# Influence of Monensin and Virginiamycin on *In Vitro* Ruminal Fermentation of Ammoniated Rice Straw[a]

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**ABSTRACT** : The object of this study was to determine the influence of monensin and virginiamycin (VM) on *in vitro* ruminal fermentation of rice straw or ammoniated rice straw. Rumen fluid was collected from 4 wethers fed 200 g of concentrate supplement with 400 g of untreated (U) or ammoniated (A) rice straw once daily for 28 days. Mixed ruminal microorganisms were incubated in anaerobic media that contained 20% (vol/vol) ruminal fluid and 0.3 g of either U or A rice straw. Monensin and/or VM, dissolved in ethanol, were added in centrifuge tubes at final concentrations of 0, 15, 30, 15+15 and 30+30 ppm of culture fluid. The addition of monensin and VM combination to A rice straw fermentation decreased ($p<0.05$) the acetate to propionate ratio, total VFA and lactate production, but increased ($p<0.05$) pH. Total gas production tended to be decreased by the addition of monensin plus VM. Antimicrobial agents decreased $NH_3$ N concentration and dry matter digestibility. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 4 : 544-547*)

Key Words : Monensin, Virginiamycin, *In Vitro* Fermentation, Ammoniated Rice Straw

## INTRODUCTION

Antimicrobial feed additives are routinely incorporated into ruminant diets to improve rate and efficiency of growth. One of the most widely used antimicrobial agents in beef cattle diet is the ionophore monensin. Increased animal performance is mostly due to the ability of monensin to alter ruminal fermentation; it increases the ratio of propionate to acetate and inhibits ruminal methanogenesis (Van Nevel and Demeyer, 1977; Chen and Wolin, 1978; Russell and Martin, 1984). Changes in ruminal fermentation patterns are attributed to the disruptive action of monensin on the permeability of ions across bacterial membranes (Russell and Strobel, 1989). Virginiamycin (VM), an antibiotic produced by *Streptomyces virginiae* has been used for many years as a performance enhancer for beef cattle (Rogers et al., 1995) and is approved for therapeutic use in the prevention of liver abscesses in feed lot cattle. *In vitro* studies have shown that VM inhibits the growth of lactate-producing ruminal bacteria (Nagaraja and Taylor, 1987; Nagaraja et al., 1987). However, the effects of monensin in combination with VM have not been reported. Also, when untreated (U) rice straw or ammoniated (A) rice straw is used as a fermentation substrate, information on the relative efficacy of the antimicrobial agents is not available.

*In vitro* fermentations with mixed ruminal microorganisms were conducted to determine whether the effects of monensin and VM on the ruminal fermentation of U rice straw or A rice straw are additive.

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## MATERIALS AND METHODS

Ruminal contents were collected from four 35 kg ruminally fistulated wethers fed 200 g of commercial concentrates which contained neither monensin nor VM with 400 g of U or A rice straw for 28 days. Rice straw was stacked and covered with polyethylene film (0.1 mm thickness) and 3% $NH_3$ (DM base of rice straw) was injected into the stack and preserved for eight weeks (Sundstøl et al., 1978). The ruminal contents obtained 1.5 h after feeding were 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℃ water bath, permitting large feed particles to rise to the top of the flask. Particle-free fluid from the flask was transferred anaerobically (20% vol/vol) to a medium (pH 6.75) 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 $MgSO_4$ $7H_2O$, 64 mg of $CaCl_2$ $2H_2O$, 4,000 mg of $Na_2CO_3$, and 600 mg of cysteine-HCl per liter (Russell and Martin, 1984; Russell and Strobel, 1988). Thirty ml of the mixed particle-free fluid with medium was transferred anaerobically to 4 of 50 ml centrifuge tubes per each treatment containing 0.3 g of either U or A rice straw. Monensin (Sigma) and/or VM (Smithkline) were dissolved in ethanol (0.45 to 0.90 mg/ml) and added (1.0 ml) to centrifuge tubes to achieve final concentrations of 15 and 30 ppm, respectively, and control tubes received an equal amount of ethanol. Tubes were sealed with rubber stoppers fitted with 60 ml syringes; their plungers were lubricated with glycerol.

After 72 h of incubation, total gas production was measured by reading the displacement of the syringe plungers. The tubes were then uncapped, and the pH was measured immediately. Tubes were then centrifuged (10,000×g, 4℃, 15 min), and cell-free supernatant and sediment were stored at -20℃.

