## PRODUCTION TECHNICAL NOTES

# Effect of the Combination of Monensin and Isoacids on Rumen Fermentation In Vitro[1,2]

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### ABSTRACT

Effects of isoacids, monensin, or a combination of them on fermentation by mixed rumen bacteria were investigated using a continuous culture technique. The culture was allowed to stabilize for 4 d before treatments were imposed. Comparisons between treatments were made on d 11 and 12 of the culture. Isoacids (equal proportions of isobutyric, 2-M-butyric, isovaleric, and valeric acids) at 15 mg/dl of culture media increased acetate (6.17 vs. 5.48 meq/dl) and total VFA production (8.93 vs. 7.87 meq/dl) compared with that of controls. Monensin at 150 μg/dl reduced acetate (3.74 vs. 6.02 meq/dl) and VFA (6.84 vs. 8.54 meq/dl) but increased propionate (2.28 vs. 1.74 meq/dl) relative to control. The combination of isoacids and monensin increased acetate relative to monensin alone (5.24 vs. 3.74 meq/dl) but did not alter the effect of monensin on propionate concentration (2.32 vs. 2.28 meq/dl). It is concluded that monensin decreases acetate production by 35% and when isoacids are added to the cultures containing monensin, acetate production is restored.

### INTRODUCTION

Monensin has been used extensively in diets for feedlot cattle for several years. Recently, isoacids have been commercialized as a nutritional supplement for lactating dairy cattle (9). Both monensin and isoacids affect rumen fermentation. However, little is known about the effects of the combination of the two compounds in the rumen. In vitro rumen fermentations have shown that monensin decreases the acetate:propionate ratio (5, 10). These results have been attributed to a toxic effect of the ionophore on ruminococci (8). Unlike monensin, isoacids (isobutyric, isovaleric, 2-methylbutyric, and valeric acids) increase rumen acetate production, probably due to an enhanced growth of ruminococci (1, 3, 6).

The mode of action of the ionophore is thought to be through an interruption in $Na^+/K^+$ transport at the membranes (2). Isoacids are carbon sources for the synthesis of branched chain amino acids and higher fatty acids (9).

The objective of this study was to examine the effects of adding isoacids to an in vitro rumen fermentation system containing monensin.

### MATERIALS AND METHODS

Ruminal fluid was obtained 3 h postfeeding from a mature nonpregnant and nonlactating rumen-fistulated Holstein cow that weighed 550 kg. Samples from different parts of the rumen were strained through two layers of surgical gauze into 1-L glass bottles kept at 40°C.

The semicontinuous culture technique of Short (11) was used with minor modifications. The culture was maintained in the same flask throughout the experiment. The substrate was timothy hay.

On the 1st d of culture establishment, 1 L of medium was prepared according to standard anaerobic procedures outlined by Bryant (4) (Table 1). The incubation vessel was a 250-ml Erlenmeyer flask with a liquid port for culture sampling and a gas sampling port. In addition, each fermentor was fitted with a graduated manometer. Inoculum and medium were mixed under $CO_2$. A 100-ml aliquot of this mixture was then transferred anaerobically into

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TABLE 1. Composition of basal media.

| Item | |
|---|---|
| Timothy hay,[1] | 20 |
| Urea, g | .40 |
| Ammonium sulfate, g | .06 |
| Mineral solution 1,[2] ml | 80 |
| Mineral solution 2,[3] ml | 80 |
| Micromineral solution,[4] ml | 20 |
| Sodium bicarbonate solution (6.32%), ml | 110 |
| Sodium sulfide solution (2.5%), ml | 5 |
| Vitamin solution[5] | 1 |
| Distilled water | 685 |

[1]Nutrient content (% of DM): CP, 6.8; NDF, 66.8; ADF, 40.5; lignin, 8.5 cellulose, 34.8; hemicellulose, 24.8; cell contents, 34.7; nitrogen, 1.5.

[2]Composition (g/L): $K_2HPO_4 \cdot 3H_2O$, 12.5.

[3]Composition (g/L): $KH_2PO_4$, 12.5; $MgSO_4 \cdot 7H_2O$, 3.0; NaCl, 12.0; $caCl_2 \cdot 2H_2O$, 1.6.

[4]Composition (g/L): $Na_2EDTA$, 5.00; $FeSO_4$ $7H_20$, 2.00; $H_3BO_3$, .03; $CoCl_2 \cdot 6H_2O$, .02; $ZnSO_4$ $7H_2O$, .01; $MnCl_2 \cdot 4H_2O$.

[5]Composition (g/L): pyridoxamine HCl, 2; riboflavin 2; thiamine HCl, 2; nicotinamide, 2; Ca pantothenate, 2; lipoic acid, 1; PAB, .1; folic acid, .05; biotin, .05; coenzyme $B_{12}$, .05.

