Rumen Modifiers in Today's Dairy Rations

Jeffrey L. Firkins[1] and Kelly E. Mitchell
Department of Animal Sciences
The Ohio State University

Abstract

We have carefully researched modes of action and provided background context on rumen and microbial function to help nutrition advisors understand when to consider various rumen modifiers for their dietary conditions. We have evaluated various microbial products that enhance the uptake and potentially even the synthesis of ruminal lactate. Lactate accumulation, not uptake, is the problem to avoid, especially when it promotes problems or decreases ruminal NDF digestibility. We also have selected some potential methane-suppressing additives for potential usage in dairy rations, i.e., to decrease methane production without decreasing milk production. Finally, we have summarized our recent research in branched chain volatile fatty acids to help maintain adequate growth factors for cellulolytic bacteria in diets with decreasing contribution of rumen-degraded supply of branched chain amino acid precursors.

Introduction

Dairy rations must balance the desire for increasing rumen available starch to provide energy for the high producing cow with the opposing need to prevent acidotic conditions in the rumen. Sub-acute ruminal acidosis (**SARA**) should be avoided. However, even before the ratio of starch:effective fiber is high enough to promote SARA, the extra starch can depress NDF digestibility compared with its potential or depress milk fat percentage. We have lessened these negative associative effects in the past few decades through monitoring particle size, sorting behavior, ruminal starch digestibility, etc. Of course, rumen modifiers cannot replace sound dietary formulation and feeding management, but some rumen modifiers also can be useful.

There are many feed additives and rumen modifiers, and our goal is not to substitute for recommendations made by those such as Dr. Mike Hutjens, who has numerous reports with which we do not intend to compete. We will follow a key recommendation made by Al Shultz in a 2014 Hoard's Dairyman article that showed a pyramid of getting information. The bottom had anecdotal information, whereas the top of the pyramid had controlled studies that were peer-reviewed. Our goal will be to summarize various scientific literature to explain modes of action for rumen modifiers within our scope so that dairy nutrition advisors can ask better questions when considering using them under their conditions.

Review of Electron Flow in Fermentation

To explain modes of action for many additives, you need to understand some basics of chemistry. If you do not want to use the full decision pyramid, you can skip this section.

[1]Contact at: 2029 Fyffe Ct, 223 Animal Science Bldg., Columbus, OH 43210-1095, (614) 688-3089, Fax: (614) 292-1515, Email: firkins.1@osu.edu.

Metabolism, including in fermentation, involves flow of electrons down a pathway analogous to a battery-powered motor. Electrons flow through a wire from one pole to complete the circuit when the wire is attached to the other pole. The other pole is the "sink", which collects electrons until the battery is dead. In fermentation, sugars from degraded carbohydrates (and proteins to a lesser extent) are sources of electrons that need a "sink". The main electron sinks are typically the electron acceptors leading to production of methane or propionate. Similar to how a battery's current can pass through and turn a motor, electrons in metabolism (including fermentation) pass through certain reactions that produce ATP. We're just finding now how much more ATP is made in interesting ways that weren't known 10 years ago. They also can spill energy (waste ATP) when it couldn't be harnessed, which allows them to keep some processes going or simply to just keep substrate away from competitors.

In metabolism, electrons typically travel in pairs down a metabolic pathway and are countered with pairs of protons ($\mathbf{H^+}$). These 2 pairs of electrons and protons are commonly referred to as metabolic hydrogen, which often is abbreviated as [2H], with the brackets indicating usage of one of several forms of electron carriers/acceptors. The various reactions are collapsed in Figure 1 to show the importance of [2H] in cellular metabolism. Importantly, the [2H] is not synonymous with dihydrogen ($\mathbf{H_2}$), as sometimes wrongly depicted in some papers. Many fibrolytic and amylolytic microbes use a hydrogenase to convert [2H] to H_2, which is released into rumen fluid. Then, the H_2 can be converted back to [2H] for metabolic purposes. This form of "interspecies hydrogen transfer" is embedded in the entire rumen microbial ecosystem. The H_2 is mainly harnessed by hydrogenases (producing [2H] again) when methanogens produce methane (CH_4). However, there is some conversion of H_2 to [2H] by hydrogenases from other microbes, including some that produce propionate or those that reduce nitrates. Some microbes do not encode a hydrogenase (i.e., they cannot make H_2) and sink their electrons to propionate.

Figure 1 is patterned after that of Ungerfeld (2020). However, we need to remember that these reactions are summative over all types of microbes, each with a mix of these various pathways. Acetate is produced about 3 times more than propionate in the rumen of dairy cattle. Because the acetate pathway generates more [2H] than is used, that extra [2H] must be sunk to some other reduced end-product, such as methane or propionate. One sink for [2H] is cellular products, such as amino and fatty acids. Growing cells therefore naturally consume more [2H] in chemically reduced cellular constituents. When [2H] produced in fermentation builds up beyond what can be used, fermentation and ATP production stalls and fiber degradation decreases. The additives we will be discussing have been shown at least in bacterial cultures to help prevent these stalls in [2H] cycling and often involve lactate and succinate.

Lactate as an Intermediate

The typical lactate dehydrogenase is: pyruvate + NADH $\leftrightarrows$ lactate + NAD^+. For simplicity, left off is a proton associated with NADH. When cells produce lactate, they are disposing of [2H] from NADH by sinking electrons in the more reduced product, lactate (see reaction 1 in Figure 1). This reaction could be rewritten as $C_3H_4O_3$ + [2H] $\leftrightarrows\leftrightarrows$ $C_3H_6O_3$ (note the gain of 2 H on lactate). Not all isoforms of this enzyme use the NAD/NADH cofactor, but there still is transfer of [2H] via some other cofactor. The lactate racemase (see reaction 2 in Figure 1) probably allows more lactate to be produced because L- and D-racemers would

each feedback-inhibit their respective lactate dehydrogenase reactions. In contrast with lactate producers, when a lactilytic (lactate consuming) microbe takes up lactate as its substrate, the conversion back to pyruvate will build up [2H] that must find a new sink. Some probiotics or prebiotics are thought to help lactilytic microbes sink that [2H] to consume more lactate.

Lactate is a stronger acid than the volatile fatty acids (**VFA**) by one log factor. That is, at the same concentration, lactate would want to drop pH by one whole pH unit (10-fold higher proton concentration) compared with the VFA. That's why lactate is associated with SARA. Not only does lactate accumulation drop pH, but the root cause (i.e., greater glucose availability) can trigger the lactate producers to make more lactate rather than VFA. They are more resilient against decreasing pH than are the lactate consumers.

To start with the concept that some lactate-producing probiotics might be considered in dairy rations, let's examine dosing lactate itself. Dosed ammonium lactate was catabolized primarily to propionate almost completely within 1.5 hours in continuous cultures (Wagner et al., 2018). Because we controlled pH above 6.0, lactate had minor effect on bacterial populations. Similar increases in molar proportions of propionate (and butyrate) were detected in cows fed this lactate source (Caputo Oliveira et al., 2019), and resultant metabolic indicators suggested that supplemental lactate increased liver supply of propionate in transition cows (Caputo Oliveira et al., 2020). Those authors noted improved milk efficiency resulting from decreased DMI while maintaining similar milk production. An improvement in feed efficiency also was noted when dosing a propionate-producing probiotic (Weiss et al., 2008). Propionate can decrease DMI by dairy cows, as shown by Mike Allen, Barry Bradford, and colleagues. However, lactate production per se should not be a problem in dairy nutrition so long as it is fermented, SARA does not result, and milk production does not decrease.

