

Can the Heat of Ruminal Fermentation be Manipulated to Decrease Heat Stress?

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Summary

- Because maintenance, energy spilling and even growth are all heat generating processes, the ability of nutritionists to manipulate the heat production of ruminal microorganisms is very limited.
- Given these constraints, the most effect means of decreasing the heat of ruminal fermentation is to decrease food intake, and this is the strategy that the cow uses.

Introduction

Ruminal fermentation gives cattle the ability to metabolize cellulose materials that would otherwise be indigestible, utilize non-protein nitrogen and harvest microbial protein as source of amino acids (Hungate, 1966). However, this fermentation also produces ammonia, methane and heat. These latter end-products represent losses of nitrogen and energy, respectively. The heat of ruminal fermentation can be advantageous in cool climates, but it represents yet another heat load on the animal if the ambient temperature is high. The question then arises, can the heat of ruminal fermentation be manipulated to decrease heat stress? Blaxter (1962) noted that “temperature measurements in the rumen certainly show that heat is evolved during microbial digestion, but such records do not allow any quantitative estimates of heat to be made. Estimates made many years ago, based on the ratio of carbon dioxide to the methane produced during the fermentation, indicated that the fermentation heat was probably 5-10% of the heat of combustion of food.” Blaxter (1962) concluded that “fermentation heat exceeds the heat loss which takes place in the gut of non-ruminants by a factor of at least five.” In the 1980’s, we used microcalorimetry measure the heat of fermentation directly and used these results to assess the efficiency of bacterial growth (Russell, 1986). By relating heat with basic concepts of ATP formation, growth and energy spilling, it was possible to develop a theoretical and more quantitative scheme of bacterial energetics.

ATP Formation

Ruminal microorganisms are not altruists, and only a teleologist would ever make the claim that ruminal fermentation evolved to aid the animal. A more biologically sound explanation of the symbiosis involves independent schemes of exploitation. Ruminal microorganisms evolved mechanisms to facilitate their own growth and development, and the animal exploited this capacity by creating a habitat where the microbes could grow. The ability of the microbes to grow is dependent on their ability to form ATP under anaerobic conditions and use this ATP to synthesize macromolecules that can be assembled into cells.

Biosynthetic reactions are driven by the free energy (ΔG) of ATP hydrolysis, but free energies are relatively difficult to estimate in biological systems (Harold, 1986). Another energetic measurement is enthalpy (ΔH). Enthalpy is the estimated by combustion, and it is heat capacity of the system. Free energy and enthalpy are positively

correlated and the difference is entropy ($T\Delta S$): $\Delta H = \Delta G + T\Delta S$. Entropy is often defined as “randomness,” but this simple definition is not very informative. A better definition for entropy is exhaustion. “The more as system has lost its capacity for spontaneous change, the more its capacity has been exhausted, the greater is the entropy” (Klotz, 1967).

Catabolic pathways differ in their ability to conserve energy as ATP, and this conservation can be related to the ratio of ΔH to ATP. Assuming approximately 1 ATP/methane (Blaut et al., 1990), a typical mixed ruminal fermentation would have an enthalpy to ATP ratio of 10 cal/mmol (Russell and Strobel, 1993). Pathways that maximize acetate, methane and ATP decrease the enthalpy of ATP production, but simple calculations indicate that the only a drastic diet shift (90% grain to 100% hay; Russell, 1998) would have a significant impact on enthalpy (11.6 versus 5.3 cal/ATP, respectively). Given these observations, the manipulation of ruminal fermentation schemes to decrease heat production of lactating dairy cattle is not practical.

ATP Use in Growth

Polymerization reactions are inherently inefficient. A peptide bond has an enthalpy content of only 3 cal/mmol, but it takes 4 ATP to synthesize the bond. If one assumes 10 cal/mmol ATP, less than 8% of the total enthalpy change would be trapped in the peptide bond (92% would be dissipated as heat). Polysaccharide synthesis is more efficient because glycosidic bonds have 4.5 cal/mmol and formation only requires 2 ATP/bond. However, even in this case, the efficiency of energy trapping is less than 23%. Since protein synthesis accounts for nearly 2/3 of the total ATP requirement for growth (Stouthamer, 1973) an overall efficiency of 12% for cell synthesis is probably reasonable. The remainder (88%) would be dissipated as heat.

