Strategies to Improve Rumen Microbial Efficiency

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Introduction

Though hidden from view, the rumen and its microbes hold a central role in feeding of cattle. During ruminal fermentation, microbes break down fiber and other feed components and produce volatile fatty acids (**VFA**). In the process, microbes generate adenosine-tri-phosphate (**ATP**, energy) for themselves, then harness part of this ATP to produce microbial protein. The VFA so produced meet up to 70% of the animal's energy needs (Bergman, 1990), and microbial protein meets 60 to 85% or more of protein needs (Storm et al., 1983).

Microbial fermentation may be essential to cattle, but microbes charge a fee for their services. Microbial metabolism causes 4% of gross energy to be lost as heat (Czerkawski, 1986), and methane production from methanogens causes an additional 2 to 12% loss of gross energy (Johnson and Johnson, 1995). Feeding more concentrate and fat can help curtail these losses (Russell, 2007a, Hristov et al., 2013), but this practice may not always be economical. Few other strategies have been developed that are successful in the long term, and these losses seem to be unavoidable in a healthy fermentation.

Even if we cannot reduce the energetic fee that microbes collect, we may be able to make microbes spend those fees more efficiently. At present, microbes are not particularly efficient with the energy they harvest from fermentation, directing as little as 1/3 ATP towards synthesis of protein. By increasing ATP directed to protein synthesis, we could increase production of microbial protein, gaining more value out of the energy we feed.

The Rumen Microbial Ecosystem at Glance

Hundreds of trillions of microbes can be found in a single, 20-gallon rumen of an average cow. Bacteria represent probably more than 98% of all cells (Lin et al., 1997), but owing to their small size, they may account for barely over half of the microbial biomass (see below). About 200 species of bacteria have been cultured in the lab (Mackie et al., 2002), but molecular techniques (sequencing of the 16s rRNA gene) suggest may be thousands more uncultured "species" exist (Kim et al., 2011). Collectively, these microbes form a complex consortium to degrade fiber, protein, starch and other components of feed.

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In addition to bacteria, the rumen teems with protozoa, fungi, methanogens, and viruses. Protozoa are a fraction of a percent (~0.01%) of total cells in the rumen (Lin et al., 1997). They are much larger than bacteria - some protozoa can be seen with the naked eye - and thus contribute around 5 to 40% of the microbial biomass despite their low numbers (Williams and Coleman, 1992, Sylvester et al., 2005). They degrade feed components much the same as bacteria, except they also engulf bacteria as a food source (for growth factors) (Williams and Coleman, 1992).

Fungi also represent a fraction of a percent of total cells and contribute up to 8% of the biomass (Orpin, 1984). By breaking apart plant tissues with their powerful rhizoid cell structures, they help degrade recalcitrant fiber (Dehority, 2003).

Methanogens have earned infamy for producing methane. Though methanogens in a typical cow rumen collectively produce up to 500 liters per day (Johnson and Johnson, 1995), they account for only perhaps 2% of total microbial cells (Lin et al., 1997). In producing methane, methanogens remove hydrogen that would otherwise inhibit a normal fermentation (Russell, 2002).

Viruses, which are not true organisms, infect and can lyse bacteria. Nearly 1/4 of rumen bacteria harbor a virus, though viruses usually exist in a dormant (lysogenic) state (Klieve et al., 1989). Viruses do not contribute directly to degradation of feed.

Sources of Waste

For more than 40 years, we have recognized that microbes grow (synthesize microbial protein) with far from perfect efficiency (Stouthamer, 1973). For mixed rumen microbes in vivo, actual growth efficiency ranges from only 1/3 to 2/3 of the theoretical maximum (calculated from biochemical pathways) (Table 1). That is, microbes spend as little as 1/3 of ATP on growth. This phenomenon is not restricted to microbes growing in the cow, as the efficiencies are roughly as low in vitro for mixed and pure cultures of bacteria (Table 1).

For microbes both in the cow and lab, ATP not spent on growth is instead directed towards functions such as maintenance, energy storage, and energy spilling (Figure 1). Maintenance encompasses “housekeeping” required for the cell to simply stay alive, such as maintaining crucial ion balances across the cell membrane (Russell and Cook, 1995). Energy storage refers to energy preservation in the form of glycogen and other compounds synthesized during period of energy excess (Preiss and Romeo, 1989). Energy spilling refers to energy dissipated as heat when ATP exceeds needs for growth, maintenance, and storage (Russell, 2007b). Simply put, it is burning energy for the sake of burning energy. It can be likened to water flowing over the brim of an overfilled bucket (Figure 2).

Maintenance

Because it is required for cell survival, maintenance is an unavoidable source of waste. Despite its requirement by microbes, it is indeed wasteful from the cow's perspective because its net product is heat (no microbial protein).

