Effects of a *Saccharomyces cerevisiae* Culture on In Vitro Mixed Ruminal Microorganism Fermentation

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ABSTRACT

Previous research has shown that *Saccharomyces cerevisiae* culture increases lactate utilization and cellulose digestion by pure cultures of ruminal bacteria. Based on these pure culture results, in vitro mixed ruminal microorganism fermentations were conducted to determine the effects of 0.35 and 0.73 g/L of *Sacc. cerevisiae* culture on the fermentation of ground corn, maltose, alfalfa hay, bermudagrass hay, and lactate. In addition, experiments were performed to evaluate the effects of *Sacc. cerevisiae* culture and monensin on the mixed ruminal microorganism fermentation. In the presence of ground corn, both concentrations of *Sacc. cerevisiae* culture had little effect on final pH or fermentation products, except the 0.35 g/L treatment increased valerate concentration. *Saccharomyces cerevisiae* culture had little effect on final pH or fermentation products in maltose or lactate fermentations. When alfalfa hay was the substrate, 0.73 g/L of *Sacc. cerevisiae* culture increased propionate concentration and both treatments decreased the acetate to propionate ratio. In the case of Coastal bermudagrass hay, 0.73 g/L *Sacc. cerevisiae* culture increased concentrations of acetate, propionate, CH_4, butyrate, isovalerate, valerate, and decreased the acetate to propionate ratio, whereas both treatments increased total volatile fatty acid concentrations. Similar to alfalfa hay, in vitro dry matter disappearance of Coastal bermudagrass hay was numerically increased in the presence of *Sacc. cerevisiae* culture. Monensin altered the fermentation by decreasing concentrations of CH_4 and lactate and increasing concentrations of propionate. There was no interaction between *Sacc. cerevisiae* culture and monensin. In conclusion, the incorporation of *Sacc. cerevisiae* culture into mixed ruminal microorganism fermentations of ground corn, maltose, or lactate had little effect on final pH and fermentation products. However, in the presence of alfalfa hay or Coastal bermudagrass hay *Sacc. cerevisiae* culture increased concentrations of several fermentation products and numerically increased in vitro dry matter disappearance of forage fiber.
(**Key words:** *Saccharomyces cerevisiae* culture, rumen, microorganisms, in vitro)

Abbreviation key: IVDMD = in vitro dry matter disappearance.

INTRODUCTION

Saccharomyces cerevisiae culture has been used as a dietary supplement in production ruminants for many years. However, interest in *Sacc. cerevisiae* culture as a potential alternative to antimicrobial feed additives has increased within the past 10 yr. Some of the benefits associated with *Sacc. cerevisiae* include increased DM and NDF digestion (4), increased initial rates of fiber digestion (24), and increased milk production in dairy cattle (8, 9, 17, 24). In vitro experiments have also reported that, in some cases, *Sacc. cerevisiae* culture favorably altered the mixed ruminal microorganism fermentation and stimulated lactate uptake and cellulose digestion by pure cultures of predominant ruminal bacteria (2, 10, 15, 16). Unfortunately, in vivo and in vitro effects of *Sacc. cerevisiae* culture are not always consistent (10).

Recent research (2) demonstrated that a filter-sterilized filtrate of Diamond V XP (Diamond V Mills, Inc., Cedar Rapids, IA) *Sacc. cerevisiae* culture stimulated growth of the predominant ruminal bacteria *Selenomonas ruminantium* and *Megasphaera elsdenii* on lactate. In addition, *Sacc. cerevisiae* culture stimulated growth of the cellulolytic bacteria *Fibrobacter succinogenes* and *Ruminococcus albus* on cellobiose in medium that did not contain yeast extract or Trypticase (2). *Sacc. cerevisiae* culture increased the initial rate but not the extent of cellulose digestion by *F. succinogenes* and *Ruminococcus flavefaciens* (2). Based on these pure culture results, one objective of this study was to evaluate the effects of *Sacc. cerevisiae* culture on the in vitro mixed ruminal microorganism fermentation of ground corn, maltose, lactate, alfalfa hay, or Coastal bermudagrass hay. Because previous research showed that monensin and malate had an additive effect on the mixed ruminal microorganism fermentation (3) and because *Sacc. cerevisiae* culture contains malate (2), experiments were

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also conducted to evaluate the effects of *Sacc. cerevisiae* culture and monensin on the mixed ruminal microorganism fermentation.

