

How Does Sucrose Level Affect The Nutrient Yield From Fermentation?

February 2000

Mary Beth Hall, Department of Animal Sciences, University of Florida

Benefits

If we understand factors that affect whether ruminal microbes convert sucrose to microbial mass (including protein) or to microbial glycogen ("starch") we will be able to more successfully formulate rations and predict animal performance when using feeds high in sucrose and similar sugars. There is much talk about feeding sugars of late; if we develop a factual basis for managing their feeding, the rations are more likely to work well, and more sugar sources to be used.

Objectives

To examine the relationship between sucrose concentration and the change over time in:

- ◆ microbial crude protein yield
- ◆ α -glucan (microbial glycogen) storage in bacteria
- ◆ fermentation products
- ◆ fermentation rate

Microbes are capable of storing a variety of carbohydrates, including sucrose, as α -glucan (microbial glycogen or "starch"). Previous studies have indicated that ruminal microbes accumulate glycogen during rapid growth on soluble carbohydrates (Lou et al., 1997) and then use it to survive once the soluble carbohydrate falls to low levels in their environment (Thomas, 1960). Lou et al. (1997) have suggested that such glycogen storage and use represents a microbial survival strategy that may have implications for availability of microbial protein in ruminant nutrition. In vitro studies in our laboratory indicate that mixed ruminal microbes provided with sucrose + isolated bermudagrass NDF (iNDF) gave a lower maximum yield of microbial crude protein from their fermentation than did cultures provided with starch + iNDF (Herejk and Hall, unpublished, see attached figure). Whereas fermentations of iNDF with starch or pectin followed a distinctive growth curve that defined the lag phase - rapid growth - peak - and decline of crude protein in the cultures, sucrose fermentations showed a plateau in protein yield from hours ~12 through 20 of a 24 hour fermentation. The lower yield of crude protein and plateau period suggest that the microbes stored glycogen rather than growing microbes from all the sucrose. They then used these reserves to maintain themselves from hours 12 through 20. If we understand the factors that shift the microbes from growth to maintaining themselves on stored carbohydrates, we can capitalize on the pattern of microbial protein and carbohydrate yield to deliver protein and energy to the animal.

The objective of this study is to examine how the amount of sucrose in an in vitro fermentation with mixed ruminal microbes affects the yield of microbial crude protein, storage of microbial glycogen, yield of organic acids, and fermentation rates. This information will be used to better describe the yield of microbes and metabolizable nutrients from sugars in order to better predict animal performance and improve ration formulation.

Lou, J., K. A. Dawson, and H. J. Strobel. 1997. Glycogen formation by the ruminal bacterium *Prevotella ruminicola*. *Appl. Environ. Micro.* 63:1483-1488.

Thomas, G. J. 1960. Metabolism of the soluble carbohydrates of grasses in the rumen of sheep. *J. Agric. Sci.* 54:360-372.

Substrates

Three levels of sucrose blended with a fixed number of milligrams of isolated Bermudagrass NDF per fermentation vial. For example:

iNDF: 130 mg
Sucrose: 65, 130, and 195 mg sucrose

Fermentation

According to the batch culture method of Goering and Van Soest (1970) using a gas production measurement system, and destructive sampling of fermentation tubes at each hour in a separate fermentation. Both ammonium bicarbonate (nonprotein nitrogen) and hydrolyzed casein (milk protein to supply amino acids) will provide N sources. Fermentation blanks will be included in all fermentations. pH of each vial will be checked at each destructive sampling point.

Two fermentations (24 h incubations) each of the gas production and destructive sampling fermentation will be run.

Sampling

At 4 h intervals including 0 h (total of 7 time points), six fermentation vials for each substrate and blanks will be destructively sampled for analysis of:

- ◆ pH, fructose, glucose, organic acids determined on the medium
- ◆ Trichloro acetic acid-precipitable CP (TCACP) to provide an estimate of microbial crude protein.
- ◆ Glycogen content (on freeze-dried contents of fermentation vials). Glycogen will be analyzed enzymatically as α -glucan.

A total of 112 samples for each analysis listed above.

Gas production will be measured on a minimum of three fermentation vials per substrate during a 24 hour incubation. Gas production will be recorded and rates of fermentation determined.

Additional Analyses: Substrates will be analyzed for NDF, CP, OM, and DM.

Statistical Analyses

Rates of fermentation (from gas production and protein yield curves), and maximal yields of glycogen and TCACP, will be compared among substrates including fermentation run, substrate, and their interaction term. Linear and quadratic effects will be examined.

Changes in yield of glycogen, TCACP, fructose, and organic acids with time will be compared among substrates using time as a continuous, independent variable. Correlations among fructose, free glucose, and glycogen will be examined.

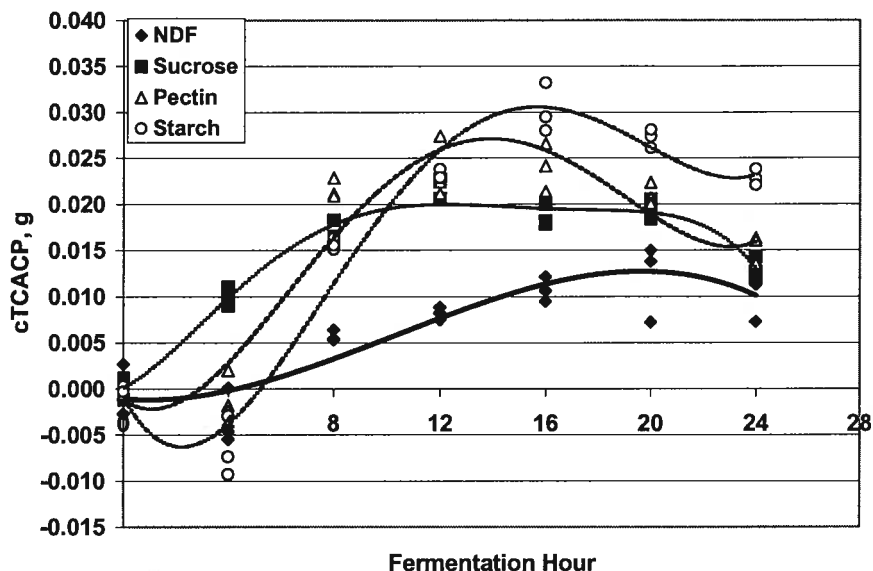
Budget

Reagents	680
Fermentation materials & runs	800
Analyses	1295
Sample preparation	225
Travel	1650
Total	4650

Labor for performing the fermentation, etc. is contributed by the researchers and is not included in the budget. Approximate value of labor: \$800. Of equipment to be used in trial and not included in budget: \$300. The experiments will be performed in Dr. Paul Weimer's laboratory at the US Dairy Forage Research Center in Madison, WI by myself, my technician, and Dr. Weimer's staff. This will allow simultaneous execution of gas production and destructive sampling fermentations.

The intent is to publish the results of this experiment in the peer reviewed literature and popular press.

Figure 1. Microbial crude protein (TCA-precipitable crude protein) yield curves from the fermentation of isolated bermudagrass NDF, and 60:40 blends of the NDF and sucrose, citrus pectin, or corn starch. Data from one fermentation. (Herejk and Hall, unpub.).



Once we have the results of this trial, some other questions that need to be addressed are:

- ◆ What is the effect of pH on protein and glycogen yield of sucrose-consuming microbes?
- ◆ Does protein source (nonprotein nitrogen vs. peptides) affect the microbes' response?