

Effect of Forage Quality and Monensin on the Ruminal Fermentation of Fistulated Cows Fed Continuously at a Constant Intake

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Introduction

Monensin improves the feed efficiency of ruminants, but decreases in methane and increases in propionate cannot explain all of this benefit. Early research indicated that monensin decreased ruminal ammonia *in vivo*, and this finding was corroborated by mixed culture and pure culture studies. The amino acid-sparing effect of monensin has not been well documented. Monensin increased passage of feed protein, but this increase was offset by decreases in bacterial protein. In some cases, monensin had no effect on ruminal ammonia, and it appeared that monensin-dependent improvements in nitrogen retention were a result of energy utilization rather than the protein sparing *per se*. Previous work indicated that cattle fed timothy hay and soybean meal 12 times per day had steady-state ruminal fermentation patterns; and, under these conditions, monensin decreased ammonia concentration, specific activity of deamination and most probably the number of obligate amino acid-fermenting bacteria. Preliminary experiments indicated that cattle fed alfalfa hay did not respond in a similar fashion, and the present research was designed to examine the relationship between forage quality and monensin-dependent amino acid sparing.

Materials and Methods

Two nonlactating cows (685 ± 59 kg) fitted with ruminal cannulas were fed forage 12 times per day. Dietary treatments consisted of three combinations of timothy and alfalfa hays (100:0, 50:50, and 0:100) and two levels of monensin (0 and 350 mg cow⁻¹ d⁻¹). Monensin (350 mg) was dissolved in 36 mL of ethanol, and 3 mL of the solution were added on the top of each meal. Water was offered free choice. No salt or other minerals were provided.

Ruminal fluid (500 ml) was squeezed through four layers of cheesecloth and analyzed for pH with a combination electrode. Volatile fatty acids

in cell-free samples were measured by high-performance liquid chromatography. Ammonia in cell-free supernatant fluid was measured by colorimetry. Ruminal K and Na concentrations in cell-free supernatant fluid were determined by flame photometry. Bacterial protein was assayed by the method of Lowry. Bacterial pellets were digested with 3 N HNO₃ (room temperature, 24 h) before analyzing for K by flame photometry.

Separate samples of ruminal fluid were collected from the rumen and allowed to incubate anaerobically in a 39 °C water bath for 60 min. Feed particles were buoyed to the top of the flask by gas production and protozoa sedimented to the bottom of the flask. Mixed ruminal bacteria (10 mL) from the center of the flask were transferred in triplicate to 15 mm x 180 mm tubes. At time zero, an anaerobic solution of Trypticase was added to the tubes (15 g/L final concentration). The tubes were incubated at 39 °C for 6 h. The incubation was terminated by a centrifugation step (10,000 x g, 20 min, 0 °C) that removed the bacteria. The bacterial pellet was washed with .9% NaCl (wt/vol) and stored at -15 °C. The cell-free supernatant fluid was stored separately at -15 °C. Ammonia and bacterial protein were measured as described previously. Preliminary experiments indicated that ammonia production was first order with respect to time and cell concentration so long as the incubation period was less than 10 h.

Cows (n = 2) were the experimental units for the statistical analysis. Each experimental unit was the mean of pooled data from four sample units (4 d of collection). All statistical analyses were performed with the GLM procedures of Minitab. Data were analyzed as a randomized complete block design. Sources of variations included animals (blocks), alfalfa (0% vs. otherwise), alfalfa level (50 vs. 100% alfalfa), monensin, alfalfa x monensin, and alfalfa level x monensin. The residual error term (block x treatment

effects) was used for testing treatment effects and interactions at $\alpha = .05$. If the F test indicated that a model interaction was significant ($P < .05$), interactive treatment means were analyzed by the method of Tukey.