The question then becomes, why is growth so inefficient? As reviewed by Harold (1986), growth and reproduction is not a series of random biosynthetic reactions; it is an assemblage of information contained within the biomolecules and organization of the cell. The relationship between information and thermodynamics was pondered by James Maxwell in 1867 in a proposition that has since been called “Maxwell’s demon.” The proposal was that information and energy are inter-convertible. While this concept cannot be tested experimentally, “it appears that you don’t get something for nothing-not even information” (Morowitz, 1968).

ATP Use for Maintenance Energy

With the advent of continuous culture techniques in the 1950’s, it became apparent that bacteria had lower growth efficiencies (yields) at slower growth rates, and the idea of a bacterial maintenance energy requirement was introduced. Pirt (1965) presented a maintenance derivation based on double reciprocal plots of yield and growth rate. Maintenance, the slope of the plot, was defined as a time dependent function that was proportional to cell mass. The theoretical maximum yield is defined as the yield that one would obtain if there were no maintenance energy requirement. These non-growth related functions have never been precisely defined, but they are essential for cell survival even though they do not directly result in cell mass increases. Ion balance across the cell membrane is probably most important (Russell and Cook, 1995).

When bacteria grow slowly, a large proportion of the energy is used to maintain the cells, and maintenance energy is analogous to overhead in a business. One can only

make a profit (growth) after the overhead (maintenance) is met, but if cash flow is large (rapid rates of energy utilization), the overhead will make up a small proportion of the total budget. Isaacson et al. (1975) grew mixed ruminal bacteria in continuous culture and determined a maintenance energy requirement of 0.26 mmol glucose/g bacteria/h and a theoretical maximum growth yield of 0.089 g cells/mmol glucose. Within the rumen, bacterial growth rates often range from 0.20 to 0.05 h⁻¹, and under these conditions maintenance energy would account for 10 to 31% of the total energy consumption, respectively.

ATP Use in Energy Spilling

Maintenance is determined under energy-limiting conditions. If energy is in excess, and growth is limited by some other factor (e.g. nitrogen), the rate of 'resting cell metabolism' can exceed the maintenance rate by as much as 18-fold (Russell and Cook, 1995). For example, when *S. bovis* was incubated in a nitrogen free-medium with an excess of glucose, the fermentation rate was 90 mmol glucose/g bacterial protein/h, but the maintenance rate (as measured under carbon-limitation) was only 1.6 mmol glucose/g bacterial protein/h (Russell and Strobel, 1990). Based on these results, it appeared that *S. bovis* had a third avenue of energy expenditure that could be classified as "energy spilling."

Maintenance and energy spilling are physiologically distinct. When bacteria are grown at slow growth rates under energy limitation, intracellular ATP concentrations are low, but bacteria spilling energy can have ATP concentrations that are 2 to 3 fold higher (Russell and Strobel, 1990). Energy spilling is most easily demonstrated when cells are limited for nutrients other than energy source, but it is clear that even rapidly growing cells can spill significant amounts of energy. Only cells limited for energy do not seem to spill energy.

In *S. bovis*, energy spilling can be explained by increased membrane bound ATPase activity, and a futile cycle of protons through the cell membrane (Russell and Strobel, 1990). The regulation of the futile cycle is caused by a cascade of effects. When glucose is in excess, and the glycolytic rate is faster than the rate at which ATP can be used for growth, fructose 1,6-bis phosphate accumulates (Bond and Russell, 1996), and this accumulation is associated with a decrease in intracellular phosphate (Bond and Russell, 1998). When the intracellular phosphate concentration decreases, the ΔG of ATP hydrolysis increases, and this latter effect increase allows the membrane bound ATPase to pump more protons and create a large protonmotive force (Bond and Russell, 2000). When protonmotive force increases, the membrane becomes more permeable to protons, and as protons are cycled through the cell membrane, and excess ATP is dissipated.

