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Divergent utilization patterns of grass fructan, inulin, and other nonfiber carbohydrates by ruminal microbes[1]

M. B. Hall[2] and P. J. Weimer
US Dairy Forage Research Center, USDA-Agricultural Research Service, Madison, WI 53706

ABSTRACT

Fructans are an important nonfiber carbohydrate in cool season grasses. Their fermentation by ruminal microbes is not well described, though such information is needed to understand their nutritional value to ruminants. Our objective was to compare kinetics and product formation of orchardgrass fructan (phlein; PHL) to other nonfiber carbohydrates when fermented in vitro with mixed or pure culture ruminal microbes. Studies were carried out as randomized complete block designs. All rates given are first-order rate constants. With mixed ruminal microbes, rate of substrate disappearance tended to be greater for glucose (GLC) than for PHL and chicory fructan (inulin; INU), which tended to differ from each other (0.74, 0.62, and 0.33 h^{-1}, respectively). Disappearance of GLC had almost no lag time (0.04 h), whereas the fructans had lags of 1.4 h. The maximum microbial N accumulation, a proxy for cell growth, tended to be 20% greater for PHL and INU than for GLC. The N accumulation rate for GLC (1.31 h^{-1}) was greater than for PHL (0.75 h^{-1}) and INU (0.26 h^{-1}), which also differed. More microbial glycogen (+57%) was accumulated from GLC than from PHL, though accumulation rates did not differ (1.95 and 1.44 h^{-1}, respectively); little glycogen accumulated from INU. Rates of organic acid formation were 0.80, 0.28, and 0.80 h^{-1} for GLC, INU, and PHL, respectively, with PHL tending to be greater than INU. Lactic acid production was more than 7-fold greater for GLC than for the fructans. The ratio of microbial cell carbon to organic acid carbon tended to be greater for PHL (0.90) and INU (0.86) than for GLC (0.69), indicating a greater yield of cell mass per amount of substrate fermented with fructans. Reduced microbial yield for GLC may relate to the greater glycogen production that requires ATP, and lactate production that yields less ATP; together, these processes could have reduced ATP available for cell growth. Acetate molar proportion was less for GLC than for fructans, and less for PHL than for INU. In studies with pure cultures, all microbes evaluated showed differences in specific growth rate constants (μ) for GLC, fructose, sucrose, maltose, and PHL. *Selenomonas ruminantium* and *Streptococcus bovis* showed the highest μ for PHL (0.55 and 0.67 h^{-1}, respectively), which were 50 to 60% of the μ achieved for GLC. The 10 other species tested had μ between 0.01 and 0.11 h^{-1} with PHL. Ruminal microbes use PHL differently than they do GLC or INU.

Key words: rumen, fermentation, fructan, nonfiber carbohydrate

INTRODUCTION

Fructan is the generic name for linear and branched polymers of D-fructose comprised primarily of fructose, but often containing a terminal glucose. These are nonstructural carbohydrates produced by many plants as an energy storage material, often ancillary to starch production (Hendry and Wallace, 1993). The bonding between the sugar molecules can differ by source, such as β-(2,1) linkages predominating in chicory (*Cichorium intybus*) inulin and β-(2,6) linkages in phlein of cool season grasses (e.g., *Dactylis glomerata*; Lewis, 1993). Among commonly used forages, fructans are found in cool season grasses. They may account for a small (<1% of DM) or substantial (>20% of DM) portion of the nonfiber carbohydrate (Mackenzie and Wylam, 1957). Unlike the monosaccharides, sucrose, or starch, fructans are not digestible by mammalian enzymes, but are used by ruminal microbes. Thus, fructans can provide an important energy source for ruminal microbiota.

Relatively little has been done to characterize the fermentation of grass fructans by mixed ruminal microbes. Thomas (1960) found that Italian ryegrass (*Lolium italicum*) fructan was fermented to VFA and lactic acid, and was also converted to microbial glycogen by both protozoa and bacteria. The protozoal, and to a lesser

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[2]Corresponding author: marybeth.hall@ars.usda.gov

extent, bacterial fractions of mixed ruminal microbes have been shown to possess β-fructofuranosidase (EC 3.2.1.26) activity (Czerkawski and Lumsden, 1971), and this activity has been demonstrated in 6 species of ruminal bacteria (Ziolecki et al., 1992). More recently, Piknova et al. (2008) described a new bacterial species, "*Treponema ziolecki*i" that is capable of fermenting fructan from timothy (*Phleum pratense*), as well as pectin and several soluble sugars. However, little quantitative information is available regarding growth of ruminal microbes on fructan or overall fates of the substrate in short-term fermentations. Here we examine grass fructan as compared with other nonfiber carbohydrates as they affect fermentation kinetics and product formation of mixed ruminal microbiota in vitro, and specific growth rates among different individual species of ruminal bacteria. The mixed culture study was performed in vitro because the ruminal system in vivo is too complex to allow evaluation of the measures investigated.