Results

The alfalfa hay had 1.4 times less NDF than the timothy hay, and the concentration of VFA in the ruminal fluid was greater ($P < .05$) when alfalfa was substituted for timothy. The substitution of alfalfa for timothy did not affect ruminal pH or the acetate:propionate ratio ($P > .05$). Alfalfa decreased bacterial protein in the fluid phase ($P < .05$). The alfalfa had 1.5 times as much K as the timothy hay. Alfalfa increased ($P < .001$) the K concentration ($K / [K + Na]$) of the ruminal fluid and decreased the K content of the bacteria ($P < .05$). The alfalfa had 1.4 times as much CP as the timothy hay, and total ruminal ammonia increased ($P < .05$) when alfalfa was substituted for timothy (Table 2). Dissociated ammonia, as predicted from pH and the Henderson-Hasselbalch equation ($pH = pK_a + \log [NH_3] / [NH_4^+]$), was unaffected by alfalfa substitution ($P > .05$). Substitution of timothy with alfalfa hay increased ($P < .001$) the specific activity of deamination (deamination rate).

At all combinations of timothy and alfalfa hays, monensin caused an increase ($P < .001$) in total VFA and a decrease ($P < .05$) in ruminal pH. Acetate increased to a smaller extent than propionate, but the decrease in acetate:propionate ratio was only significant ($P < .05$) when alfalfa was 50% or less of the forage. Monensin caused a small but significant ($P < .001$) increase in the K concentration of the ruminal fluid, but it did not have an effect on the K content of bacteria in ruminal fluid ($P > .05$). Monensin did not decrease total ammonia when alfalfa was 0 or 50% of the diet and even increased it in 100% alfalfa. The total ammonia, however, was not corrected for changes in ruminal pH. Monensin decreased ($P < .05$) the dissociated ammonia concentration when only timothy hay was fed, but monensin had no impact ($P > .05$) on dissociated ammonia if

alfalfa was 50 or 100% of the diet. Monensin alleviated alfalfa-dependent decreases in bacterial protein ($P < .05$) and decreased ($P < .001$) the specific activity of deamination (deamination rate) at all combinations of alfalfa and timothy.

Discussion

Because monensin did not decrease total ammonia accumulation when animals were fed 100% alfalfa, it seemed that some component in alfalfa was counteracting potential decreases in deamination. Because monensin catalyzes the efflux of K from monensin-sensitive ruminal bacteria, high ruminal potassium may inhibit monensin action. Our animals were not given supplemental Na, and the $K \div (K + Na)$ values were high (.56 to .68), and animals fed alfalfa had a greater K intake and higher ruminal K ($K \div [K + Na]$) than animals fed only timothy.

Animals fed monensin had lower ruminal pH values than untreated controls, and these differences could have influenced the absorption rate of ammonia from the rumen. Dissociated ammonia is a much more lipophilic substance than ammonium ion, and it would be more rapidly absorbed. Based on ruminal pH and the pK_a of ammonia, it was possible to estimate the ruminal concentration of dissociated ammonia. Because monensin decreased steady-state dissociated ammonia when timothy was 100% of the diet, it seemed that monensin had the potential to spare amino acids from deamination. Monensin did not decrease dissociated ammonia when alfalfa was fed, but it was able to alleviate alfalfa-dependent decreases in bacterial protein.

The observation that monensin decreased the specific activity of deamination at all combinations of alfalfa and timothy further supports the idea that monensin had the potential to spare amino acids. The effect of monensin on deamination is consistent with its ability to inhibit Gram-positive, highly active, obligate amino acid-fermenting ruminal bacteria in vitro and in vivo. Monensin is clearly an antimicrobial substance, but ruminal VFA concentrations were

always greater when monensin was added to the diet. Increases in ruminal VFA were correlated with the increases in bacterial protein. Monensin (across all three diets) caused a 20% increase in total VFA and a 20% increase in bacterial protein in the fluid phase. This comparison indicated that: 1) monensin enhanced VFA production, 2) the bacteria had more ATP for growth, and 3) the animal would have more bacterial protein.

The rumen often has a dense population of protozoa, but protozoal counting procedures are

confounded by the propensity of protozoa to associate with feed particles and by the difficulty in obtaining representative samples. In vitro results indicated that protozoa were sensitive to monensin, but this effect has been difficult to document in vivo. Because ruminal protozoa store large amounts of carbohydrate as intracellular glycogen, appear to recycle bacterial nitrogen, and decrease microbial flow from the rumen, it is conceivable that a monensin-dependent decrease in protozoa caused the increase in bacterial protein and VFA that we observed.