointestinal community, we can begin to understand what a “more productive” microbial community looks like, thus making it possible to create tailored or prescriptive probiotic approaches, finally allowing us to “hit a moving target” as we improve production efficiency and sustainability.

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