Fructose 1,6-bis phosphate accumulation is characteristic of low G+C gram-positive bacteria like *S. bovis* (Russell et al., 1995), but some bacteria spill energy in mechanisms that are not directly linked to fructose 1,6-bis phosphate or a futile cycle of protons. In *E. coli*, energy spilling is facilitated by the low affinity potassium proton symporter (Mulder et al., 1986; Buurman et al., 1991). When potassium or ammonium ion limits growth, the high affinity ATP-driven potassium (ammonium) uptake system is induced. Potassium is then allowed to move out of the cell by a reversible low affinity potassium (ammonium) transporter.

Ruminally Degraded Protein

Most ruminal bacteria can use ammonia and other non-protein nitrogen sources, but their growth efficiency (yield) is stimulated by amino nitrogen. In the Cornell Net Carbohydrate Protein System, maintenance corrected yields are subjected to a peptide stimulation function that is as large as 17% (Russell et al., 1992), but the cause of this stimulation was not explained. Stouthamer (1979) presented calculations on the amount of ATP that was needed to synthesize bacterial biomass. His calculations indicated that it costs nearly as much to transport an amino acid as does to synthesize one *de novo*. The question then arose, how were amino acids stimulating growth efficiency?

The impact of amino N (peptides) on bacterial yield is most easily explained by energy spilling and the balance of anabolic and catabolic rates (Russell and Cook, 1995). When bacteria are forced to use ammonia, they grow more slowly than they would if amino nitrogen was available, and the anabolic rate can be lower than the catabolic rate. This imbalance of anabolic and catabolic rates leads to an accumulation of “excess” ATP that is then dissipated by energy spilling. This latter use (waste) of ATP leads to a decrease in growth efficiency. Conversely, if amino nitrogen is available the bacteria can grow faster, the imbalance of anabolic and catabolic reactions is less, and the bacteria spill less ATP in reactions that only generate heat.

Ruminal Acidosis

The rumen is well buffered by the bicarbonate of saliva, but starch and sugar fermentation can be so rapid that ruminal pH declines (Owens, 1998). This decline is aggravated by inability of non-fiber carbohydrate to stimulate rumination, enhance saliva production and buffer the rumen. When saliva production and fluid dilution rate from the declines, the flow of fermentation acids from the rumen to the abomasum decreases, ruminal volatile fatty acid concentrations increase, and pH declines (Allen, 1997; Russell, 2002; Allen et al., 2006)). Rumen bacteria differ in their ability to tolerate acid pH, and this difference appears to be mediated by their strategy of intracellular pH regulation (Russell and Diez-Gonzalez, 1998).

Acid tolerant (typically starch-fermenting) ruminal bacteria have metabolisms that can withstand a decrease in intracellular pH, and the decline in intracellular pH prevents an influx and an accumulation fermentation acids as undissociated anions. By contrast, acid-sensitive bacteria ruminal bacteria do not let intracellular pH decline as a function of extracellular pH, and they have much greater pH gradients across their cell membranes when the extracellular pH is acidic. The ability of undissociated fermentation acids to pass across the cell membrane and dissociate in more alkaline interior leads to large logarithmic and toxic accumulations of fermentation acid anions (Russell, 1992; Russell and Diez Gonzalez, 1998).

Intracellular pH regulation can play a role in the efficiency of bacterial growth and heat production. Because acid-sensitive bacteria do not let their intracellular pH decline, and declines in ruminal pH do not trigger either an imbalance in anabolic and catabolic rates (energy spilling). In this case, the toxicity is caused by osmotic effects due to the accumulation of fermentation acid anions and potassium. Acid-resistant bacteria let their intracellular pH decline so fermentation acid anions do not accumulate, but this decline in intracellular pH affects the anabolic rate to a greater extent than the catabolic rate. This imbalance causes energy spilling and additional heat production.

