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Nitrogen source and concentration affect utilization of glucose by mixed ruminal microbes in vitro[1]

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ABSTRACT

The availability of rumen-degradable protein (RDP) changes the use of carbohydrates by ruminal microbes. However, the effects of RDP on the simultaneous use of carbohydrate and formation of microbial products are not well described, although such information is needed to understand the potential effect on nutrient supplies for ruminants. The objective of this in vitro study was to compare the effects of different levels of RDP (0.15, 0.31, 0.46 g of N/L) from tryptone (Tryp) or urea (Ur) on product formation from glucose in fermentations with mixed ruminal microbes. The study had a randomized complete block design with 2 replicated fermentation runs and destructive sampling at 0, 0.5, 1, 2, 3, 4, and 5 h. All rates given are first-order rate constants. Glucose disappearance rates and organic acid carbon (C) production rates tended to be or were greater for Tryp (0.64 and 0.58 h^{-1}) than for Ur (0.51 and 0.22 h^{-1}), respectively, but did not differ by N level. Maximum detected microbial N production was 67% greater for Tryp (2.35 mg) than for Ur (1.41 mg), which did not differ from the basal medium (1.47 mg). The pattern of glycogen accumulation over time tended to differ between Tryp and Ur: glycogen peaked and declined earlier in the fermentations with Tryp, resulting in less glycogen remaining at 5 h with Tryp (7.2 mg) than with Ur (11.0 mg). At the point of maximum microbial N accumulation, Tryp and Ur did not differ in the amount of glucose C used (29.4 and 28.9 mg), but did differ in the amounts of cell C (10.1 and 6.0 mg), organic acid C (17.4 and 13.8 mg), glycogen C (3.81 and 6.07 mg), and total microbial product C (35.4 and 29.6 mg) present. This resulted in increased efficiency for Tryp compared with Ur for cell C produced per used glucose C, corrected for glycogen C (0.40 and 0.27 mg/mg), and it resulted in a tendency for increased yield of cell C per organic acid C (0.59 and 0.44 mg/mg). Total product C exceeded used glucose C for Tryp, likely because of incorporation or fermentation of C from the provided AA. Overall, RDP source altered the temporal patterns of glucose use and the patterns and amounts of microbial product formation.
Key words: protein, glucose, rumen, fermentation

INTRODUCTION

Glucose is a monosaccharide commonly found in fresh legume and grass forages (1 to 5% of DM; Smith, 1973), molasses (1 to 11%; Dionex Corp., 2003), dextrose (100%), and at varying concentrations in byproduct feedstuffs (e.g., 4 to 9% of DM in almond hulls; M. B. Hall, unpublished data). It is reported to be among the most rapidly fermented carbohydrates, with ruminal rates of disappearance of up to 734% h^{-1} in vivo (Weisbjerg et al., 1998). However, disappearance may not equate to fermentation. Both ruminal protozoa (Oxford, 1951) and bacteria (Gong and Forsberg, 1993) can convert glucose to the intracellular storage polysaccharide, glycogen. Production of this α-(1,4), α-(1,6)-linked glucan delays fermentation of the substrate and requires input of 1 ATP per glucose for synthesis (Stouthamer, 1973). Increases in glycogen formation could reduce microbial protein yield because of reductions in ATP available for protein synthesis.

The availability of RDP alters how ruminal microbes utilize readily available carbohydrate and energy from feeds. The α-dextran (glycogen) content of ruminal bacteria declined by 33 to 45% when dietary protein was increased by adding casein or urea to the hay/straw/flaked maize diets of weaned bull calves (McAllan and Smith, 1974). Lactic acid, which is associated with more rapid flux of carbohydrate through glycolysis (Counotte and Prins, 1981), was increased in the rumens of lactating cows that received a greater proportion of dietary CP as RDP (Hall, 2013). The provision of AA increased the growth efficiency of *Streptococcus*

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bovis and decreased the specific rate of heat production (energy spilling; Russell, 1993). However, despite these associations, little quantitative information is available about the effects of different levels and types of RDP on the time courses of carbohydrate utilization and product formation, and the balance between them.