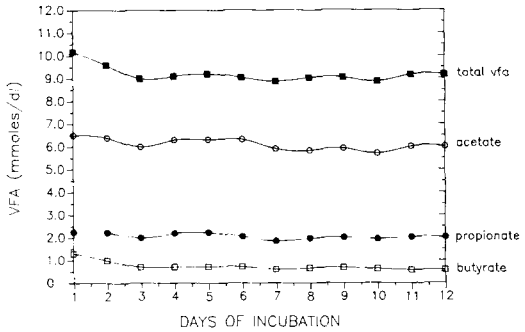


Figure 1. Daily rumen VFA concentration in vitro using timothy hay as substrate.

the incubation flasks. The fermentation flasks were gassed with $N_2$ and then placed in a shaking water bath maintained at 39°C.

Isobutyric, 2-methylbutyric, isovaleric, and valeric acids were mixed at equimolar concentrations and neutralized with sodium hydroxide. The final concentrations of the isoacid mixture in the incubation flasks were 10, 15, and 20 mg/dl of media. Monensin (from Sigma Chemical Company, St. Louis, MO) was first dissolved in 10 ml of methanol and then diluted with water. The final medium concentrations were 100, 150, and 200 μg/dl. The culture flasks contained sampling ports through which isoacids and monensin were added to the media. The treatments were imposed on d 4 of the incubation.

Fifty milliliters of a 24-h culture were collected daily from each digestor and replaced with 50 ml of fresh media. Volatile fatty acids were determined as previously described (6).

Every 24 h, .5-ml gas samples were collected and allowed to remain in the Pressure-Lok A2 gas syringes until injection into the gas chromatograph. A Hewlett-Packard 5750 gas chromatograph (Avondale, PA) equipped with a thermal conductivity detector was used for the analysis. Ratios between gases were calculated, based upon peak height. Experiment 1 investigated the effect of isoacids at 10, 15, and 20 mg/dl of final incubation media. Experiment 2 investigated the effect of monensin at 100, 150, and 200 μg/dl of final incubation media. For Experiment 3, monensin was fixed at 150 μg/dl, and isoacids were added to the incubation media at 10 or 15 mg/dl of the final incubation.

All statistical analysis were carried out using the SAS. Data were analyzed for the 2 last d (d 11 and 12) of the fermentation by analysis of variance using split-plot for repeated measurements. Differences between treatment means were tested by Tukey's test for all experiments (7).

## RESULTS AND DISCUSSION

Establishment of a rumen-like fermentation was confirmed by low hydrogen and normal VFA concentrations (Figure 1). The effects of different concentrations of isoacids on the fermentation are summarized in Table 2.

Isoacids at 10 and 15 mg/dl increased acetate, total VFA, and propionate concentration, but the increase was not as great at 20 mg/dl. As expected, branched-chain fatty acid concentrations increased in proportion to the amounts added in the medium. In contrast to VFA production, $CH_4$, $CO_2$, and $H_2$ were not affected by isoacids.

The effects of different concentrations of monensin are in Table 3. In contrast to isoacids, acetate concentration decreased at all concentrations of monensin tested. Propionate concen-

TABLE 2. Effect of isoacids on rumen VFA concentration and gas composition in vitro using inoculum from a cow fed timothy hay.

| Variable | Isoacid concentration, mg/dl | | | | SEM |
|---|---|---|---|---|---|
| | Control | 10 | 15 | 20 | |
| | (mmol/dl) | | | | |
| VFA | 7.87[c] | 8.72[a] | 8.93[a] | 8.41[b] | .08 |
| Acetate | 5.48[b] | 6.07[a] | 6.17[a] | 5.61[b] | .07 |
| Propionate | 1.55[b] | 1.69[a] | 1.63[a] | 1.52[b] | .02 |
| Butyrate | .60 | .58 | .66 | .65 | .03 |
| Isoacids | .25[d] | .40[c] | .49[b] | .59[a] | .01 |
| Isobutyrate | .03[d] | .06[c] | .07[b] | .08[a] | .001 |
| 2-Methylbutyrate | .06[d] | .10[c] | .13[b] | .15[a] | .003 |
| Isovalerate | .04[d] | .10[c] | .12[b] | .13[a] | .003 |
| Valerate | .12[c] | .14[c] | .16[b] | .22[a] | .006 |
| | (%) | | | | |
| $CH_4$ | 35.15 | 36.15 | 34.38 | 33.08 | 1.99 |
| $CO_2$ | 49.02 | 54.94 | 49.03 | 47.37 | 3.15 |
| $H_2$ | 3.83 | 4.00 | 3.67 | 3.83 | .2 |

[a,b,c,d]Means in a row with different superscripts differ ($P<.05$).

tration was significantly increased, whereas butyrate concentration decreased at all concentrations tested. Monensin decreased methane production. Neither $CO_2$ nor $H_2$ was affected. The results from the monensin experiment agree with similar studies conducted by Chalupa (5). Monensin consistently decreased acetate and butyrate but increased propionate production.

The effects of the combination of isoacids and monensin are in Figures 2 to 3. The addition of monensin caused a decrease in acetate and propionate after 24 h of incubation, but after 48 h, propionate was higher than control values, whereas acetate remained lower than the controls. The addition of isoacids at 10 and 15 mg/dl to flasks containing monensin increased acetate concentration compared with monensin alone ($P<.05$) (Figure 2). However, the addition of isoacids to flasks containing monensin did not alter propionate concentration (Figure 3).