MATERIALS AND METHODS

Experiment 1. Mixed Culture Fermentations

Substrates. Purified glucose (G7021, Sigma, St. Louis, MO), inulin from chicory (Orafti-HP, BENEO, GmbH, Mannheim, Germany), and purified phlein were used as substrates. Phlein isolated from orchardgrass (*Dactylis glomerata*) was a gift from P. Harrison, USDA-ARS Forage and Range Research Laboratory, Logan, Utah. The phlein was isolated using initial extractions with 80% ethanol to remove low molecular carbohydrates, water extraction to extract the phlein, then removal of coloring material and proteins from the water extract using anion exchange medium (diethylaminoethyl cellulose) and precipitation of protein using $ZnSO_4$. The phlein was further purified to remove lower molecular weight carbohydrates through ultrafiltration under pressure with stirring (Amicon stirred cell model 8200, EMD Millipore, Darmstadt, Germany) using ultrafiltration discs with a nominal molecular weight cutoff of 1 kDa (PLAC07610, EMD Millipore, Darmstadt, Germany). For ultrafiltration, solutions of the phlein in ultrapure water were stirred at 4°C under N_2 gas at 193 kPa (28 lb/in^2).

Fermentations. Duplicate fermentation runs were performed using Goering and Van Soest (1970) medium in sealed borosilicate glass fermentation tubes (121 mm long, 28 mm outer diameter, 2.8 mm wall thickness, the ends of the tubes were formed to be sealed with crown caps; custom made by Wilmad-LabGlass, Vineland, NJ). Each vessel contained 20 mL of medium, 1 mL of reducing solution, and 5 mL of ruminal inoculum. The medium plus reducing solution supplied 6.54 mg of N from tryptone (pancreatic digest of casein, T-9410, Sigma-Aldrich Co.), 3.54 mg of N from ammonium bicarbonate, and 0.56 mg of N from cysteine-HCl in each tube. Vessels were incubated in tube racks in an incubating orbital shaker at 39°C and 160 rpm (Innova 40 bench top incubator shaker, 19 mm orbit, New Brunswick Scientific, Edison, NJ). Tubes were secured within racks which were set on their sides within the incubator so that the long axis of the tubes was positioned parallel to the platform and motion of the shaker to continuously mix substrate, inoculum, and medium. Glucose, inulin, and phlein substrates (78 mg of DM per tube, SD of sample weights = 0.3 mg) were weighed into 3 replicate vessels each for each sampling time after 0 h. The replicates were used for analysis of accumulated microbial N, glycogen, and organic acids/residual carbohydrate; 3 pairs of tubes with no substrate (fermentation blanks) collected at 0 h were subject to the same 3 analyses. Two fermentation blanks were included at each time point for organic acid/residual carbohydrate analysis. Fermentation runs were performed 1 yr apart.

Inoculum for each fermentation was obtained from 2 lactating Holstein cows maintained under protocols approved by the University of Wisconsin College of Agriculture and Life Sciences Animal Care and Use Committee. In the 2 separate years, donor cows were fed a TMR consisting on a DM basis of 25 to 29% corn silage, 19 to 24% alfalfa haylage, 6% whole linted cottonseed, and 44 to 45% mixed concentrate supplemented with vitamins and minerals to meet NRC (2001) recommendations; in the second year, 1% wheat straw was included in the diet. For each cow in each year, 50 g of inulin (Orafti-HP) and 50 g of dextrose were mixed into the TMR and the diet was top-dressed with ~0.1 kg of timothy (*Phleum pratense*) hay in the 15 d before inoculum collection. Ruminal contents obtained from each cow within 2 h postfeeding were strained through 4 layers of cheesecloth and the ruminal liquor maintained under CO_2. Equal volumes of ruminal liquor from each cow were measured and filtered through an additional 4 layers of cheesecloth with ruminal fluid from both cows blended together in a common flask maintained at 39°C in a water bath with CO_2 bubbled continuously through the liquor. Inocula pH values in the fermentation runs were 5.98 and 5.73, which were approximately the average ruminal digesta pH of the 2 cows used in each run.

Fermentation vessels were destructively sampled at 0, 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, 6, and 8 h, except for the phlein substrates for which 0.5 and 1.5 h time points were omitted due to limitations in amount of available substrate. At each sampling hour, harvested tubes were placed immediately on ice and chilled for a minimum of 10 min to stop the fermentation. One tube for each