Maximizing Microbial Protein with Branched-Chain Volatile Fatty Acids

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Introduction

In recent years, margins across the dairy industry have evolved to a point where they are routinely tight and oftentimes negative. For example, since 2000 income over feed costs has averaged only $5.26/cwt and farm profitability was actually -$3.04/cwt when accounting for all costs (Brouk, 2020). Feed costs comprise approximately 60% of the operating costs (Brouk, 2020) so it is no surprise that dairy producers and nutritionists focus on ways to control this major input cost while striving to maintain production when margins are unfavorable.

Nitrogen conversion to milk protein is extremely inefficient in dairy cattle. Huhtanen and Hristov (2009) found milk N efficiency averages only 24.7% (range = 14 to 41.1%) across 1,737 research diets from North America and Northern Europe. This is worth noting because protein supplementation also tends to be the most expensive component in a typical dairy ration. Furthermore, animal performance may be impacted as energy is required to convert excess ammonia to urea in the liver (12 kcal/g of N; Tyrrell et al., 1970).

The dairy industry has been under increasing pressure to reduce N excretion in both urine and feces as they are potential pollutants for water sources and air (gaseous N emissions). Urinary N excretion is of particular concern (Hristov et al., 2019). Localized regulations, particularly near watersheds and urban populations, will continue to become more restrictive and will have an impact on nutritional practices on dairies. Huhtanen and Hristov (2009) concluded that reducing dietary protein was the most efficient tool to reduce N losses from dairy operations.

Rumen degradable protein (RDP) typically comprises 60 to 67% of dietary protein and is converted to microbial protein (MicP), a highly digestible protein with an amino acid (AA) profile that closely matches the needs for milk protein synthesis. Unfortunately, MicP typically only provides approximately 50% of the metabolizable protein (MP) supplied to the dairy cow so part of the strategy to reduce protein supplementation is to maximize MicP yield and efficiency. Mixed rumen microbes have a growth efficiency of only 1/3 to 2/3 of their theoretical maximum (Hackmann and Firkins, 2015) so there are opportunities to improve this efficiency with the right nutritional strategies.

Why Focus on Microbial Protein?

Even though the rumen environment has some inefficiencies, ruminants do have some advantages over monogastric animals when microbial growth is maximized. Most notable, ruminants can utilize forages as an energy source via microbial fermentation of carbohydrates and conversion to volatile fatty acids (VFAs; acetate, propionate, and butyrate). These VFAs are used for energy by the animal. Second, the rumen microbes can detoxify mycotoxins and lessen their effect on the animal (Upadhaya, et al., 2010). Next, rumen microbes increase P availability due to endogenous production of phytase (Yanke et al., 1998). The rumen microbes are also major contributors to b-vitamins supply (Schwab et al., 2006), reducing the need to supplement relative to monogastric animals. Lastly, microbes can convert lower cost non-protein nitrogen (NPN) sources, like urea, to a superior source of MP thereby reducing protein supplementation costs.

Maximizing production in dairy cattle requires one to supply the correct array of AA via MP. For example, a 1500 lb Holstein cow producing 95 lbs of milk (3.8% fat and 3.2% true protein) in her second lactation requires over 3000 grams (6.6 lbs) of MP per day to sustain this level of production (Van Amburgh et al., 2015). In order to supply this copious amount of MP, one needs to prioritize maximizing MicP yield in the rumen and then correct the remaining MP deficit with ruminally-undegradable protein (RUP).

It should be noted that meeting the MP needs of the cow does not necessarily mean that her AA needs are being met. The true MP requirement of the animal is dependent upon the AA profile. The amount of protein fed in the diet can be lowered if the MP has an AA profile that is more reflective of the animal's AA requirements. As noted previously, MicP is a highly digestible protein source that has the most favorable AA profile for meeting the needs for milk protein synthesis relative to commonly used protein sources (Schingoethe, 1996). Thus, neglecting the RDP needs of cattle in favor of over-supplementing RUP may increase the amount of dietary protein that needs to be fed, especially if the RUP source is of poor or variable quality. Santos et al. (1998) summarized 88 lactation trials with 127 direct comparisons in which soybean meal, was replaced with protein sources containing high concentrations of RUP. In that review, only 17% of the comparisons showed that added RUP increased milk yield. This finding was attributed to: 1) poor essential AA profile of the RUP source, 2) low digestibility of the RUP source in the small intestine, 3) control diets may have had sufficient RUP, and 4) a decrease in rumen MicP synthesis.