We need to consider fermentation intermediates (i.e., those produced by one microbe but fermented by another) more carefully with respect to methane suppression. Fermentation of propionate via succinate likely involved *Prevotella* and *Selenomonas* in goats (Denman et al., 2015), and fermentation of lactate to propionate and butyrate likely occurred via *Megasphaera* in sheep (Kamke et al., 2016). Both cases were associated with suppressed methane production. If you look at Figure 1 (modes 3 and 4), you can see that both ways of producing propionate consume [2H] that is not converted to H_2, thus circumventing methane production. Even butyrate production yields less H_2 compared with acetate, and many researchers have documented that butyrate fuels the rumen epithelium to increase VFA absorption rate. Butyrate supplementation might have value for calves (Aschenbach et al., 2019) but won't be further discussed.

Lactate Accumulation

Why does lactate production get unbalanced with respect to lactate usage, i.e., increasing accumulation of lactate? First, this will only happen when there is plenty of substrate, i.e., starch, that is extensively converted to glucose. Additionally, greater glucose availability triggers lactate producers to make more lactate rather than VFA. Cornell researchers noted that glucose conversion to lactate yields only 2 ATP/glucose, but this process speeds up glucose usage by 5 fold. Thus, they gain ATP about 2.5-fold faster, and it helps out these lactate producers because they are more resilient to lactate's role to decrease pH. There also is some indication that lactate

is absorbed more slowly from the rumen than the VFA, exacerbating this acidotic situation. The net effect is the well-known spiral that can promote SARA (Jouany, 2006).

We can see swings in growth of these microbes followed by death ("lysis"). The lipopolysaccharide (**LPS**) components (among other endotoxins) of their cell walls are associated with rumen inflammatory responses (Zebeli et al., 2012a). Those authors' threshold for potential systemic inflammation was 44% concentrate (or higher) and 39% NDF (or lower), documenting practical consequences for dairy nutrition. To make things worse, apparently LPS itself increases lactate production without affecting lactate consumption (Dai et al., 2020). Thus, any managerial factors to prevent swings in starch availability helps to keep lactate production from spiraling.

The rumen epithelium is already likely microaerophilic (some dissolved O2 diffusing from the blood). Microbes have adapted to these microaerophilic conditions (Mann et al., 2018). Much more work is needed to understand these epithelium-associated microbes' role in rumen function because they seem to be related to differences among animals such as feed efficiency (Bickhart and Weimer, 2018). Future research could justify a greater mode of action for probiotics to aid in these microbes' roles under SARA conditions (Petri et al., 2020).

Redox and Rumen Ecology

The oxidation/reduction potential (**ORP**; sometimes called redox potential) technically measures the tendency of a chemical species to accept or donate electrons to an electrode. It typically has been measured in a similar way as pH using a hand-held device but now is being measured in intra-ruminal cow monitors. A lower value (more reduced rumen environment) indicates a lower affinity for electrons, and a higher value (more oxidized) indicates a higher affinity for electrons. Ruminal function has been associated with ORP, e.g., NDF digestibility (Huang et al., 2018). Cellulolytic specialists produce end-products, such as succinate (how *Fibrobacter succinogenes* was named) or the H_2 from acetate production (*Ruminococcus*). Succinate and H_2 are converted primarily to propionate and methane, respectively (modes 4 and 7 in Figure 1). Both the production and usage of succinate and H_2 seem to be favored under a more reduced environment. The mechanism is not fully clear, but key enzymatic reactions probably require cofactors that are very sensitive to O_2. Increasing O_2 entry with feed and water raises ORP, whereas facultative anaerobes respiring that O_2 makes ORP more negative. The affinity of methanogens for H_2 is so strong that methanogenesis actually pulls an otherwise unfavorable reaction by the starch and fiber degraders to produce H_2, gaining them more ATP. Most of the aqueous H_2 winds up in methane unless we try to inhibit methanogenesis using additives like nitrate (Mode 6, Figure 1) to compete for that aqueous H_2.

We can only measure ORP in rumen fluid (extracellular), but this measurement reflects intracellular redox state of enzymes and the likelihood to direct fermentation pathways (Dijkstra et al., 2020). In Figure 2, we fed the same diet but manipulated pH using buffers (Panel A). In Panel B, we manipulated two forage:concentrate ratios while maintaining very similar pH response curves. Decreasing pH (Panel A) inherently makes ORP more positive (higher proton concentration is related to the measurement of ORP); however, increasing concentrate (Panel B) also increases ORP independent of pH. The net of increasing concentrate and assuming it will decrease pH is a 10 to 15% increase in ORP from Figure 2. The positive association

of ORP with propionate (Huang et al., 2018) is overly simplistic because more starch increases amylolytic microbes that encode the propionate pathway. Trying to unbundle these sources of multiple correlation (more starch and lower pH) is difficult experimentally (Broudiscou et al., 2014). From our standpoint in this paper, this tendency for a more positive ORP would be exacerbated when dietary conditions increase SARA risk because of the risk for O_2 leakage from blood into the rumen.

A more positive ORP probably aids in establishing a healthy relationship between the rumen microbes and the rumen wall. Although outside of our scope, part of a calf's rumen development is for anaerobes to supplant facultative anaerobes and increase in dominance as the ORP declines with increasing age. Probably hastening this response should get them to degrade fiber better and sooner. Even with adults, some O_2 diffuses from blood across the rumen epithelium. Mann et al. (2018) noted that those bacteria associated with the epithelium have a high ability to quench O_2. For cows with SARA, repeated exposure of the rumen epithelium to low pH can wear away the barrier of the membrane and allow further O_2 diffusion. Thus, the already higher susceptibility to a more positive ORP could be exacerbated and help tilt the epithelium to a more inflammatory state when facultative anaerobes that promote inflammation (able to live in higher ORP) increasingly displace the desirable fermentative bacteria (Friedman et al., 2017). Even in a healthy state, the ORP partially regulates fermentation pathways that are affected by intracellular NAD:NADH ratio; in particular, ORP probably has a role in regulating acetate:propionate and methanogenesis (Dijkstra et al., 2020). If we can further reduce variability associated with measuring ORP, then maybe it will help explain variability among animals per se.

Probiotics and Organic Acids to Stimulate Lactate Consumption

Although entodiniomorphid protozoa can consume lactate, *Megasphaera elsdenii* and the lactilytic group of *Selenomonas ruminantium* have been the best characterized in the scientific literature. There probably also are other lactilytic bacteria that are predominant, but they have not yet been cultured and characterized. Even so, *Megasphaera* and *Selenomonas* are useful models to describe the 2 pathways that consume the [2H] by producing propionate through acrylate and succinate, respectively (modes 3 and 4 in Figure 1). Both clearly have evolved ways to use electron carriers to produce ATP (Ungerfeld, 2020) and therefore require highly reduced conditions (highly negative ORP). Because production of propionate is a reductive process (electrons and [2H] are sinked), a lactilytic microbe producing propionate via the succinyl CoA pathway would have even more [2H] to dispose of when lactate is converted to pyruvate.

There are many papers on stimulation of lactate uptake by the lactilytic *Selenomonas* strains from the 1990's. Malate and fumarate stimulated lactate uptake and fermentation to propionate, especially with decreasing pH below 6.0 (Martin, 1998). Those authors explained that those dicarboxylic acids can be in feeds such as some forages. However, they also are in feed additives directly. Moreover, they can be produced by live yeast or are in yeast extract (Jouany, 2006). Malate and fumarate provide 2 critical roles: 1) providing an electron sink (see [2H] consumption to make succinate) and 2) filling carbon needed to balance fermentation and cell synthetic reactions (see modes 4 and 5 in Figure 1). The critical compound oxaloacetate (OAA; mode 5) is at the juncture of propionate synthesis and for critical anabolic roles such as producing amino acids and nucleic acids.