The sediment was filtered through a sintered crucible

**Table 1.** Effects of monensin and virginiamycin on VFA production (molar %) *in vitro* when untreated (U) or ammoniated (A) rice straw was the fermentation substrate

| Antimicrobial compounds | Concentration (ppm) | VFA production | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total VFA (mM) | | Acetate (A) | | Propionate (P) | | Butyrate | | A:P ratio | |
| | | U | A | U | A | U | A | U | A | U | A |
| Control | 0 | 56.22$^{a}$ | 67.92$^{ag}$ | 66.8$^{a}$ | 51.9$^{af}$ | 26.6 | 35.3$^{cf}$ | 6.5 | 12.9$^{bcg}$ | 2.57$^{a}$ | 1.48$^{af}$ |
| Monensin | 15 | 35.07$^{c}$ | 32.82$^{ag}$ | 64.2$^{ab}$ | 49.7$^{bg}$ | 27.5 | 36.9$^{cg}$ | 8.3 | 13.4$^{abg}$ | 2.34$^{abc}$ | 1.35$^{abg}$ |
| | 30 | 35.50$^{c}$ | 33.34$^{af}$ | 64.2$^{ab}$ | 49.2$^{bg}$ | 27.7 | 37.1$^{cg}$ | 8.1 | 13.7$^{ag}$ | 2.32$^{abc}$ | 1.33$^{bg}$ |
| Virginiamycin | 15 | 51.92$^{b}$ | 68.25$^{ag}$ | 66.8$^{a}$ | 53.4$^{ag}$ | 26.7 | 36.8$^{cg}$ | 6.5 | 9.7$^{dg}$ | 2.51$^{ab}$ | 1.45$^{abg}$ |
| | 30 | 52.66$^{ab}$ | 69.26$^{ag}$ | 61.1$^{bc}$ | 48.3$^{bcg}$ | 31.1 | 43.6$^{ag}$ | 7.7 | 8.1$^{c}$ | 1.96$^{cd}$ | 1.11$^{cg}$ |
| Monensin+ | 15+15 | 35.45$^{c}$ | 36.22$^{c}$ | 58.8$^{c}$ | 47.1$^{cg}$ | 32.2 | 40.1$^{bg}$ | 8.9 | 12.8$^{bcg}$ | 1.82$^{d}$ | 1.17$^{cg}$ |
| Virginiamycin | 30+30 | 38.37$^{c}$ | 41.98$^{b}$ | 62.5$^{bc}$ | 44.9$^{dg}$ | 29.7 | 42.9$^{ag}$ | 7.8 | 12.3$^{cg}$ | 2.10$^{bcd}$ | 1.05$^{cg}$ |
| SEM[h] | | 4.44 | 1.25 | 5.37 | 1.16 | 3.38 | 1.56 | 0.49 | 0.14 | 0.06 | 0.01 |

[a,b,c,d,e] Mean values with different superscripts within the same column are significantly different ($p<0.05$).
[f] Maens differ from corresponding mean of untreated rice straw (U) ($p<0.05$).
[g] Means differ from corresponding mean of untreated rice straw (U) ($p<0.01$).
[h] Standard error of the mean.

by vacuum, and the DM digestibility was calculateded (AOAC, 1990). The VFA in the supernatants was measured by GLC using a Varian GC-3400 (Walnut Creek, California, 94598, USA) gas chromatograph (column temperature = 120℃ to 170℃ at 10℃/min, injector temperature = 170℃, detector temperature = 190℃) equipped with an autosampler and Stabilwax-DA (30 m×0.5 mm I.D.×0.5 film) column (Restek). Lactate was analyzed with the GC as previously described (Song and Kennelly, 1989). Ammonia was measured by the colorimetric method of Chaney and Marbach(1962). All incubations were performed on duplicate days with two replicates per day (n=4). Data were analyzed using a GLM procedure (SAS, 1996) for a completely randomized block design with seven levels (0, 15, 30, 15+15, 30+30) of monensin and/or VM and two treatments (U and A) of rice straw. Least square means for monensin and/or VM levels and for U and A treatments are reported and significance was tested at the $p<0.05$ level. The LSD method was used to determine differences between seven levels means and to determine differences between any two treatment means.

## RESULTS AND DISCUSSION

When mixed ruminal microorganisms were incubated *in vitro* with either U or A rice straw, both monensin and VM caused significant ($p<0.05$) decreases in VFA production except when VM was added to U straw at 30 ppm and at 15 and 30 ppm to A rice straw (table 1). Effects of the antimicrobials did not increase with dose rate and were not additive. When A rice straw was the substrate, greater amounts of VFA were produced in most treatments than U rice straw. Similar effects were reported earlier for monensin and VM (Nagaraja et al., 1987) due to considerably lowered microbial growth (Van Nevel et al., 1984). When A rice straw was the substrate, the well established effects of monensin and VM in reducing acetate and increasing propionate production resulting in a decreased acetate to propionate ratio (Richardson et al., 1976; Nagaraja et al., 1987) were confirmed (table 1). When U rice straw was the substrate, 30 ppm VM and both monensin plus VM treatments caused decreases ($p<0.05$) in acetate production. There was little change in butyrate production ($p>0.05$) with either antimicrobial compounds.

Generally, the acetate to propionate ratio in both U and A rice straws tended to be lower with higher amounts of monensin and/or VM. However, significant ($p<0.05$) reductions were observed with VM 30ppm and both monensin and VM combinations irrespective of the substrate used.