Santos et al. (1998) noted that in 29 comparisons from 15 metabolism trials, in which soybean meal was replaced by a RUP supplement, MicP synthesis decreased in 76% of the comparisons. This finding shows that supplementing RUP at the expense RDP is a further hindrance to meeting the MP needs of dairy cattle. Furthermore, several studies have demonstrated that commonly used RUP sources, including blood meal (Paz et al., 2014), distillers grains

(Kleinschmit et al., 2006; Li et al., 2012), meat and bone meal and corn gluten meal (Maiga et al., 1996) can be variable in RUP content and digestibility. In addition to having a superior AA profile, rumen microbes have been estimated to be comprised of 82.4% true protein (Sok et al., 2017) and to be 90% digestible (Sniffen et al., 1992).

Supplementing dairy diets with rumen-protected AA (RPAA) has become a widely accepted practice as nutritional models have become more accurate in predicting dietary AA requirements and supply. Balancing with RPAA have allowed nutritionists to lower CP content of diets (<16.5%) thus lowering N excretion and improving N efficiency (Broderick et al., 2008; Broderick et al., 2009). The quality of many of these products are quite good and more predictable in nutrient content relative to other RUP sources. However, these products are costly, and are often removed from lactating diets when the price for milk protein drops. Improving microbial yield and efficiency can reduce, though not necessarily eliminate, one's reliance on RUP and RPAA sources.

Branched-Chain Volatile Fatty Acids (BCVFA)
Cellulolytic bacteria (fiber digesters; Fibrobacter succinogenes, Ruminococcus albus, and Ruminococcus flavefaciens) require BCVFA (isovalerate, isobutyrate, and 2-methylbutyrate; Figure 1), but these VFAs are often limiting in the rumen. BCVFA are derived from branched-chain amino acids (BCAA; valine, leucine, and isoleucine) within the RDP fraction that have been decarboxylated and deaminated in the rumen. The first function of BCVFA in cellulolytic bacteria is the formation of MicP. Cellulolytic bacteria require BCVFA and ammonia for de novo synthesis of BCAA. In vitro research has shown that supplementing BCVFA increased ruminal MicP synthesis (Cummins and Papas, 1985; Russell and Sniffen, 1984). Gorosito et al. (1985) showed that supplementing wheat straw cell wall with individual or a combination of BCVFAs in a batch culture system improved cell wall digestion and lowered ammonia -N concentration, which indicated improved N uptake by rumen microbes. More recently, Roman-Garcia et al. (2019b) found, within an in vitro batch culture system, that rumen bacteria preferentially utilized BCVFA compared to BCAA and observed a corresponding reduction in ammonia-N concentrations and improved NDF digestibility.

BCVFA are also required by cellulolytic bacteria to synthesize branched-chain long chain fatty acids (BLFA). BLFA and odd-chain fatty acids provide fluidity (Figure 2) to the lipid membrane of the bacterial cell for adaption to environmental changes (Russell, 2002). Roman-Garcia et al. (2019d) demonstrated that a higher rumen pH (6.3 to 6.8 vs. 5.7 to 6.2) increased the requirement for 2-methylbutyrate. Furthermore, Roman-Garcia et al. (2019c) showed that microbes preferentially utilized BCVFA vs BCAA for fatty acid elongation.

Dietary factors affect the need for supplemental BCVFA. Cellulolytic bacteria have a disadvantage to amylolytic microbes (starch and sugar digesters) because amylolytic bacteria

have a faster rate of growth and preferentially utilize BCVFA over other de novo methods for BCAA synthesis (Allison et al., 1984). As a result, a diet containing high levels of rumen fermentable carbohydrates are often limiting in BCVFA for cellulolytic bacteria. DeFrain et al. (2004) observed a reduction in ruminal molar proportions of BCVFA as increasing levels of lactose was added to lactating dairy diets. Similarly, Oba and Allen (2003) found that feeding a high starch diet (31.6%) versus a low starch diet (21.2%) lowered ruminal concentrations of isovalerate and isobutyrate.