The precursor OAA thereby limits growth just like OAA is typically a limiting metabolite linked with a dairy cow's ketotic liver! Second, those bacteria using lactate need to dispose of [2H], as mentioned previously, so malate or fumarate provide electron sinks to do that. In addition, because *Selenomonas* is well known to ferment succinate released by bacteria, such as the cellulolytic *Fibrobacter* succinogenes to propionate, *Selenomonas* (and related bacteria) could potentially aid fiber degradation by keeping succinate concentration low and thereby stimulating succinate production (Sawanon et al., 2011).

Many of the important fermentative bacteria do not adjust well to low pH conditions. Jim Russell and co-workers at Cornell University documented that low pH (more extracellular protons) naturally increases diffusion of protons and their various accompanying anions, such as acetate, into the cell until the anion concentration becomes toxic. Fumarate reductase is the key enzyme needed for propionate producers to make ATP via proton (H^+) or sodium (Na^+) gradients (Hackmann et al., 2017). This enzyme's activity decreased as pH declined below 6.0 (Asanuma and Hino, 2000), which helps explain why Martin (1998) inferred that the benefit from providing malate or fumarate was important as pH declined below 6.0. Preventing downswings in ruminal pH (which wants to increase redox) should help improve efficiency of lactate fermentation to propionate and therefore decrease inhibition by accumulated lactate. A more negative ORP from yeast was associated with more lactate consumption in dairy cows (Marden et al., 2008).

Megasphaera elsdenii has been studied as a probiotic to "seed" the rumen based on numerous results from previous research using culture-based approaches. There has been some conflicting discussion whether or not increasing *M. elsdenii* activity increases trans-10, cis-12 concentration or if this lactilytic bacterial species increases concomitantly with conditions that promote this "trans-10 shift" that predisposes milkfat depression. Most likely, *M. elsdenii* does not contribute significantly in a direct way (Dewanckele et al., 2020). However, in 2 dairy studies with relatively high amounts of starch (30 and 31%) that was provided by highly available sources, such as wheat and barley, 2 rather different trends were detected. When DMI numerically decreased by about 4 lb/day by *M. elsdenii* dosing, propionate concentration in the rumen was increased (Aikman et al., 2011). When DMI was not affected by M. elsdenii dosing, propionate was unchanged but butyrate increased (Zebeli et al., 2012b). This latter study had much more NDF and forage NDF than the previous study. In general, *M. elsdenii* is thought to produce propionate more from lactate, which it prefers as substrate, but produces more butyrate from glucose if lactate decreases (Weimer and Moen, 2013). Because propionate is well known to potentially suppress intake and to be insulinogenic (potentially partitioning energy to adipose instead of mammary tissues), these types of very high rumen-available starch situations with less NDF might be riskier for this additive because of the potential for depressing DMI than for depressing milkfat. In Ohio State University studies with moderate dietary starch, those animals that are more likely to respond (i.e., higher in lactation number) were more likely to benefit from dosed *M. elsdenii* (Stevens et al., 2017, Ye and Eastridge, 2018).

Yeast Products and Ionophores

Live yeast and yeast extract have found their places in many dairy rations to help optimize fiber degradation and improve DMI (Adesogan et al., 2019). We note that fungal extract appears to have some of the same properties as yeast extract, but we could not

find adequate review summaries to include in this report. Jouany (2006) suggested oxygen scavenging by live yeast that congregated to newly ingested feed (which includes O_2) benefited those nearby fibrolytic bacteria. Oxygen scavenging was supported as a major role for live yeasts and potential boosting of cellulolytic bacterial abundance (Pinloche et al., 2013) and probably for pH control via increased lactate uptake (Marden et al., 2008). Although isotrichid (and perhaps other) protozoa and facultative anaerobic bacteria also can help remove O_2, live yeast would be more likely to respire O_2 compared with yeast culture. Newbold et al. (1996) discussed relatively little benefit expected from malate derived from yeast, although their continuous cultures' pH was buffered near 7.0 (which might have limited the response; see earlier discussion). Even extract that was experimentally collected from live yeast stimulated lactate fermentation to help maintain pH (Rossi et al., 1995) or stimulate growth of *Selenomonas*, *Ruminococcus*, and *Fibrobacter* strains (Callaway and Martin, 1997). The authors suggested that extract included soluble growth factors because yeast extract is a common addition to enriched media for culturing microbes. However, the extract also might have some indirect benefit to help keep ORP low. For example, cysteine is a natural O_2 quencher for batch cultures (Broudiscou et al., 2014) and compounds such as glutathione (containing cysteine) might be high in yeast extract.

Live yeast or yeast extract probably have additional roles still being worked out. Petri et al. (2020) recently suggested that yeast extract stimulated expression of transporters in the rumen epithelium that could increase rate of VFA absorption. Yeast extract has improved feeding frequency (DeVries and Chevaux, 2014; Yuan et al., 2015; Dias et al., 2018), although rumination bouts decreased in another study (Longuski et al., 2009). A similar spread in feeding frequency has been documented for monensin in dairy cattle (Mullins et al., 2012). Further research is needed to explain mechanisms and potential sources of variation, but more frequent meal consumption would help prevent pH swings and help stabilize rumen function.

Ionophores have been very well studied and are strongly recommended. Among other modes of action, they are thought to increase propionate while inhibiting lactate-producing bacteria and bacteria that enhance proteolysis and deamination (McAllister et al., 2011; Firkins and Yu, 2015). Monensin's role in feed efficiency is so well entrenched that we will spend little time discussing this additive other than to make a few points. After its legalization, monensin was noted to tilt toward the trans-10 biohydrogenation pathway in cows fed diets high in fermentable starch and unsaturated fats (particularly with free oils such as in distillers grains), but most of these types of situations have since been well worked out in the field. Increasing dosage for beef cattle (on a dose/DMI basis) relative to dairy likely decreases acetate:propionate and methanogenesis, but increasing dosage would likely equalize differences between dairy and beef cattle (Appuhamy et al., 2013). The slight decrease in DMI (part of the feed efficiency response) probably also has a role in suppression of methanogenesis (Patra et al., 2017), particularly when expressed per unit of energy-corrected milk (Hristov et al., 2013). Increasing propionate has obvious ramifications for post-absorptive function (e.g., decreasing risk for ketosis), but monensin's role might extend beyond propionate (Mullins et al., 2012).

Ionophores have many benefits related to stabilization of ruminal fermentation and to improved nitrogen and energetic efficiency (Firkins and Yu, 2015). As we explained in

that paper, microbial adaptation explains why major inhibitions of gram-positive bacteria in the landmark pure culture studies are lessened in the rumen of adapted animals. Protozoa also adapt to monensin, although their generation time might be lengthened (Ye et al., 2018). Many of the methanogens associated with protozoa adjust to alternative bacterial H2 producers if protozoa are suppressed by rumen modifiers, and protozoa certainly have benefits besides just negatives (Firkins and Yu, 2015). Hence, many suppression efforts had side effects, such as depressed DMI or NDF degradation, that should be considered along with their role to increase methanogenesis (Tapio et al., 2017). In fact, many other feed additives suffer the same variability and potential for adaption such that suppression of protozoal counts has an uncertain role in methanogenesis (Hristov et al., 2013). Thus, a greater understanding of the rumen community is needed.