It is interesting to speculate on the mode of action of the combination of isoacids and mo-

TABLE 3. Effect of monensin on rumen VFA concentration and gas composition in vitro using inoculum from a cow fed timothy hay.

| Variable | Monensin, μg/dl | | | | SEM |
|---|---|---|---|---|---|
| | Control | 100 | 150 | 200 | |
| | (mmol/dl) | | | | |
| VFA | 8.54[a] | 7.22[b] | 6.84[b] | 6.88[b] | .14 |
| Acetate(A) | 6.02[c] | 4.28[b] | 3.74[a] | 3.60[a] | .11 |
| Propionate(P) | 1.73[c] | 2.27[b] | 2.28[b] | 2.42[a] | .03 |
| Butyrate | .52[b] | .46[b] | .42[a] | .35[a] | .02 |
| Isoacids | .18 | .16 | .16 | .19 | .01 |
| A:P | 3.50[a] | 1.90[b] | 1.75[bc] | 1.61[c] | .07 |
| | (%) | | | | |
| $CH_4$ | 36.33[a] | 27.07[b] | 26.77[b] | 26.00[b] | .91 |
| $CO_2$ | 49.83 | 48.33 | 42.50 | 47.50 | 3.2 |
| $H_2$ | 3.50[b] | 3.5[b] | 3.5[b] | 4.00[a] | |

[a,b,c]Means in row with different superscripts differ ($P<.05$).

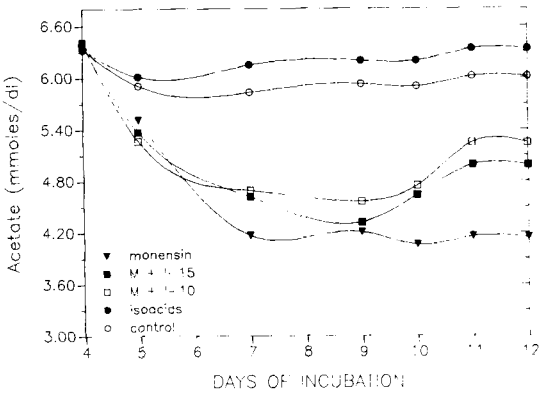


Figure 2. Effect of the combination monensin and isoacids on daily rumen acetate concentration in vitro using timothy hay as substrate. M = Monensin; I-10 and I-15 = isoacids at 10 or 15 mg/dl of media.

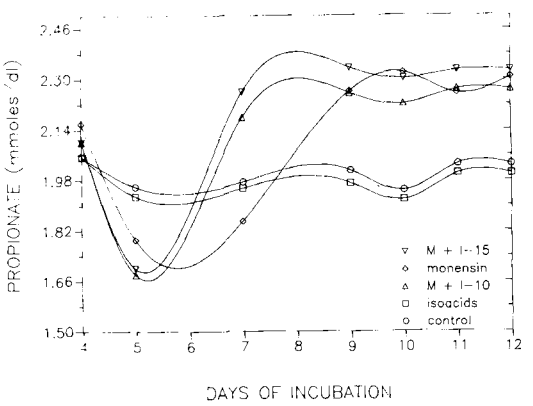


Figure 3. Effect of the combination monensin and isoacids on daily rumen propionate concentration in vitro using timothy hay as substrate. M = Monensin; I-10 and I-15 = isoacids at 10 and 15 mg/dl of media.

nensin. According to Wolin and Miller (12), monensin affects the rumen fermentation by selecting for organisms that participate in the production of relatively more propionate and against those that contribute to the production of acetate, butyrate, and precursors of methane. The growth of ruminococci and butyrivibrio is inhibited by very low concentrations of monensin. These genera are important producers of acetate, butyrate, and the substrate for methanogens, $H_2$ and $CO_2$. Selenomonads are very insensitive, whereas bacteroides, although sensitive, rapidly become resistant to the ionophore. Both organisms are important in the production of propionate. The addition of isoacids to the culture containing monensin may cause an outgrowth of bacteroides and perhaps ruminococci and methanogenic bacteria, which require isoacids, resulting in more acetate, succinate, and formate. Succinate would be decarboxylated to propionate by selenomonads and formate would be used as an energy source by methanogens. The end result would be an increase in acetate and probably more substrate degraded as indicated by the higher VFA in Experiment 3.

It is proposed that the combination of isoacids and monensin may be of practical application in cattle rations. The addition of monensin alone to the diet of growing steers increases the efficiency of growth by increasing propionate production. Because of the importance of propionate in glucose metabolism and insulin secretion in ruminants, the increase in ruminal propionate production will promote growth. Isoacids increase acetate, total VFA, and microbial synthesis. Because the addition of isoacids to cultures containing monensin does not alter the effect of monensin on propionate production, the additional energy and microbial protein may further improve growth.

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