Scenarios that increase ruminal passage rate may reflect a need for BCVFA supplementation. It is not uncommon to observe lactating dairy cows consume >60 lbs of dry matter each day. Increased dry matter intake corresponds with greater ruminal passage rate of feed, which lowers potential digestibility of RDP and availability of BCAA to be converted to BCVFA. Roman-Garcia et al. (2019d) showed a greater need for 2-methylbutyrate to support NDF digestibility when passage rate in a continuous culture system was increased from 2.5 to 5%/h.

Diets with adequate to excessive RDP may not limit the supply of BCVFA. Mir et al. (1986) evaluated the effect of each individual BCAA, the corresponding BCVFA + urea, combination of all three BCAA, or all three BCVFA + urea on the DM digestibility of barley straw or alfalfa hay with in vitro batch culture. Interestingly, DM digestibility of alfalfa hay was not improved with any of the treatments. In contrast, all treatments, with the exceptions of valine and leucine, improved the DM digestibility of barley straw. The authors concluded that the lack of response in alfalfa hay was due to this substrate providing adequate RDP (BCAA) to rumen microbes. More recently, Roman-Garcia (2019a) demonstrated that supplementing various combinations of BCVFA did not improve in vitro NDF digestibility when the substrate contained ground corn and alfa lfa hay (high protein). However, NDF digestibility was improved when these same treatments were applied to ground corn and orchard grass (low protein).

Supplementation of Branched-Chain Volatile Fatty Acids
BCVFA have been shown to increase MicP synthesis (Cummins and Papas, 1985; Russell and Sniffen, 1984) and reduce rumen ammonia -N concentrations (Gorosito et al., 1985; Roman-Garcia et al., 2019b). Thus, supplementing BCVFA in conjunction with an NPN source, like urea, may potentially replace a portion of the supplemental protein (soybean meal, canola meal, etc.), reducing feed costs. This strategy also shows promise at lowering dietary CP levels and improving N efficiency. Felix et al. (1980) observed a reduction in milk yield when urea replaced soybean meal in a lactating dairy cow diet. However, a portion of the milk was recovered when BCVFA were added to the urea -based diet.

Several lactating studies have shown the positive benefits of supplementing lactating dairy cattle diets with BCVFA. Felix et al. (1980) found a 5.9% improvement in milk yield when supplementing a 14% CP diet with BCVFA. In addition, this study showed a 11.3% reduction in

plasma urea -N and a 22.7% reduction in rumen ammonia-N. Similarly, a three-university trial demonstrated a 12.7% increase in milk yield across the entire 305 -d lactation period (Papas et al., 1984). Peirce-Sandner et al. (1985) also showed that providing BCVFA increased yields of milk and 4% fat-corrected milk by 6.8 and 7.1%, respectively.

Best responses may be dependent on dietary factors, as previously discussed. Otterby et al. (1990) summarized a four-university trial that evaluated BCVFA being fed throughout the entire lactation. An interesting finding with this summary was that the best response in yield of milk, fat, and protein was observed in cows during the late lactation (225 – 305 days in milk) when corn silage-based diets contained less than 14% CP. Similar findings were observed by Peirce-Sandner et al. (1985), in which the best response was observed when diets contained 15% (mid-lactation) vs. 16.8% CP (early-lactation).

Nutritional models, such as the NRC (2001) and the CNCPS (Van Amburgh et al., 2015) have improved the precision of ration balancing. As we look to the next step of ration balancing, perhaps balancing for the needs of BCVFA by the rumen microbes is logical. Roman-Garcia et al. (2019e) conducted a meta-analysis on milk production responses to BCAA and found isoleucine to be the most important BCAA for milk production. Isoleucine is the corresponding BCAA to 2-methylbutyrate. Interestingly, Roman-Garcia (2019d) found this BCVFA to be most critical for supporting NDF digestibility in high passage-rate scenarios, similar to what is seen with high producing dairy cattle and is utilized more heavily in higher pH environments. In dairy diets, one of the objectives is to maintain a rumen pH above 6 to maximize NDF digestibility.