Yeast products have some potential for young calves to resist inflammation-based diseases (Alugongo et al., 2017). As those authors noted, competitive exclusion of pathogens and improved gut barrier function need more research but justify further research. Although outside of the scope of this paper, if these additives can survive the acidity of the abomasum and the detergent action of bile salts, they could potentially outcompete potential pathogens in the hindgut of lactating cows. Whether the rumen or hindgut, inflammation and lost production occurs in dairy cattle fed higher grain diets (Zebeli et al., 2015).

Feeding Nitrate to Suppress Methanogenesis

Lactilytic strains of bacteria in the rumen also are likely important nitrate and/or nitrite reducers (mode 6 in Figure 1). Japanese researchers noted that the lactilytic *Selenomonas* strains represented only a small portion of the total *Selenomonas* isolates, yet the large majority of those lactilytics also could reduce nitrate and nitrite (Yoshii et al., 2003). Microbiologists studying lactate dehydrogenase by lactilytic bacteria have noted a large thermodynamic hill to climb to regenerate pyruvate from lactate by coupling NADH conversion to NAD+ (Weghoff et al., 2015). They assumed that most of the anaerobic lactate users have developed a way to break up that large hill into 2 smaller hills by using an electron carrier intermediate. Surprisingly, little research has characterized if stimulation of lactate production could increase efficiency of nitrite reduction and whether or not redox influences these interactions.

Moderate amounts of dietary nitrate could provide a useful alternate electron sink to decrease H_2 production (and therefore methane). Practical nutritionists are well aware that a buildup of nitrite can lead to methemoglobin formation by animals consuming forages recently harvested during drought conditions. In contrast, this risk for feeding nitrate is lessened by ruminal adaptation through stepping up nitrate inclusion rates. Lee and Beauchemin (2014) concluded that nitrate was likely persistent in effectiveness. Along with those authors' expectations, OSU researchers also noted that decreased DMI dampened nitrate's effectiveness to decrease methane per unit of milk (Meller et al., 2019). Beef cattle fed diets with nitrate at ad libitum naturally spread out their meals and exhibited more of a "nibbling" pattern of eating compared with those that did not have nitrate, whereas those that were restricted in intake consumed larger meals even when nitrate was added (Lee et al., 2015). Feng et al. (2020) noted that increasing DMI and use of slow-release nitrate sources enhanced the anti-methanogenic response. Because they did not directly assess if nitrate dose suppressed DMI, further work is needed to ascertain if these compounds also

help mask a potential palatability issue. If so, the final product, ammonia, could be used for bacterial synthesis in lower protein diets. There is potential for using additives to simulate lactilytic nitrate and especially nitrite reducers, especially those related to *Selenomonas*, but live yeast did not seem to enhance this response (Meller et al., 2019). *Megasphaera elsdenii* encodes the *nir*A gene, which could help prevent nitrite accumulation, but to our knowledge, no one has dosed this probiotic with animals fed nitrate. There likely are other nitrate and/or nitrite users in the rumen that have not been studied and could be stimulated by other means.

3-Nitrooxypropanol (3-NOP)

International efforts to evaluate the efficacy, persistency, and safety of 3-NOP have largely been successful (Løvendahl et al., 2018). Dijkstra et al. (2018) noted that increasing dietary NDF decreased the efficacy to suppress methanogenesis, although the product worked well in dairy studies. In contrast with 3-NOP, some other products that have a similar mode of action have had questionable persistency. Three recent studies (Melgar et al., 2020a; Melgar et al., 2020b; Melgar et al., 2021) from Penn State University documented that 3-NOP had no negative effects on palatability and resultant milk quality while supporting its efficacy to suppress methanogenesis by approximately 25% (which increased with increasing dose in one study). However, they also show divergent effects on energy-corrected milk/DMI (ECM efficiency). Results ranged from 1) significantly improved ECM efficiency to 2) no effect at all to 3) an improvement in ECM efficiency but with a numerical (not significant) decrease in body weight gain. The need to accommodate potential changes in body weight and factors such as ruminal passage rate need more attention to explain changes in feed efficiency (Løvendahl et al., 2018). As those authors explained, selecting for decreased methanogenesis might also select animals for reduced NDF degradability.

The metabolizable energy spared by decreased methanogenesis associated with nitrate was not expected to offer much improvement in ruminant animal production (Lee and Beauchemin, 2014), and these conclusions have been noted for other methane-suppressing agents (Ungerfeld, 2018). Further research is needed using direct or indirect calorimetry to better understand this facet because the typically observed increased emission of H_2 just doesn't seem enough to explain conflicting results. Some potential differences in metabolism (e.g., decreased insulin or increased mammary de novo synthesis of fatty acids) from the Penn State research need further verification for 3-NOP but could indicate nutrient partitioning toward the mammary gland. Clearly, 3-NOP is consistently effective at methane suppression and potentially improves feed efficiency, although those latter results have not been consistent.

Lactate-Producing Probiotics

If we borrow from the concept that lactate is only bad if it accumulates, there is some potential to gain a benefit by administering lactate-producing probiotics (McAllister et al., 2011). Those authors explained that there are strain variations likely behind the variable responses in the published literature. There is some potential for lactate producers to help maintain a healthy hindgut by inhibiting potential pathogens, such as *E. coli* 0157 (Doyle et al., 2019). Those authors also discussed a potential role for probiotic lactic acid bacteria to decrease methanogenesis. The mechanistic evidence seems sound, although there is the caveat that these probiotics probably should only be considered when there is not a SARA risk. A lactate-producing probiotic is probably more effective to suppress methanogenesis when

combined with a lactilytic probiotic (Philippeau et al., 2017) as has been noted for increased populations of both commensal lactate producers and consumers in naturally low methane emitters (Ungerfeld, 2020). Ferraretto and Shaver (2015) noted no major differences in lactation performance when a probiotic combining a lactate producer and consumer was added to the TMR. They discussed some studies in which ECM efficiency was improved. However, they speculated that high starch availability might have attenuated their study's potential response. More research is needed to explain these sources of variation among studies, including when methanogenesis results are different than expectations (Jeyanathan et al., 2019).

Branched-Chain Volatile Fatty Acids

We have known since the 1950's that some prominent cellulolytic bacteria in the rumen require branched chain volatile fatty acids (**BCVFA**), which are produced only when microbes remove the amino group and the carboxyl group from the branched chain amino acids in RDP. The BCVFA are well known for producing branched chain amino and fatty acids needed by cellulolytic bacteria (top left of Figure 1). Numerous papers were published from research supporting an 'isoacid' product developed for dairy cattle in the 1980's (Andries et al., 1987). Those authors noted 4 to 8% increase in milk production. However, that product went down the same path as did film-based cameras. With increasing milk yield and increasing pressure to limit N excretion in waste by decreasing RDP in today's climate, these isoacids are being investigated with a fresh light.

Ohio State researchers have several projects that have been published in full or preliminary form. We have noted 3 to 5% unit increases in NDF digestibility in batch cultures (Roman-Garcia et al., 2021) and in two continuous culture studies (Roman-Garcia et al., unpublished data; Mitchell et al., unpublished data; both reported as abstracts at ADSA in 2019 and 2020). Microbial N flow was not affected in one of those, but it increased by 7% in another. RDP was moderate, but urea was supplied in the buffer in those studies. When RDP apparently was not limiting in lactating dairy cows, we did not see a benefit in NDF digestibility (Copelin et al., 2021), but the isoacids helped prevent milkfat depression apparently by stimulating milk fatty acid synthesis in the mammary gland (Lee et al., 2021). Thus, there could be both a ruminal and a post-ruminal benefit to isoacids. Supplemental valine (degraded to isobutyrate) stimulated milk production (Hultquist and Casper, 2016), potentially through a post-ruminal response (i.e., thyroxine concentration increased). Supplementing all 3 BCVFA increased DMI and production of milk components, potentially mediated by an increase in growth hormone (Wang et al., 2019).

