Understanding rumen microbial growth effectiveness to improve digestive efficiency of ruminants

Katie Backus

backus.36@buckeyemail.osu.edu

Animal Sciences

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Faculty Advisor

Dr. Jeff Firkins, Professor

Department of Animal Sciences

2029 Fyffe Ct

Columbus, OH 43210

firkins.1@osu.edu

Abstract:

Ruminant animals, such as sheep, cattle and goats, are important livestock animals and acquire much of their protein from microbes growing in the rumen. These microbes do not use all energy for growth, but probably direct some energy towards storage (glycogen synthesis), maintenance, and production of heat alone(energy spilling). Whereas stored energy (glycogen) can be used later for growth, energy directed towards maintenance and spilling cannot. Energy spilling and maintenance thus depress efficiency of microbial growth, decrease microbial protein available to the ruminant, and cause need for more protein to be fed to ruminants. Although glycogen synthesis, maintenance, and energy spilling have been measured using pure cultures of bacteria, they have not been simultaneously measured in mixed rumen microbes. The purpose of this study was to measure how much energy mixed rumen microbes direct towards glycogen synthesis, maintenance, and energy spilling. Washed suspensions of mixed microbes were prepared from rumen fluid of a dairy cow. Microbes were dosed with either 5 mM or 20 mM glucose and aliquots of culture sampled at various time increments. Heat production (using a microcalorimeter), free glucose, cell glycogen, and cell protein were measured. Results showed that mixed rumen microbes initially direct energy entirely towards maintenance and glycogen synthesis, but, over time, they spilt progressively more energy. This shows that the growth efficiency of microbes is not perfectly efficient and if we can reduce the amount of energy spilled my mixed microbes, we would be able to improve the energy efficiency of ruminant livestock operations.

Introduction

Cattle, sheep, and goats are all ruminant animals that are capable of converting fibrous feedstuff such as hay into meat and milk. They are capable of digesting this fibrous feedstuff with the help of microbes growing in their rumen (forestomach). These microbes anaerobically degrade the feedstuff and use the energy to synthesize cellular protein. Much (>50%) of the protein digested by the ruminant animals comes from this microbial protein (18). Improving our understanding of how these microbes use the energy from fermentation for growth and for factors that lower efficiency will allow us to better predict the microbial growth efficiency and thus the amount of microbial protein available to the ruminant animal (6,16). The ability to better predict the growth efficiency of microbes and the availability of microbial protein should decrease the amount of protein needed in ruminant livestock feed. Decreasing the amount of protein fed to ruminant animals would decrease the cost of feed and decrease the amount of nitrogen waste excreted from the animal into the environment.

Problem Identification and Justification

Microbes do not grow with perfect energetic efficiency. Some of the energy is used for maintenance, or upkeep of the cells, and energy spilling, both non-growth functions that detract energy from growth (11). Energy spilling is energy dissipated as heat when the amount of ATP available from fermentation of feedstuff exceeds the amount used for growth and maintenance (17). Energy spilling can be thought of as water spilling over a bucket (Fig. 1). Energy spilling was demonstrated in rumen bacteria when they were pulse-dosed with glucose and were shown to ferment excess glucose and produce heat rapidly with no detectable growth (15). Energy spilling can be a major detraction from efficient growth in bacteria. Those bacteria that spill energy fermented glucose 10-fold faster than those that did not (25). Energy spilling diverts energy away from growth, decreasing the efficiency of the microbial growth and thus the amount of microbial protein available for digestion.

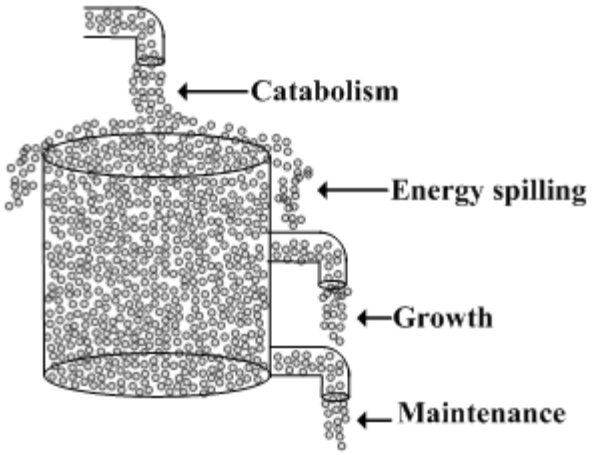

Fig. 2. Bucket model of energy spilling. After (51).