Roman-Garcia (2019a) concluded that best responses in NDF digestibility was achieved with isobutyrate and 2-methylbutyrate together or when all three BCVFA were fed. Isovalerate, which is derived from leucine, may not need to be supplemented in many of the dairy diets fed in North America. Dairy cattle diets in North America are largely based on corn grain, silage, and coproducts containing high concentrations of leucine. If one can better predict the requirements of individual BCVFA, supplementation may be achieved more economically, and allows one to better predict likelihood of response.

Conclusion
In conclusion, dairy producers have both an economical and environmental incentive to improve N efficiency. Much of the dairy industry's focus on improving N efficiency has been on providing higher quality RUP and RPAA. This strategy has been effective a s it is common to have diets with < 16.5% CP and it wasn't that long ago, that 18% CP was more common. The next stage in improving N efficiency is to maximize rumen microbial efficiency. The supplementation of BCVFA is an effective tool to achieve this objective that, together with the adoption of sophisticated dairy ration software, shows promise in meeting the needs of predicted BCVFA for rumen microbes, making this strategy profitable for dairy producers.

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Figure 1. Molecular structure of branched-chain volatile fatty acids.

Isobutyrate

$C_4H_8O_2$

2- methylpropanoic acid

Isovalerate

$C_5H_{10}O_2$

3- methylbutanoic acid

2- methylbutyrate

$C_5H_{10}O_2$

2- methylbutanoic acid

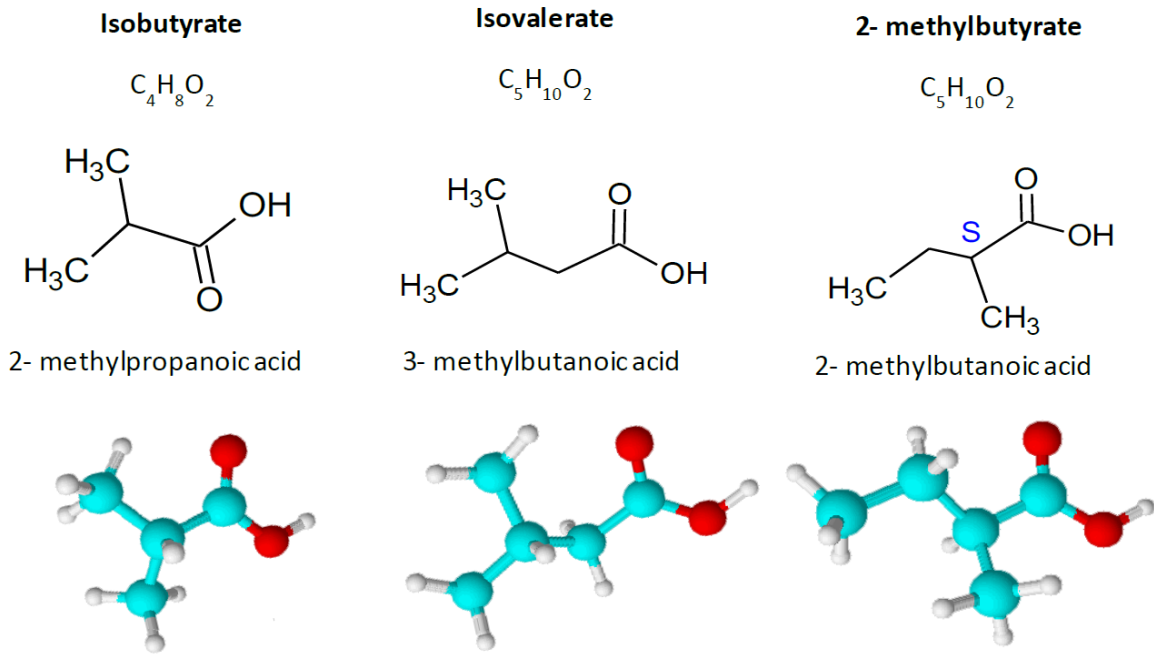**Figure 2.** Diagram showing change of membrane fluidity by incorporation of unsaturated and branched-chain long chain fatty acids.