

# The Endogenous Metabolic Rate of Mixed Ruminal Bacteria and the Effect of Energy Starvation on Ruminal Fermentation Rates

J.S. Van Kessel and J.B. Russell

## Introduction

Grazing ruminants sometimes consume food continuously, but domestic livestock usually eat larger, less frequent meals. The impact of meal interval on ruminal fermentation has not been studied in a systematic fashion. The rumen usually has an abundance of insoluble feed materials, but soluble carbohydrates are only available 1 to 2 h post feeding. Soil and marine bacteria can remain viable for long periods of time, but long term survival is not a ubiquitous feature of bacteria. Many ruminal bacteria are very sensitive to energy starvation *in vitro*, and direct counts can be 10-fold higher than viable counts. Some ruminal bacteria store large amounts of polysaccharide that resembles glycogen, but glycogen does not always prevent starvation. Viability is a highly operational definition that is based solely on bacterial reproduction. Given the fact that bacteria seem to survive better in dense populations than dilute ones and can suffer from a variety of stresses including substrate accelerated death, "viable" is not always a synonym for metabolically active. The following experiments sought to: 1) measure the endogenous metabolic rate of mixed ruminal bacteria, 2) correlate the endogenous metabolic rate with fermentation capacity, and 3) assess the effect of feeding frequency on the initial rate of ruminal fermentation.

## Materials and Methods

Ruminal contents were collected 2 h or 24 h post-feeding from the ventral and anterior sections of the rumen. Contents were squeezed through 8 layers of cheese cloth and the pH was determined immediately. Feed particles and protozoa were removed by centrifugation (325 x g, 5 min) and ruminal fluid was anaerobically transferred to a flask that was flushed with oxygen-free carbon dioxide (39°C). Samples were removed from the flask at 4 h intervals. The specific rate of soluble carbohydrate fermentation (final concentration per ml: 0.42 mg of soluble starch, 0.175 mg of cellobiose, 0.105 mg of sucrose, 0.125

mg of xylose, 0.125 mg of arabinose, and 0.05 mg of pectin) was estimated from the rate of heat production. The specific degradation rate for ball-milled cellulose was estimated from the reciprocal of digestion time needed for complete digestion, the amount of cellulose degraded (2 mg/ml) and the initial concentration of bacterial protein. The specific activity of ammonia production from Trypticase (15 mg/ml) was estimated from the increase in ammonia. The specific activity of methane production from hydrogen and carbon dioxide (1 atm each) was estimated from the increase in methane. ATP was extracted from the cells by perchloric acid-EDTA treatment after separation of the cells from the growth medium. ATP was measured by the luciferin-luciferase method. Total cellular polysaccharide was measured by the anthrone method. Utilizable polysaccharide was estimated from the difference in total carbohydrate and the amount of carbohydrate that was still present after 48 h of starvation. Bacterial protein was measured by the method of Lowry. RNA and DNA were measured using an orcinol reaction.

## Results

When mixed ruminal bacteria were starved *in vitro* for 24 h, cellular ATP decreased, but there was little decline in cell protein. Starved ruminal bacteria utilized nucleic acids (primarily RNA), but the decline in ATP was more closely correlated with decreases in polysaccharide. Because polysaccharide declined at a first order rate of 23% per h, it was possible to estimate the endogenous metabolic rate at various stages of starvation. The bacteria were initially able to ferment soluble carbohydrates at a rate of 700 mg of hexose equivalent per mg of protein per h. Starvation had little impact on the rate of soluble carbohydrate fermentation until 8 to 12 h, and the endogenous metabolic rate was less than 10 mg of hexose per mg of protein per h. The bacteria digested ball-milled cellulose at a rate of 25 mg of hexose per mg of

protein per h for 8 to 12 h. Even bacteria that had been starved for 24 h digested cellulose at a rate of 16 mg of hexose per mg of protein per h. The bacteria produced methane from hydrogen and carbon dioxide at a rate of 70 nmol of methane per mg of protein per min. Short periods of starvation (< 12 h) had little impact on methane production, but longer times caused an almost complete inhibition of methanogenesis. The bacteria deaminated amino acids at a rate of 30 nmol per mg of protein per min, and the critical phase of starvation was again 8 to 12 h. Ruminal bacteria that were harvested 24 h after feeding had 10-fold less polysaccharide than bacteria 2 h after feeding, but this polysaccharide supported high rates of soluble carbohydrate fermentation, cellulose degradation, deamination and methane production.

## Discussion

The maintenance rate of animals is frequently estimated from the fasting (basal) metabolic rate, but bacteria can have different rates of maintenance and endogenous metabolism. With bacteria, maintenance is defined as the non-growth energy dissipation of growing cells, and, in this case, energy is derived from exogenous sources. Endogenous metabolism is a characteristic of starved, non-growing cells, and this energy is derived from cellular reserves. Mixed ruminal bacteria have a maintenance rate of 100 mg hexose per mg of bacterial protein per h, but starved ruminal bacteria had endogenous metabolic rates that were 2 to 50 fold lower.

Based on the observation that the decline in cellular polysaccharide was highly correlated with the decrease in cellular ATP, it appeared that the specific rate of polysaccharide degradation was a reasonable estimate of endogenous metabolic rate. The endogenous metabolic rate declined logarithmically at a simple first order rate of 23% per h. Starvation did not reach its critical phase until 8 to 12 h when the

endogenous metabolic rate was less than 10 mg hexose per mg of bacterial protein per h. Even highly starved (as long as 24 h) bacteria were able to ferment soluble carbohydrates at a rapid rate. Cellulose-digesting bacteria were even less sensitive to starvation than bacteria that fermented soluble carbohydrates. Methanogens only account for a small portion of the total ruminal population, and they must depend on the hydrogen that arises from the endogenous metabolism. Even ruminal bacteria that had been starved for 24 h could still deaminate amino acids at a rate of 20 nmol per mg of protein per min, and this result indicated that polysaccharide reserves were not a critical feature of deamination.

The idea that mixed ruminal bacteria were not particularly sensitive to energy source starvation *in vivo* was supported by the observation that mixed ruminal bacteria obtained from the rumen 24 h post feeding had nearly as high a rate of soluble carbohydrate fermentation, cellulose degradation, methane production and amino acid deamination as bacteria that were obtained soon after feeding. The 24 h post-feeding bacteria had 10-fold less utilizable polysaccharide, but even this amount of polysaccharide supported a rate of endogenous metabolism that did not compromise subsequent fermentation rates. The notion that the rumen has a large population of non-viable or dead bacteria is probably nothing more than an artifact of "culturability." If the ruminal bacteria were indeed metabolically inactive and unable to grow, they would soon be washed out of the rumen and be replaced by ones that could grow.

## Conclusion

Ruminal bacteria may be more susceptible to starvation than other bacteria, but they clearly seem to have enough cellular reserves to withstand the relatively brief periods of starvation imposed by normal feeding practices.