Energy spilling has been measured in rumen bacteria but not rumen protozoa (which make up 10-50% of the microbe biomass (3, 23)) or mixed populations of microbes and is thus incompletely understood (8,15). Protozoa likely spill less energy and reduce energy spilling by other microbes. Protozoa store catabolic substrate and use it much more slowly at a rate better

matched with growth (4, 5, 24, 26). This prevents bacteria from catabolizing substrate very rapidly and spilling much of the energy. Improving our understanding of the energy use in rumen protozoa and mixed populations of rumen microbes should allow us to better predict the growth efficiency of rumen microbes and decrease the amount of protein needed in ruminant livestock feed.

Decreasing the amount of protein in the feed would reduce the amount of nitrogen waste excreted into the environment. Ruminants excrete 70-90% of total nitrogen in feed protein (14). Ruminant livestock worldwide excrete $70*10^8$ kg N/year, including $7.9*10^8$ kg N/year for U.S. dairy cattle alone (19). Nitrogen excreted by ruminants and other livestock leads to 2/3 of anthropogenic output of NO_2 and NH_3 (7, 20). Along with a decrease in nitrogen waste, decreasing the amount of protein in feed would decrease the cost to livestock producers.

Hypothesis and Objective

The central hypothesis is that rumen protozoa are capable of reducing energy spilling by bacteria when protozoa and bacteria are present together. The overall objective is to gain a better understanding of energy spilling in mixed populations of protozoa and bacteria after a pulse dose of either 5m*M* or 20m*M* catabolic substrate (glucose). Multiple variables relevant to energy spilling will be measured including heat production, free glucose, cell glycogen, and cell protein.

Materials and Methods

Mixed microbes (protozoa and bacteria) were prepared. Aliquots of either 5m*M* or 20m*M* glucose were dosed. Heat production, free glucose, cell glycogen, and cell protein were measured. Energy spilling was quantified from baseline heat production before dosing and calculated heat production from glycogen synthesis to account for maintenance and growth.

Detailed procedure: Microbes were collected by collecting rumen fluid from a cannulated cow fed a typical dairy ration (50:50 corn silage: concentrate). Mixed microbes (protozoa and bacteria) were prepared through a combination of filtration (protozoa) and centrifugation (bacteria). Glucose (5mM and 20 mM) was dosed. Heat production was measured using a microcalorimeter.

Aliquots (1mL) were collected from parallel cultures at -1200 s, -60 s, +0 s, +1200 s, +2400 s, +3600 s, +4800 s, and +6000 s, relative to dose. Supernatant and cell pellet were separated by centrifugation (10 000 g for 10 min and 4° C, followed by re-suspending pellet in 0.9% NaCl, followed by 10 000 g spin)(16).

Cell-free supernatant was analyzed for free glucose using the glucose oxidase-peroxidase method of Karkalas (1). Cell pellets were analyzed for protein using a Pierce BCA kit after hydrolyzing the pellet in NaOH (0.2 M final concentration, 100 C, 15 min). Bovine serum

albumin, also boiled in NaOH, served as the standard. Glycogen was analyzed using the anthrone method (5).

Data Analysis:

Energy spilling was calculated as heat production not accounted by glycogen synthesis or maintenance. Heat released by glycogen synthesis was calculated from measured increases in glycogen (g/L) and the heat theoretically released per gram of glycogen synthesized (166 J; T.J. Hackmann, unpublished calculation). Maintenance was defined as baseline heat production before pulse dose of glucose. Energy spilling was total heat production minus heat from glycogen synthesis and maintenance.

Disappearance of catabolic substrate (glucose) associated with increased heat production will verify that catabolic substrate is being consumed. A lack of significant growth in addition to the consumption of substrate will verify that energy is being spilt.

Results

The results showed (Figure 1) that, regardless of dose (5m*M* or 20m*M*), protein concentration remains relatively stable, unaffected by excess glucose. Glucose in the supernatant spikes when dosed but is quickly consumed and returns to almost zero. Glycogen concentration increases after the glucose dose showing the storage of energy. Heat production spikes dramatically with the dose of glucose and slowly returns to baseline heat production. Figure 2 shows the heat production of microbes in response to dosed glucose. Heat production due to endogenous metabolism (or maintenance) is determined from baseline heat production before dosing. Heat production due to glycogen synthesis is calculated from known values of heat production per mol of ATP consumed during glycogen synthesis. Any heat production above this is considered energy spilling. Results show that there is very little spilling with small (5m*M*) glucose, but with larger doses (20m*M*), energy spilling is significant and increases over time.