In another OSU study (Mitchell et al., unpublished data; reported at 2020 ADSA), we noted that feed efficiency improved by over 5% when isobutyrate and 2-methylbutyrate were supplemented to Jersey cows. Most of our work suggests that there is some substitution effect among the BCVFA, but only isobutyrate and 2-methylbutyrate are likely to be needed. This situation would be especially likely with high corn silage and corn grain in diets because of corn protein's higher leucine, which degrades to isovalerate. As with some of the other additives reported herein, this improved feed efficiency was partially driven by a modest decrease in DMI without any decrease in milk energy output and with no changes in rate of body weight gain. We expect that cows with potential to improve DMI would receive a benefit from BCVFA if RDP is modest, whereas those later in lactation that are not limited by DMI would harvest the improved NDF digestibility to support milk production and therefore need less DMI.

Plant Components and Extracts

Many studies have explored various plant components and extracts that inhibit 1) methanogens to suppress enteric methane production and 2) protozoa and the bacteria that rely on amino acids as energy sources ("hyper-ammonia producers") to improve efficiency of protein usage in the rumen (Cobellis et al., 2016; Patra et al., 2017; Hartinger et al., 2018). Much of the efficacy is projected from interactions with susceptible microbes' cell walls (Benchaar and Greathead, 2011). As with some microbes adapting to monensin by changing the architecture of their cell wall, microbes appear to adapt to many of these plant bioactive compounds. Current research in our lab is suggesting that bacteria adapt to environmental stressors against their cell wall by modifying their fatty acid profile in phospholipids and potentially in plasmalogen fatty aldehydes. The BCVFA have long been known to support the integrity of fibrolytic bacterial cell walls (Andries et al., 1987), so perhaps isoacids should be studied in combination with some of these plant bioactive compounds to help decrease unintended depressions in NDF degradation. More longer term in vivo studies are needed for plant bioactives (Beauchemin et al., 2020).

Each class of compound has various chemicals involved in their modes of action and have been discussed in the literature. Tannins have had positive and negative results to inhibit undesirable bacteria and protozoa. However, those microbes also probably can adapt by modifying their cell wall. Tannins also can reduce protein degradability in the rumen but also the intestinal digestibility of RUP (Hartinger et al., 2018). Those authors also reviewed saponins, which also had variable responses (e.g., to inhibit protozoa) in the literature, including that they also sometimes have decreased DMI. Essential oils have received considerable study (Cobellis et al., 2016) and offer some potential in dairy nutrition. Ye et al. (2018) hypothesized that combining monensin and an essential oil would inhibit protozoa and suppress methane in continuous culture. There were apparent benefits in protozoal suppression, but the chemicals themselves limited typical methodologies from further explaining results.

Combinations of plant bioactives might limit the general inhibition by each individual compound while gaining more consistency and efficacy (Beauchemin et al., 2020). Belanche et al. (2020) reported that a combination of compounds in one product documented persistency and, in fact, a greater response with increasing time. Feed efficiency was improved by about 4%, and methane production per unit of energy- and protein-corrected milk was decreased by about 10%. This is important because some compounds might oxidize or dissipate (evaporate) over time. Using a baseball analogy, rumen-active compounds have been approached too many times as swinging for home runs that sometimes lead to strikeouts in studies in which large doses of single compounds were fed. In contrast, swinging for successive singles (smaller doses of multiple compounds) also scores runs while minimizing strikeouts that could otherwise result from perturbing the complicated microbial web in the rumen. Hence, more research is needed to evaluate combinations of additives.

Conclusions

Rumen modifiers should not be used to substitute for good feeding management. They can reduce variability among animals to improve feed efficiency, lessen risk for lowering dietary protein, and decrease rumen methane production with less risk for decreased milk production. Monensin is well known to increase feed efficiency. However, monensin might have

more beneficial responses than just increasing propionate, so it should have further study when combined with other rumen modifiers. There is some evidence that compounds that increase ruminal propionate might enhance feed efficiency as does monensin. When using rumen bioactives, we need to understand their modes of action to better justify their usage but also to understand what variables can limit their benefits. We have not tried to cover all modifiers in the field, but we hope that our efforts to explain mechanisms in the rumen will help nutrition advisors gain better information needed for today's high producing dairy cows.

References

Adesogan, A.T., K.G. Arriola, Y. Jiang, A. Oyebade, E.M. Paula, A.A. Pech-Cervantes, J.J. Romero, L.F. Ferraretto, and D. Vyas. 2019. Symposium review: Technologies for improving fiber utilization. J. Dairy Sci. 102:5726-5755.

Aikman, P.C., P.H. Henning, D.J. Humphries, and C.H. Horn. 2011. Rumen pH and fermentation characteristics in dairy cows supplemented with Megasphaera elsdenii NCIMB 41125 in early lactation. J. Dairy Sci 94:2840-2849.

Alugongo, G.M., J. Xiao, Z. Wu, S. Li, Y. Wang, and Z. Cao. 2017. Review: Utilization of yeast of Saccharomyces cerevisiae origin in artificially raised calves. J. Anim. Sci. Biotechnol. 8:34.

Andries, J.I., F.X. Buysse, D.L. de Brabander, and B.G. Cottyn. 1987. Isoacids in ruminant nutrition: Their role in ruminal and intermediary metabolism and possible influences on performances--A review. Anim. Feed Sci. Technol. 18:169-180.

Appuhamy, J.A. D.R.N., A.B. Strathe, S. Jayasundara, C. Wagner-Riddle, J. Dijkstra, J. France, and E. Kebreab. 2013. Anti-methanogenic effects of monensin in dairy and beef cattle: A meta-analysis. J. Dairy Sci. 96:5161-5173.

Asanuma, N. and T. Hino. 2000. Activity and properties of fumarate reductase in ruminal bacteria. J. Gen. Appl. Microbiol. 46:119-125.

Aschenbach, J.R., Q. Zebeli, A.K. Patra, G. Greco, S. Amasheh, and G.B. Penner. 2019. Symposium review: The importance of the ruminal epithelial barrier for a healthy and productive cow. J. Dairy Sci. 102:1866-1882.

Beauchemin, K.A., E.M. Ungerfeld, R.J. Eckard, and M. Wang. 2020. Review: Fifty years of research on rumen methanogenesis: Lessons learned and future challenges for mitigation. Animal 14:S2-S16.

Belanche, A., C.J. Newbold, D.P. Morgavi, A. Bach, B. Zweifel, and D.R. Yáñez-Ruiz. 2020. A meta-analysis describing the effects of the essential oils blend agolin ruminant on performance, rumen fermentation and methane emissions in dairy cows. Animals 10:620.

Benchaar, C. and H. Greathead. 2011. Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Anim. Feed Sci. Technol. 166:338-355.

Bickhart, D.M. and P.J. Weimer. 2018. Symposium review: Host–rumen microbe interactions may be leveraged to improve the productivity of dairy cows. J. Dairy Sci. 101:7680-7689.

Broudiscou, L.P., A. Offner, and D. Sauvant. 2014. Effects of inoculum source, pH, redox potential and headspace di-hydrogen on rumen in vitro fermentation yields. Animal 8:931-937.

Callaway, E.S. and S.A. Martin. 1997. Effects of a Saccharomyces cerevisiae culture on ruminal bacteria that utilize lactate and digest cellulose. J. Dairy Sci. 80:2035-2044.

Caputo Oliveira, R., S.J. Erb, R.S. Pralle, H.T. Holdorf, C.R. Seely, and H.M. White. 2020. Postpartum supplementation with fermented ammoniated condensed whey altered nutrient partitioning to support hepatic metabolism. J. Dairy Sci. 103:7055-7067.