Energy directed towards maintenance accounts for a large proportion of the energy expended when growth rates are low. At low growth rate of 5%/h, it accounts for more than 30% total energy expended. At high growth rate of 20%/h, by comparison, it accounts for only 10% (Russell, 2007a). This may be explained by analogy to a company's profit, overhead, and sales: when profits (growth) are low, overhead (maintenance) makes up a large proportion of sales (energy expended) (Russell, 2007a).

Growth rate increases with digesta passage rate in the rumen. For reasons explained above, increasing passage rate might seem a good strategy to decrease the relative impact of maintenance. Such a strategy for reducing waste may have unintended consequences, however, because increasing passage rate, such as by grinding forage, decreases digestibility (Van Soest, 1994).

Energy Storage and Spilling

Energy storage occurs when an excess of energy exists (Fig. 1, 2). Although stored energy can be later mobilized for growth (Wilkinson, 1959), storage is still somewhat wasteful because ATP is irreversibly spent to synthesize glycogen. This waste represents 20 to 50% of the available ATP in glucose, given 1 net ATP is spent on glycogen synthesis (Stouthamer, 1973) and between 2 to 5 ATP are available from glucose fermentation (Russell, 2002).

While small to moderate excesses of energy can be stored, large excesses of energy can also be simply burned off as heat through energy spilling (Fig. 1, 2). The function of spilling is not known, but it may give a microbe a growth advantage over its competitors (Russell, 2007a). Regardless of its function to the microbe, spilling may not be strictly required for its survival. Spilling produces only heat (no protein), and thus may be considered particularly wasteful. When mixed rumen microbes were given a large excess of energy (20 mM glucose), nearly 40% of heat was from spilling alone (Hackmann et al., 2013a).

Avoiding Energy Excess

Proper ration formulation is needed to avoid carbohydrate excess and thus reduce waste through spilling and storage. Carbohydrate excess occurs when rumen-degradable protein (**RDP**) is low, thereby limiting synthesis of microbial protein. The Dairy NRC (2001) reports RDP requirements between 9.5 to 11.3% of diet dry matter for lactating cattle and 8.6 to 10.8% for heifers.

A shortfall in RDP arises most commonly for feedlot-type rations with high inclusion of concentrate (Russell, 1998), but it can still occur in dairy rations with corn silage as the sole source of forage (VandeHaar, 2005). If ration formulation software or a nutritionist indicates a shortfall of RDP in a ration, this can be corrected by increasing the inclusion of high-RDP ingredients (such as soybean meal and urea). Alternatively, the carbohydrate concentration of the ration should be decreased by substituting it with fat (which is energy-rich but cannot be fermented).

Though adequate RDP may reduce energy spilling and storage, it may not completely eliminate them. Some energy storage occurs even when RDP is apparently adequate: glycogen can be detected in microbes when cattle are given a lactation diet with adequate RDP (Hackmann et al., 2013b). Additionally, mixed rumen bacteria still spilled energy in vitro when adequate RDP was given in the form of non-protein nitrogen (**NPN**) from ammonia. Bacteria grew more slowly with NPN than with true protein, and spilling resulted (Van Kessel and Russell, 1996). This finding has been corroborated in vivo, where microbial efficiency has been increased 36% by partially replacing urea (NPN) with casein (true protein) (Hume, 1970).

Wasteful Microbes

Some microbes may be inherently more wasteful than others and thus are targets for elimination from the rumen. *Streptococcus bovis* is a conspicuously wasteful microbe, though it was first recognized for causing acidosis (Russell, 2002). When cattle are abruptly switched from high-forage to high-concentrate diet, *S. bovis* feasts on the abundant carbohydrate (starch and sugars) available in the rumen. If left unchecked, it can hijack the rumen by 1) rapidly fermenting carbohydrate to lactate, 2) growing at a rate unmatched by any other rumen bacterium, and 3) decreasing rumen pH to 4.5 or lower, which few microbes besides acid-tolerant lactobacilli can survive. The result is acute rumen acidosis.

For the same reason it causes acidosis, *S. bovis* excels at spilling energy. Cells rapidly ferment carbohydrate to lactate whether they can grow or not. Because *S. bovis* lacks ability to store energy, spilling inevitably occurs when growth is limited (Figures 1 and 2). The mechanism of spilling is by a futile cycle of protons, in which protons are pumped out of the cell only to return later, with net production of heat (Russell, 2007b).

Although it proliferates best under the abrupt switch to concentrate as mentioned above, *S. bovis* is still present in low abundance regardless of the diet, even in high-forage diets (Russell, 2002). Because it is a fixture in the rumen and conspicuously wasteful, *S. bovis* is a natural target for reduction.