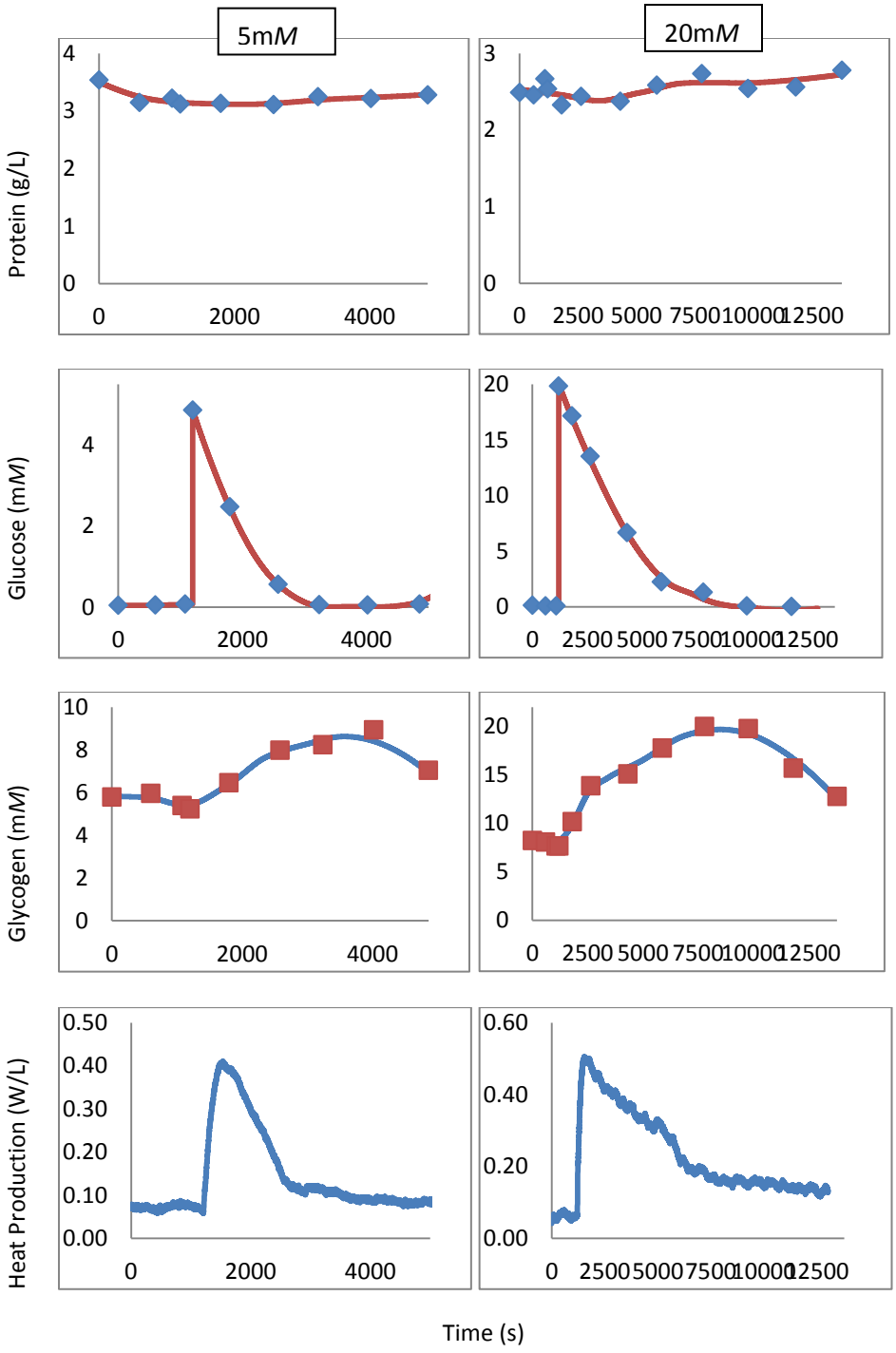

Fig. 1:
Response of mixed rumen microbes in response to 5 m*M* (a-d) or 20 m*M* (e-h) dosed at 1200 s. Data are from cow 472; data for 3 other cows is not shown. (a,e) Protein. (b,f) Glucose. (c,g) Glycogen. (d,h) Heat production (W/L).