Caputo Oliveira, R., K.J. Sailer, H.T. Holdorf, C.R. Seely, R.S. Pralle, M.B. Hall, N.M. Bello, and H.M. White. 2019. Postpartum supplementation of fermented ammoniated condensed whey improved feed efficiency and plasma metabolite profile. J. Dairy Sci. 102:2283-2297.

Cobellis, G., M. Trabalza-Marinucci, and Z. Yu. 2016. Critical evaluation of essential oils as rumen modifiers in ruminant nutrition: A review. Sci. Total Environ. 545-546:556-568.

Copelin, J.E., J.L. Firkins, M.T. Socha, and C. Lee. 2021. Effects of diet fermentability and supplementation of 2-hydroxy-4-(methylthio)-butanoic acid and isoacids on milk fat depression: 1. Production, milk fatty acid profile, and nutrient digestibility. J. Dairy Sci. 104:1591-1603.

Dai, X., T.J. Hackmann, R.R. Lobo, and A.P. Faciola. 2020. Lipopolysaccharide stimulates the growth of bacteria that contribute to ruminal acidosis. Appl. Environ. Microbiol. 86:e02193-02119.

Denman, S.E., F.G. Martinez, T. Shinkai, M. Mitsumori, and C.S. McSweeney. 2015. Metagenomic analysis of the rumen microbial community following inhibition of methane formation by a halogenated methane analog. Front. Microbiol. 6:1087.

DeVries, T.J. and E. Chevaux. 2014. Modification of the feeding behavior of dairy cows through live yeast supplementation. J. Dairy Sci. 97:6499-6510.

Dewanckele, L., P.G. Toral, B. Vlaeminck, and V. Fievez. 2020. Invited review: Role of rumen biohydrogenation intermediates and rumen microbes in diet-induced milk fat depression: An update. J. Dairy Sci. 103:7655-7681.

Dias, A.L. G., J.A. Freitas, B. Micai, R.A. Azevedo, L.F. Greco, and J.E.P. Santos. 2018. Effects of supplementing yeast culture to diets differing in starch content on performance and feeding behavior of dairy cows. J. Dairy Sci. 101:186-200.

Dijkstra, J., A. Bannink, J. France, E. Kebreab, and S. van Gastelen. 2018. Short communication: Antimethanogenic effects of 3-nitrooxypropanol depend on supplementation dose, dietary fiber content, and cattle type. J. Dairy Sci. 101:9041-9047.

Dijkstra, J., S. Van Gastelen, K. Dieho, K. Nichols, and A. Bannink. 2020. Review: Rumen sensors: Data and interpretation for key rumen metabolic processes. Animal 14:S176-S186.

Doyle, N., P. Mbandlwa, W.J. Kelly, G. Attwood, Y. Li, R.P. Ross, C. Stanton, and S. Leahy. 2019. Use of lactic acid bacteria to reduce methane production in ruminants, A critical review. Front. Microbiol. 10:2207.

Feng, X.Y., J. Dijkstra, A. Bannink, S. van Gastelen, J. France, and E. Kebreab. 2020. Antimethanogenic effects of nitrate supplementation in cattle: A meta-analysis. J. Dairy Sci. 103:11375-11385.

Ferraretto, L.F. and R.D. Shaver. 2015. Effect of direct-fed microbial supplementation on lactation performance and total-tract starch digestibility by midlactation dairy cows. Prof. Anim. Sci. 31:63-67.

Firkins, J.L. and Z. Yu. 2015. How to use data on the rumen microbiome to improve our understanding of ruminant nutrition. J. Anim. Sci. 93:1450–1470.

Friedman, N., E. Shriker, B. Gold, T. Durman, R. Zarecki, E. Ruppin, and I. Mizrahi. 2017. Diet-induced changes of redox potential underlie compositional shifts in the rumen archaeal community. Environ. Microbiol. 19:174-184.

Hackmann, T.J., D.K. Ngugi, J.L. Firkins, and J. Tao. 2017. Genomes of rumen bacteria encode atypical pathways for fermenting hexoses to short-chain fatty acids. Environ. Microbiol. 19:4670-4683.

Hartinger, T., N. Gresner, and K.-H. Südekum. 2018. Does intra-ruminal nitrogen recycling waste valuable resources? A review of major players and their manipulation. J. Anim. Sci. Biotechnol. 9:33.

Hristov, A.N., J. Oh, J.L. Firkins, J. Dijkstra, E. Kebreab, and G. Waghorn. 2013. Special topics—mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91:5045-5069.

Huang, Y., J.P. Marden, C. Julien, and C. Bayourthe. 2018. Redox potential: An intrinsic parameter of the rumen environment. J. Anim. Physiol. Anim. Nutr. 102:393-402.

Hultquist, K.M. and D.P. Casper. 2016. Effects of feeding rumen-degradable valine on milk production in late-lactating dairy cows. J. Dairy Sci. 99:1201-1215.

Jeyanathan, J., C. Martin, M. Eugène, A. Ferlay, M. Popova, and D.P. Morgavi. 2019. Bacterial direct-fed microbials fail to reduce methane emissions in primiparous lactating dairy cows. J. Anim. Sci. Biotechnol. 10:41.

Jouany, J.P. 2006. Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. Anim. Reprod. Sci. 96:250-264.

Kamke, J., S. Kittelmann, P. Soni, Y. Li, M. Tavendale, S. Ganesh, P.H. Janssen, W. Shi, J. Froula, E.M. Rubin, and G.T. Attwood. 2016. Rumen metagenome and metatranscriptome analyses of low methane yield sheep reveals a Sharpea-enriched microbiome characterised by lactic acid formation and utilisation. Microbiome 4:56.

Lee, C., R.C. Araujo, K.M. Koenig, and K.A. Beauchemin. 2015. Effects of feed consumption rate of beef cattle offered a diet supplemented with nitrate ad libitum or restrictively on potential toxicity of nitrate. J. Anim. Sci. 93:4956-4966.

Lee, C. and K.A. Beauchemin. 2014. A review of feeding supplementary nitrate to ruminant animals: Nitrate toxicity, methane emissions, and production performance. Can. J. Anim. Sci. 94:557-570.

Lee, C., J.E. Copelin, T. Park, K.E. Mitchell, J.L. Firkins, M.T. Socha, and D. Luchini. 2021. Effects of diet fermentability and supplementation of 2-hydroxy-4-(methylthio)-butanoic acid and isoacids on milk fat depression: 2. Ruminal fermentation, fatty acid, and bacterial community structure. J. Dairy Sci. 104:1604-1619.

Longuski, R.A., Y. Ying, and M.S. Allen. 2009. Yeast culture supplementation prevented milk fat depression by a short-term dietary challenge with fermentable starch. J. Dairy Sci. 92:160-167.

Løvendahl, P., G.F. Difford, B. Li, M.G.G. Chagunda, P. Huhtanen, M.H. Lidauer, J. Lassen, and P. Lund. 2018. Review: Selecting for improved feed efficiency and reduced methane emissions in dairy cattle. Animal 12:S336-S349.

Mann, E., S.U. Wetzels, M. Wagner, Q. Zebeli, and S. Schmitz-Esser. 2018. Metatranscriptome sequencing reveals insights into the gene expression and functional potential of rumen wall bacteria. Front. Microbiol. 9:43.

Marden, J.P., C. Julien, V. Monteils, E. Auclair, R. Moncoulon, and C. Bayourthe. 2008. How does live yeast differ from sodium bicarbonate to stabilize ruminal pH in high-yielding dairy cows? J. Dairy Sci. 91:3528-3535.