Ionophores

Ionophores (e.g. monensin and lasalocid) have been used as feed additives in the beef cattle industry since the 1970's, and they have increased feed efficiency (Russell and Strobel, 1989). The improvement in feed efficiency appears to be due to a combination of effects that include: 1) a decrease in hydrogen, a precursor of methane, 2) an inhibition of amino acid deamination, 3) and a more consistent and typically lower feed intake, and 4) and a decreased chance of acidosis. The effect on feed intake has been explained in two ways. When ruminal fermentation is altered to retain more energy and nitrogen, the animal needs to consume less feed (Donoho, 1984). Another explanation is toxicity. Monensin has the ability to dissipate ion gradients, and this effect can be mediated in animal tissues particularly if the dosage exceeds recommendations (Pressman, 1985). This latter effect is consistent with the observation that cattle can "learn" to avoid to monensin (Baile et al., 1979) and some animals (e.g. horses and man) cannot tolerate even low doses (Pressman, 1985). Rumensin (monensin) was recently approved by the Food and Drug Administration (FDA) for use in dairy cattle. Less is known about monensin in dairy cattle, but at least part of its effect is mediated via a decrease in feed intake. As noted by Aguililar (2005) milk production efficiency was enhanced, and there was a 4.0% improvement in solids-corrected milk per unit of feed intake.

Summary of Dietary Strategies for Decreasing Heat of Fermentation

Strategy 1: By-pass ruminal fermentation. The most straightforward way of decreasing the heat of fermentation is to bypass ruminal fermentation altogether. This strategy can be achieved by using feed materials not degradable in the rumen or treating feeds so there is a greater escape or by-pass to the lower gut. For example, fatty acids arising from triglycerides or phospholipids hydrolysis can be biohydrogenated by ruminal bacteria, but the fatty acids are not degraded or fermented *per se*. Fatty acids can also be protected from the rumen by treating them with calcium and magnesium, and some starch sources are encapsulated by proteins (e.g. zein) that are not readily hydrolyzed unless heat is provided as a feed treatment. By using starch sources that are not heat treated, it is possible to increase ruminal escape, but ruminants may have a limited capacity to digest starch in their intestines (Harmon, 1991). Post ruminal starch fermentation can also cause a variety of problems that include diarrhea, laminitis and an over-growth of *Escherichia coli*. Another avenue of enhancing the flow of feed to the lower gut is particle size reduction, but this approach may also decrease fiber digestibility.

Strategy 2: Select fermentation pathways that generate less heat. This approach sounds like a good approach, but not it is not practical. The pathways used by ruminal bacteria in vivo don't differ much in efficiency, and $\Delta H / \text{ATP}$ is already low.

Strategy 3: Minimize the impact of bacterial maintenance energy expenditures. Because fiber carbohydrate-digesting (FC) ruminal bacteria have a lower maintenance energy than non-fiber carbohydrate digesting (NFC) bacteria, one might conclude that additional fiber would decrease fermentation heat. However, the opposite is probably true. FC bacteria usually grow more slowly than NFC bacteria, and rapid growth of NFC bacteria (on starch) minimizes overall maintenance energy expenditures and heat production. The problem with starch feeding is acidosis. If ruminal starch fermentation is rapid, the animal can suffer from a variety of maladies that range from indigestion and ruminal ulcers to liver abscesses or even death (Owens et al., 1998).

Strategy 4: Minimize the impact of energy spilling. NFC ruminal bacteria can spill energy if their growth is impaired by factors other than energy availability. This can be achieved by adding ruminally degraded protein to the ration so the NFC bacteria can better match their anabolic and catabolic rates when the rate of starch fermentation is faster than the growth rate allowed by ammonia. Only down side is cost. True protein costs more non-protein nitrogen.

Strategy 5: Decrease food intake. Because maintenance, energy spilling and even growth are all heat generating processes, the ability of nutritionists to manipulate the heat production of ruminal microorganisms is very limited. Given these constraints, the most effect means of decreasing the heat of ruminal fermentation is to decrease food intake, and this is the strategy that the cow uses. This latter point is illustrated by unpublished data provided to me by Professor Peter Robinson (University of California at Davis). "We did a study with a high production group last summer on a well managed 3x milked (cooled in the parlor), and misted/fanned in the pens, dairy. In period 1, the weather was normal (highs 90+, lows in the 60's) and the average DM intake and milk yields were 27.1 and 46.8 kg. In period 2, the cows were whacked with the heat (highs in the 110 to 120 range and lows in the low 80's). Same cows 4 weeks later were 18.4 kg of DM intake and 33.3 kg/d of milk. Same TMR. Milk components were not impacted."

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