Reducing *Streptococcus bovis*

A few strategies exist for reducing *S. bovis*, most of which were developed originally to ameliorate acidosis. The antibiotic virginiamycin decreased *S. bovis* counts 10-fold or more in vivo and prevented uncontrollable accumulation of lactic acid (Coe et

al., 1999). Monensin plus tylosin were less effective. Antibiotics kill bacteria other than *S. bovis* (Nagaraja and Taylor, 1987), however, and their net effect on microbial efficiency remains unknown.

Live yeast (*Saccharomyces cerevisiae*) compete with *S. bovis* for carbohydrate, at least *in vitro* (Chaucheyras et al., 1996). However, how extensive this ruminal competition really is remains unclear (Russell, 2002)

Other strategies, still experimental, involve administering 1) antibodies, which bind to and inhibit bacteria, 2) vaccines, which stimulate production of antibodies, 3) bacteriophages, which are viruses that infect and kill bacteria, and 4) bacteriocins, which are antibiotic-like compounds produced naturally by rumen bacteria. Antibodies reduced *S. bovis* counts about 4-fold in vivo as long as they were fed (DiLorenzo et al., 2008). A vaccine reduced *S. bovis* counts by less than half, but it did protect against acidosis for the majority of animals (Shu et al., 1999). However, the efficacy of this strategy has not been tested long term (more than a week or two after the last booster).

Bacteriophages and bacteriocins can sometimes be effective in vitro, but success in animal trials is yet to be firmly demonstrated. One problem is the existence of *S. bovis* stains that are or become resistant. *Streptococcus bovis* was initially inhibited by the bacteriocin, nisin, in vitro, but resistant cells soon developed and grew as fast as untreated ones (Russell and Mantovani, 2002). Strains of *S. bovis* from the rumen were sensitive to one bacteriophage for 40 d, but afterwards resistant strains developed spontaneously (Iverson and Millis, 1977).

Identifying Other Wasteful Microbes

Streptococcus bovis is unlikely to be the only wasteful microbe. We have observed that populations of rumen microbes can spill energy even when lactate is not produced (Hackmann et al., 2013a). Because lactate production is a fingerprint of rapid fermentation and energy spilling by *Streptococcus bovis* (Russell and Strobel, 1990), other microbes must be responsible for this waste. Further, *S. bovis* does not store energy, but populations of rumen microbes can store large amounts of energy (Hackmann et al., 2013a). If we focus on energy spilling by *S. bovis* alone, we ignore microbes wasting energy through storage. Research is underway to identify those wasteful microbes, and strategies to eliminate these microbes could then follow.

Microbial Turnover

The strategy to increase microbial efficiency emphasized most by this review is increasing direction of ATP towards microbial growth. Another strategy is to decrease turnover of microbial protein to ammonia, as this effectively “undoes” microbial growth. As much as 50% of microbial protein turns over in the rumen (Wells and Russell, 1996, Oldick et al., 2000).

Protozoa promote turnover by engulfing bacteria. Although protozoa incorporate some of engulfed bacterial protein into their own cells, they break down and release some as ammonia into the rumen milieu (Williams and Coleman, 1992). Accordingly, removing protozoa from the rumen decreases turnover, increasing microbial efficiency by an average of 58% (Williams and Coleman, 1992). Removing all protozoa is not a practical strategy, however, as animals spontaneously re-acquire protozoa unless the animal is isolated.

Viruses also cause bacterial lysis and turnover. However, the majority of viruses exist in a dormant (lysogenic) state in the bacterial cell, and their lytic activity is probably low (Klieve et al., 1989). Bacteria, protozoa, and probably other microbes also autolyse (die spontaneously) (Williams and Coleman, 1992, Wells and Russell, 1996). This autolysis is not necessarily a response to starvation (Wells and Russell, 1996), and it is unclear how to reduce it.

Conclusions

Rumen microbes do not grow with perfect efficiency, but spend considerable ATP on non-growth functions (maintenance, energy storage, and energy spilling). Because they generate heat instead of microbial protein, these non-growth functions are wasteful to the cow. Waste can be reduced by including adequate dietary RDP to avoid excesses of energy. *Streptococcus bovis* is a conspicuously wasteful microbe because of its propensity to spill energy. Anti-bacterial agents, including antibiotics, antibodies, and vaccines, are effective in reducing *S. bovis* counts, though it is not clear if they increase microbial efficiency in turn. Improving efficiency will depend on identifying other wasteful microbes and further developing strategies to manipulate the microbial population.