MATERIALS AND METHODS

Ruminal contents were collected from a 700-kg ruminally fistulated Holstein steer fed 36.3 kg of wheat silage and 4.5 kg of concentrate supplement once daily (as-fed basis). The ruminal contents were obtained 1.5 h after feeding and squeezed through four layers of cheesecloth into an Erlenmeyer flask with an O_2-free CO_2 headspace. The flask was not disturbed for 30 min while being incubated in a 39°C water bath, which permitted feed particles to rise to the top of the flask. Particle-free fluid from the flask was anaerobically transferred (20% vol/vol) to a medium (pH 6.5) containing 292 mg of K_2HPO_4, 240 mg of KH_2PO_4, 480 mg of $(NH_4)_2SO_4$, 480 mg of NaCl, 100 mg of $MgSO_4 \cdot 7H_2O$, 64 mg of $CaCl_2 \cdot 2H_2O$, 4,000 mg of Na_2CO_3, and 600 mg of cysteine hydrochloride per liter (19, 20). Particle-free fluid and medium were mixed, and 40 ml was transferred anaerobically to 160-ml serum bottles that contained either no substrate, 0.4 g of ground corn, 0.4 g of maltose (Sigma Chemical Co., St. Louis, MO), 0.4 g of DL-lactate (sodium salt, Sigma), 0.4 g of alfalfa hay, or 0.4 g of Coastal bermudagrass hay. Weighed amounts of Diamond V XP yeast culture (Diamond V Mills, Inc., Cedar Rapids, IA) were added to achieve final concentrations of 0.35 and 0.73 g/L. These concentrations are consistent with current recommended feeding levels. Monensin (Sigma) was dissolved in undiluted ethanol (0.25 mg/ml) and added (0.8 ml) to serum bottles to achieve a final concentration of 5 ppm, and non-monensin treated bottles received an equal amount of ethanol as control. Incubations containing only yeast culture were also run. Bottles were sealed (CO_2 atmosphere) with butyl rubber stoppers and aluminum seals to contain the gas pressure and placed in a 39°C water bath for either 24 h (ground corn, maltose, lactate) or 48 h (alfalfa, bermudagrass) and periodically mixed.

After 24 (ground corn, maltose, lactate) or 48 h (alfalfa, bermudagrass) of incubation, a gas sample (0.5 ml) was removed from each bottle and analyzed for hydrogen (H_2) and methane (CH_4) on a Gow Mac thermal conductivity series 580 gas chromatograph (Gow Mac Instrument, Bridgewater, NJ) equipped with a Porapak Q column (60°C, 20 ml/min of N_2 carrier gas). The bottles were then uncapped, and the pH was measured immediately with a pH meter. Bottles then were emptied into centrifuge tubes, centrifuged (10,000 × *g*, 4°C, 15 min) and the cell-free supernatant fluids stored at –20°C.

To examine the effects of yeast culture on the rate of forage fiber digestion by mixed ruminal microorganisms, alfalfa and bermudagrass incubations were also conducted over time. Serum bottles were prepared as described above and incubated for 0, 24, or 48 h. After each period, bottles were uncapped and poured into centrifuge tubes and centrifuged (10,000 × *g*, 4°C, 15 min). Pellets were resuspended in deionized water and poured back into the original serum bottles and stored at 4°C. Undigested residue was collected on a preweighed oven-dried Whatman no. 1 filter (Whatman Lab Sales, Inc., Hillsboro, OR) by vacuum filtration. The filter and undigested residues were then oven-dried (105°C) for 24 h to remove excess moisture and weighed. In vitro dry matter disappearance (**IVDMD**) was calculated as original dry sample weight minus dry residue weight divided by the original sample weight. This value was then multiplied by 100 to derive IVDMD percentage.

The VFA in supernatant fluid samples were measured by GLC with a Shimadzu GC-14A (Shimadzu Scientific Instruments, Columbia, MD) gas chromatograph (column temperature = 125°C, injector temperature = 170°C, detector temperature = 175°C) equipped with an autosampler and GP 10% SP-1200/1% H_3PO_4 80/100 mesh size Chromosorb W AW column (23). Ammonia was measured by a modified colorimetric method (5, 18). Lactate was analyzed by HPLC with an organic acid column as previously described (12).

All fermentations were performed on duplicate days with two replicates per day (n = 4). Data were analyzed by a general linear model procedure (22). Incubations that contained no substrate, alfalfa hay, or Coastal bermudagrass hay were analyzed by fitting a model that contained *Sacc. cerevisiae* culture dosage (0, 0.35, 0.73 g/L). Ground corn and lactate incubations were analyzed separately by fitting a model that contained additive (ethanol or monensin), *Sacc. cerevisiae* culture dosage, and interaction between the two to the dependent variables (pH, fermentation products). Because additive effects and the interaction involving additive effects were not significant ($P > 0.05$) for any of the dependent variables, only *Sacc. cerevisiae* culture dosage was fit to the dependent variables for each substrate. Each forage (alfalfa hay, Coastal bermudagrass hay) was also analyzed separately by fitting a model that contained *Sacc. cerevisiae* culture dosage, incubation time (0, 24, 48 h), and the interaction between the two to the dependent variable (IVDMD).

RESULTS AND DISCUSSION

In the absence of added substrates, *Sacc. cerevisiae* culture had no effect on pH, isovalerate, acetate to pro-