Methods for Measurement of Reserve Carbohydrate for Mixed Rumen Microbes

Author: Bethany Keyser

Project Adviser: Dr. Jeffrey Firkins

Rumen microbes use energy sources eaten by the ruminant for maintenance, growth, spilling (wastage), and storing energy as reserve carbohydrate. The reserve carbohydrate can be used later by the microbes or ruminant for energy. Two methods for quantifying microbial reserve carbohydrate are (i) enzymatic digestion with amyloglucosidase and (ii) chemical detection with anthrone reaction; however, the two methods yielded different results in our pilot studies. After reviewing the literature, we noted that there has been little comparison of accuracy among methods to quantify reserve carbohydrate in microbes. The purpose of this study was to experimentally characterize reserve carbohydrate of mixed rumen microbes and then evaluate the resulting accuracy for subsequent experiments. Rumen fluid from Jersey cows fed a lactation diet was centrifuged and washed with buffer to harvest mixed rumen microbe samples. These samples were then dosed with 20 mM of glucose, and cell pellets were harvested from samples before and after dosing. Reserve carbohydrate was then quantified using (i) anthrone or (ii) digestion using amyloglucosidase followed by quantification of glucose by glucose oxidase peroxidase. Thin layer chromatography verified that cytoplasmic carbohydrates were primarily glycogen and minor amounts of glucose and maltose. Anthrone detected significantly greater increases (24.2%) and decreases (37.8%) in reserve carbohydrate when dosed and after glucose exhaustion respectively. Anthrone led to complete mathematical recoveries of energy (97.5%) and carbon (100.2%) when microbes were dosed glucose, whereas digestion with amyloglucosidase resulted in lower recovery of energy (88.9%) and carbon (91.6%). That the results were so close to theoretical calculations supported our conclusion that anthrone quantified more microbial reserve carbohydrate not assessed by amyloglucosidase. Using anthrone to quantify microbial reserve carbohydrate has allowed us to establish protocols for use in future studies evaluating mechanisms of microbial growth efficiency and ultimately to use dietary protein more efficiently in ruminant production.