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Table 1. Efficiency of rumen microbial growth

	Efficiency	
Organism	g microbial DM mol ATP^{-1*}	% of theoretical maximum[#]
Mixed rumen microbes, in vivo	11 to 21	34 to 66
Mixed rumen bacteria, in vitro	7.5 to 16.7	23 to 52
Pure cultures, in vitro	10 to 25	31 to 78

[*]From Russell and Wallace (1997).
[#]32 g (g microbial DM mol ATP)$^{-1}$; from Stouthamer (1973).

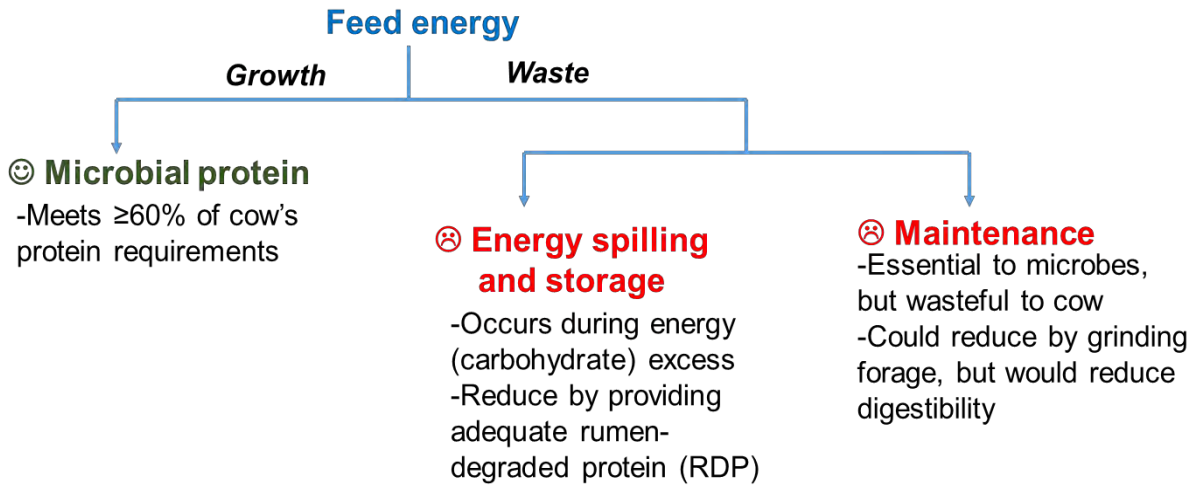

Figure 1. Use of feed energy by rumen microbes. As little as 1/3 energy that microbes harvest will be used for production of microbial protein (growth), and the rest is wasted on energy spilling, energy storage, and maintenance.

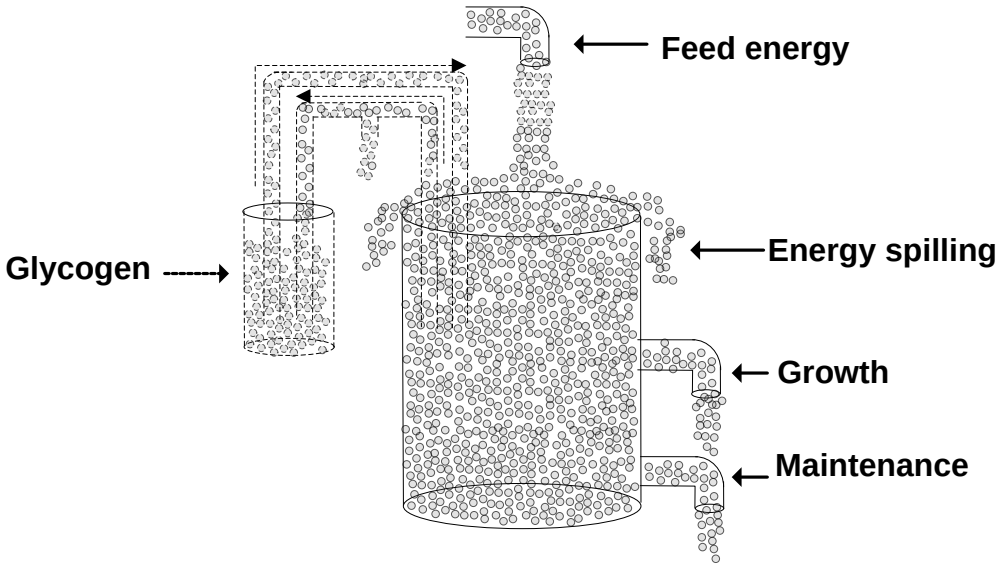

Figure 2. Bucket model of energy spilling. The large bucket represents the main pool of ATP-equivalents available to cell functions (maintenance, growth, energy storage, energy spilling). The smaller bucket represents the pool of ATP-equivalents in glycogen, which can be stored from and mobilized to the main pool by pumps. Modified from Russell (2002).

SESSION NOTES