In vitro studies are often used to investigate the utilization of substrates by microbes, but are not equivalent to in vivo studies for describing what actually happens in the animal. The utility of in vitro fermentations rests in their use as model systems to study specific questions related to the in vivo ruminal system. In vitro studies are conducted if the in vivo system is too complex to allow evaluation of the hypotheses and measures investigated; in vivo studies are typically more suitable for evaluating total diets than specific feed fractions. To put this in perspective, in vitro models may be seen as similar to mathematical models: "Essentially, all models are wrong, but some are useful" (Box and Draper, 1987). Regarding the relationship of model systems to the actual system of interest, statistician George Box commented: "Now it would be very remarkable if any system existing in the real world could be *exactly* represented by any simple model," and "For such a model, there is no need to ask the question 'Is the model true?' If 'truth' is to be the 'whole truth' the answer must be 'No.' The only question of interest is 'Is the model illuminating and useful?'" (Box, 1979). These statements about mathematical models apply to in vitro models used to explore in vivo systems. In vitro findings can provide information about specific aspects of a system to help us refine our understanding and hypotheses, but they must eventually be related to and evaluated in the context of the in vivo system they attempt to describe.

The objective of this study was to evaluate the effect of the type and amount of supplemented RDP on substrate use, product formation, and fermentation kinetics in fermentations of glucose by mixed ruminal microbes. This mixed-culture study was performed in vitro.

MATERIALS AND METHODS

Fermentations

Five treatments of different nitrogen (N) types and concentrations were applied in duplicate fermentation runs using modified Goering and Van Soest (1970) media in sealed borosilicate glass fermentation tubes as described by Hall and Weimer (2016). Each tube contained 20 mL of medium, 1 mL of reducing solution, 0.5 mL of autoclaved glucose solution or water, and 5 mL of ruminal inoculum. Purified glucose (G7021; Sigma-Aldrich, St. Louis, MO) in a 159 mg/mL autoclaved solution was used to deliver 79.5 mg of glucose per tube. Media were used to deliver the protein treatments. The basal medium was modified from Goering and Van Soest (1970) to contain no tryptone (basal; pancreatic digest of casein, T9410; Sigma-Aldrich). The basal medium plus reducing solution supplied 3.54 mg of N from ammonium bicarbonate and 0.56 mg of N from cysteine-HCl in each tube. The other 4 treatments were prepared from the basal medium: basal + 1.17 g of tryptone/L (**TrypL**), basal + 2.34 g of tryptone/L (**TrypH**), basal + 0.329 g of urea/L (**UrL**), or basal + 0.658 g of urea/L (**UrH**), where L and H indicate low and high levels of N. The basal medium and treatment media provided 0.15, 0.31, 0.46, 0.31, and 0.46 g of N/L in the 26.5-mL liquid volume in each tube, for basal, TrypL, TrypH, UrL, and UrH, respectively. 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). Fermentation vessels were destructively sampled at 0, 0.5, 1, 2, 3, 4, and 5 h. Three replicate vessels for each glucose × N treatment were included at each sampling time after 0 h. Individual replicates were analyzed for accumulated microbial N, glycogen, or organic acids/residual carbohydrate. Nine tubes with no substrate (fermentation blanks) in basal medium were collected at 0 h, and the same 3 analyses were applied to 3 tubes each. Two fermentation blanks for each treatment were included at each time point >0 h for organic acid/residual carbohydrate analysis.

Inoculum for each fermentation was obtained from 2 ruminally cannulated, lactating Holstein cows maintained under protocols approved by the University of Wisconsin College of Agriculture and Life Sciences Animal Care and Use Committee. Donor cows were fed a TMR consisting (on a DM basis) of 25.2% corn silage, 24.1% alfalfa silage, 6.4% whole linted cottonseed, and 44.3% mixed concentrate, supplemented with vitamins and minerals to meet NRC (2001) recommendations. Daily DMI averaged 23.8 ± 1.4 kg/cow per day. Dextrose as 1% of diet DM was mixed into the TMR in the 15 d before inoculum collection. Ruminal contents, obtained manually via the ruminal cannula primarily from the ventral portion of the rumen of each cow within 2 h after feeding, 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. Inoculum pH values in the fermentation runs were 5.97 and 5.89, approximately