Martin, S.A. 1998. Manipulation of ruminal fermentation with organic acids: A review. J. Anim. Sci. 76:3123-3132.

McAllister, T.A., K.A. Beauchemin, A.Y. Alazzeh, J. Baah, R.M. Teather, and K. Stanford. 2011. Review: The use of direct fed microbials to mitigate pathogens and enhance production in cattle. Can. J. Anim. Sci. 91:193-211.

Melgar, A., M.T. Harper, J. Oh, F. Giallongo, M. E. Young, T.L. Ott, S. Duval, and A.N. Hristov. 2020a. Effects of 3-nitrooxypropanol on rumen fermentation, lactational performance, and resumption of ovarian cyclicity in dairy cows. J. Dairy Sci. 103:410-432.

Melgar, A., C.F.A. Lage, K. Nedelkov, S.E. Räisänen, H. Stefenoni, M.E. Fetter, X. Chen, J. Oh, S. Duval, M. Kindermann, N.D. Walker, and A.N. Hristov. 2021. Enteric methane emission, milk production, and composition of dairy cows fed 3-nitrooxypropanol. J. Dairy Sci. 104:357-366.

Melgar, A., K.C. Welter, K. Nedelkov, C.M.M.R. Martins, M.T. Harper, J. Oh, S.E. Räisänen, X. Chen, S.F. Cueva, S. Duval, and A.N. Hristov. 2020b. Dose-response effect of 3-nitrooxypropanol on enteric methane emissions in dairy cows. J. Dairy Sci. 103:6145-6156.

Meller, R.A., B.A. Wenner, J. Ashworth, A.M. Gehman, J. Lakritz, and J.L. Firkins. 2019. Potential roles of nitrate and live yeast culture in suppressing methane emission and influencing ruminal fermentation, digestibility, and milk production in lactating Jersey cows. J. Dairy Sci. 102:6144-6156.

Mullins, C.R., L.K. Mamedova, M.J. Brouk, C.E. Moore, H.B. Green, K.L. Perfield, J.F. Smith, J.P. Harner, and B.J. Bradford. 2012. Effects of monensin on metabolic parameters, feeding behavior, and productivity of transition dairy cows. J. Dairy Sci. 95:1323-1336.

Newbold, C.J., R.J. Wallace, and F.M. McIntosh. 1996. Mode of action of the yeast Saccharomyces cerevisiae as a feed additive for ruminants. Brit. J. Nutr. 76:249-261.

Patra, A., T. Park, M. Kim, and Z. Yu. 2017. Rumen methanogens and mitigation of methane emission by anti-methanogenic compounds and substances. J. Anim. Sci. Biotechnol. 8:13.

Petri, R.M., V. Neubauer, E. Humer, I. Kröger, N. Reisinger, and Q. Zebeli. 2020. Feed additives differentially impact the epimural microbiota and host epithelial gene expression of the bovine rumen fed diets rich in concentrates. Front. Microbiol. 11:119.

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., N. McEwan, J.-P. Marden, C. Bayourthe, E. Auclair, and C.J. Newbold. 2013. The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle. PLoS ONE 8:e67824.

Roman-Garcia, Y., B.L. Denton, K.E. Mitchell, C. Lee, M.T. Socha, and J.L. Firkins. 2021. Conditions stimulating NDF degradation by dosing branched chain volatile fatty acids. I: Comparison with branched chain amino acids and forage source in ruminal batch cultures. J. Dairy Sci. (In Press).

Rossi, F., P. Cocconcelli, and F. Masoero. 1995. Effect of a Saccharomyces cerevisiae culture on growth and lactate utilization by the ruminal bacterium Megasphaera elsdenii. Annales de Zootechnie 44:403-409.

Sawanon, S., S. Koike, and Y. Kobayashi. 2011. Evidence for the possible involvement of Selenemonas ruminantium in rumen fiber digestion. FEMS Microbiol. Lett. 325:170-179.

Stevens, K.D., M.L. Eastridge, K.M. Daniels, S.K. Finney, S.N. LeShure, and J.L. Firkins. 2017. Effect of oral administration of Megasphaera elsdenii on performance of Holstein cows during early lactation. Prof. Anim. Sci. 33:120-126.

Tapio, I., T.J. Snelling, F. Strozzi, and R. J. Wallace. 2017. The ruminal microbiome associated with methane emissions from ruminant livestock. J. Anim. Sci. Biotechnol. 8:7.

Ungerfeld, E.M. 2018. Inhibition of rumen methanogenesis and ruminant productivity: A meta-analysis. Front. Vet. Sci. 5:113.

Ungerfeld, E.M. 2020. Metabolic hydrogen flows in rumen fermentation: Principles and possibilities of interventions. Front. Microbiol. 11:589.

Wagner, B.K., B.A. Wenner, J.E. Plank, G.D. Poppy, and J.L. Firkins. 2018. Investigation of ammonium lactate supplementation on fermentation end products and bacterial assimilation of nitrogen in dual-flow continuous culture. J. Dairy Sci. 101:8032-8045.

Wang, C., Q. Liu, G. Guo, W.J. Huo, Y.L. Zhang, C.X. Pei, and S.L. Zhang. 2019. Effects of rumen-protected folic acid and branched-chain volatile fatty acids supplementation on lactation performance, ruminal fermentation, nutrient digestion and blood metabolites in dairy cows. Anim. Feed Sci. Technol. 247:157-165.

Weghoff, M.C., J. Bertsch, and V. Müller. 2015. A novel mode of lactate metabolism in strictly anaerobic bacteria. Environ. Microbiol. 17:670-677.

Weimer, P.J. and G. N. Moen. 2013. Quantitative analysis of growth and volatile fatty acid production by the anaerobic ruminal bacterium Megasphaera elsdenii T81. Appl. Microbiol. Biotechnol. 97:4075-4081.

Weiss, W., D.J. Wyatt, and T.R. McKelvey. 2008. Effect of feeding Propionibacteria on milk production by early lactation dairy cows. J. Dairy Sci. 91:646-652.

Ye, D. and M.L. Eastridge. 2018. Oral administration of Megasphaera elsdenii to Jersey cows during early lactation. Prof. Anim. Sci. 34:67-74.

Ye, D., S.K.R. Karnati, B. Wagner, J.L. Firkins, M.L. Eastridge, and J.M. Aldrich. 2018. Essential oil and monensin affect ruminal fermentation and the protozoal population in continuous culture. J. Dairy Sci. 101:5069-5081.

Yoshii, T., N. Asanuma, and T. Hino. 2003. Number of nitrate- and nitrite-reducing Selenomonas ruminantium in the rumen, and possible factors affecting its growth. Anim. Sci. J. 74:483-491.

Yuan, K., T. Liang, M.B. Muckey, L.G.D. Mendonça, L.E. Hulbert, C.C. Elrod, and B.J. Bradford. 2015. Yeast product supplementation modulated feeding behavior and metabolism in transition dairy cows. J. Dairy Sci. 98:532-540.

Zebeli, Q., K. Ghareeb, E. Humer, B.U. Metzler-Zebeli, and U. Besenfelder. 2015. Nutrition, rumen health and inflammation in the transition period and their role on overall health and fertility in dairy cows. Res. Vet. Sci. 103:126-136.

Zebeli, Q., B.U. Metzler-Zebeli, and B.N. Ametaj. 2012a. Meta-analysis reveals threshold level of rapidly fermentable dietary concentrate that triggers systemic inflammation in cattle. J. Dairy Sci. 95:2662-2672.