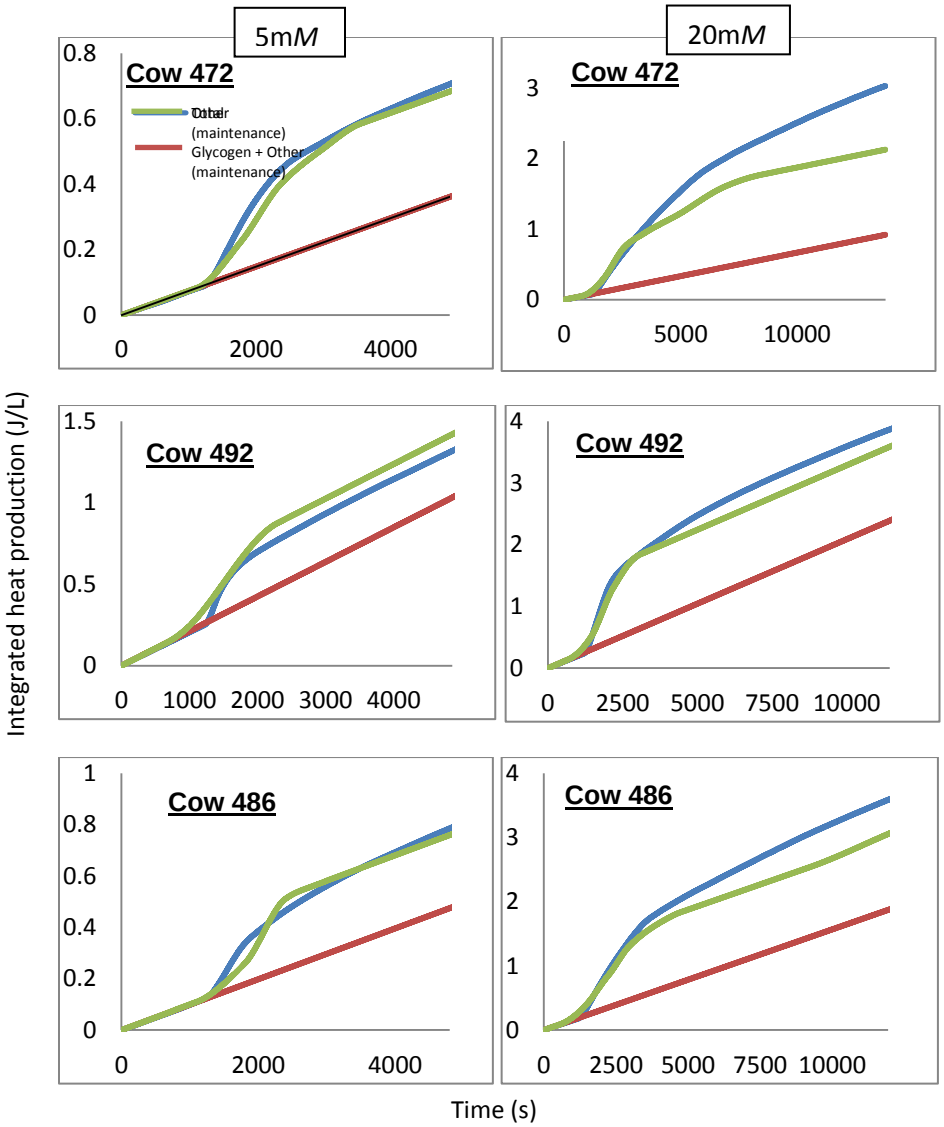

Fig. 2:
Heat production of mixed rumen microbes in response to 5 m*M* (left) or 20 m*M*(right) glucose dosed at 1200 s. Heat spilt is heat above maintenance and below total heat produced (below blue line, above green line).

Discussion

These results show how the excess energy provided by dosed glucose is being used by the microbes. The microbes very quickly consume excess energy, storing some of it as glycogen, and producing a lot of heat but without showing an increase in protein concentration. Microbes are not very efficient in utilizing excess energy. Increasing microbial efficiency in utilizing or storing excess energy could be beneficial for producing more microbial protein to be available to the ruminant animal.

Microbes appear to be able to efficiently use small amounts of energy, but when faced with excess energy, more and more of it is lost as excess heat production and less of it is put towards glycogen synthesis for later use in growth and protein production.

Conclusion

Mixed rumen microbes are not perfectly efficient in utilizing excess energy. Initially they direct all the glucose towards glycogen synthesis, but overtime they progressively divert more glucose to energy spilling. If we can improve the energy efficiency of rumen microbes and control this wasteful spilling, we can improve the energy efficiency of ruminant livestock operations.

Future Directions

Future research in this field is planned to extend these studies to different microbial populations, including bacteria or protozoa populations, to compare to these results of mixed microbial populations. The effect of different substrates, other than glucose will be examined as well. Exploring ways to control the wasteful spilling of ruminant microbes could prove very advantageous to livestock operations and have beneficial environmental effects as well.

Expected future results: More studies with different microbial populations are to be performed. It is expected that protozoa alone should spill less energy than bacteria alone because they digest substrate more slowly than bacteria. It is also expected that mixed microbes should spill less energy than bacteria alone because protozoa should deprive bacteria of substrate by quickly consuming a large amount of substrate and reduce bacterial energy spilling.

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