Zebeli, Q., S.J. Terrill, A. Mazzolari, S.M. Dunn, W.Z. Yang, and B.N. Ametaj. 2012b. Intraruminal administration of Megasphaera elsdenii modulated rumen fermentation profile in mid-lactating dairy cows. J. Dairy Res. 79:16-25.

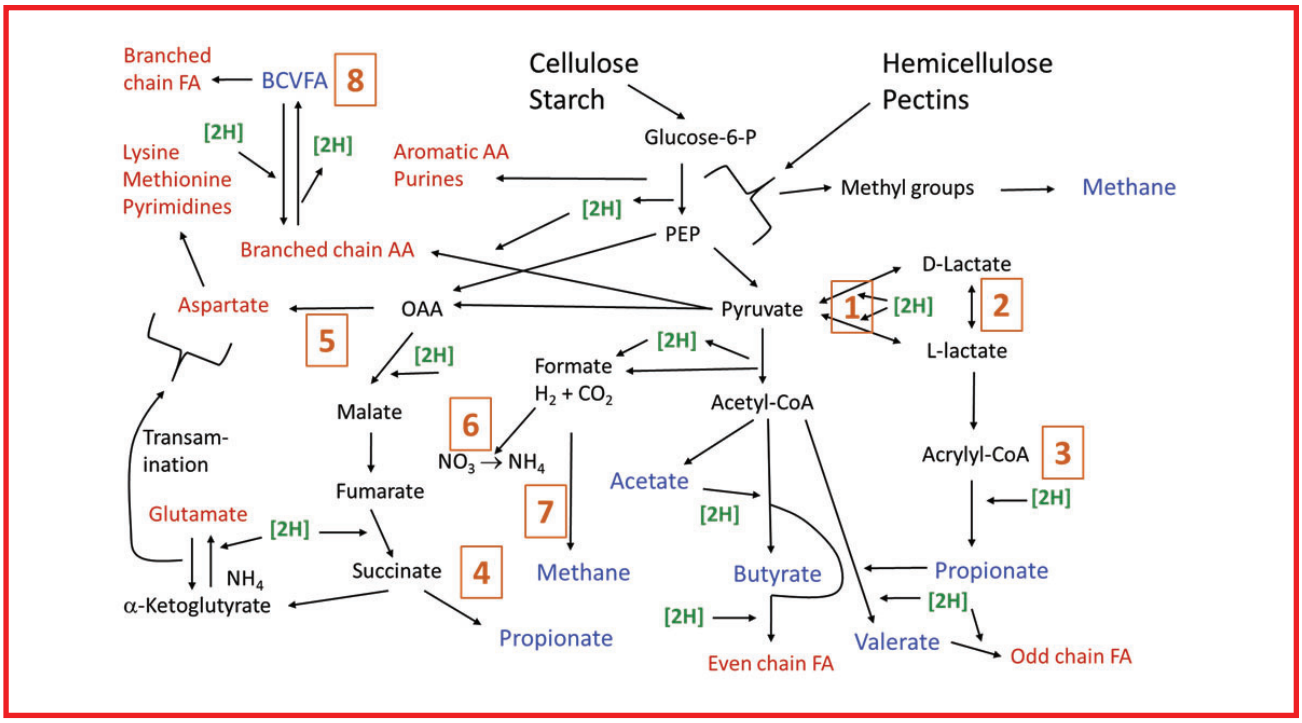

Figure 1. Schematic flow of degraded carbohydrates toward fermentation (blue) or selected amino acids and fatty acids (red) when making or producing metabolic hydrogen ([2H]). Boxed numbers represent modes of action discussed in this paper. 1 = lactate production and utilization, 2 = lactate racemization, 3 = propionate production via the acrylate pathway (*Megasphaera*), 4 = propionate production via the succinate pathway (*Selenomonas*), 5 = oxaloacetate (OAA) used for generation of the aspartate family of amino acids and pyrimidines, 6 = nitrate reduction to ammonia using hydrogen (H_2) dissolved in liquid, 7 = methane production inhibited by 3-nitrooxypropanol (3-NOP), and 8 = provision of branched chain volatile fatty acids (BCVFA) to synthesize branched chain amino acids or fatty acids (FA).

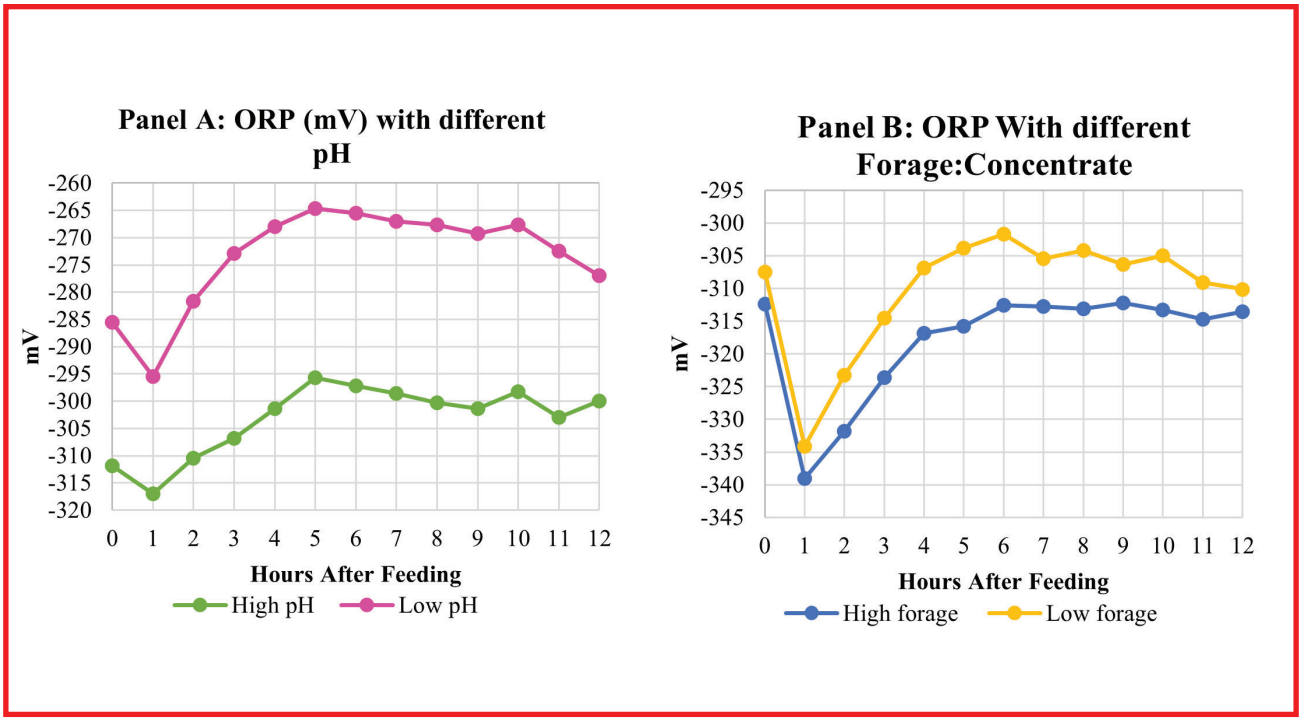

Figure 2. The effects of pH and forage:concentrate, independent from each other, on the oxidation-reduction potential (ORP) in dual flow continuous cultures. In Panel A, a 50:50 forage:concentrate was fed, but buffers were adjusted for pH to range from 6.3 to 6.8 (High pH) vs 5.7 to 6.2 (Low pH). In Panel B, forage:concentrate ratio ranged from 67:33 (High Forage) or 33:67 (Low Forage), but buffers were adjusted to have very similar pH in the cultures.