In most instances, some responses with U rice straw were not as clear as with A rice straw.

Fermentation of U and A rice straws with ruminal fluid only resulted in the tendency of lower pH than the others (table 2), and lactate concentrations of ruminal fluid only treatments were higher than when monensin and/or VM were present at the end of the 72h incubations (table 3). In all instances, the extent of lactate inhibition tended to be dose-dependent and greater ($p<0.05$) with both monensin and VM combinations.

**Table 2.** Effects of monensin and virginiamycin on pH *in vitro* when untreated (U) or ammoniated (A) rice straw was the fermentation substrate

| Antimicrobial compounds | Concentration (ppm) | Substrate | |
|---|---|---|---|
| | | U | A |
| Control | 0 | 6.61$^{b}$ | 6.55$^{d}$ |
| Monensin | 15 | 6.87$^{a}$ | 6.83$^{a}$ |
| | 30 | 6.87$^{a}$ | 6.85$^{a}$ |
| Virginiamycin | 15 | 6.70$^{b}$ | 6.49$^{e}$ |
| | 30 | 6.68$^{b}$ | 6.46$^{f}$ |
| Monensin+ | 15+15 | 6.83$^{a}$ | 6.79$^{b}$ |
| Virginiamycin | 30+30 | 6.86$^{a}$ | 6.72$^{c}$ |
| SEM[g] | | 0.01 | 0.02 |

[a,b,c,d,e,f] Mean values with different superscripts within the same column are significantly different ($p<0.05$).
[g] Standard error of the mean.

Maximal inhibition of lactate was observed with lower combination in both substrates. However, the

relatively high pH of the control and low concentration of lactate of this trial seems to be due to the fiber-rich substrates, because monensin produced a significant increase in pH and lower lactate concentration with *in vitro* studies of rumen fluid with carbohydrate stress (Dennis et al., 1980).

**Table 3.** Effects of monensin and virginiamycin on lactate concentration (mM) *in vitro* when untreated (U) or ammoniated (A) rice straw was the fermentation substrate

| Antimicrobial compounds | Concentration (ppm) | Substrate U | Substrate A |
|---|---|---|---|
| Control | 0 | $0.57^{a}$ | $0.64^{a}$ |
| Monensin | 15 | $0.31^{b}$ | $0.35^{b}$ |
| | 30 | $0.31^{b}$ | $0.34^{bc}$ |
| Virginiamycin | 15 | $0.34^{b}$ | $0.37^{b}$ |
| | 30 | $0.28^{bc}$ | $0.33^{bc}$ |
| Monensin+ Virginiamycin | 15+15 | $0.18^{d}$ | $0.23^{d}$ |
| | 30+30 | $0.22^{cd}$ | $0.27^{cd}$ |
| SEM[e] | | 0.01 | 0.01 |

[a,b,c,d] Mean values with different superscripts within the same column are significantly different ($p<0.05$).

[e] Standard error of the mean.

**Table 4.** Effects of monensin and virginiamycin on gas production (ml/0.1g DM) *in vitro* when untreated (U) or ammoniated (A) rice straw was the fermentation substrate

| Antimicrobial compounds | Concentration (ppm) | Substrate U | Substrate A |
|---|---|---|---|
| Control | 0 | $38.8^{a}$ | $52.7^{ac}$ |
| Monensin | 15 | $7.0^{e}$ | $0.6^{dc}$ |
| | 30 | $4.8^{d}$ | $0.3^{dc}$ |
| Virginiamycin | 15 | $19.6^{b}$ | $33.1^{bf}$ |
| | 30 | $24.9^{b}$ | $31.8^{bf}$ |
| Monensin+ Virginiamycin | 15+15 | $10.7^{e}$ | $2.6^{ec}$ |
| | 30+30 | $10.7^{e}$ | $7.5^{ec}$ |
| SEM[g] | | 1.04 | 0.47 |

[a,b,c,d] Mean values with different superscripts within the same column are significantly different ($p<0.05$).

[e] Means differ from corresponding mean of untreated rice straw (U) ($p<0.05$).

[f] Means differ from corresponding mean of untreated rice straw (U) ($p<0.01$).

[g] Standard error of the mean.

Total gas production was decreased ($p<0.05$) with the antimicrobial compounds. Maximal effect was shown with 30 ppm monensin while the effect of VM alone was much lower ($p<0.05$) than those of other treatments in both U and A rice straws. Schelling (1984) indicated that monensin reduced productions of methane and carbon dioxide by rumen microbes at levels of 30ppm or higher. However, methane production was reduced at less than 20 ppm of VM *in vitro* fermentation (Van Nevel et al., 1984). Decreased $NH_3$-N was observed over all antimicrobial compound treatments (table 5).

**Table 5.** Effects of monensin and virginiamycin on $NH_3$-N concentration (mg/ℓ) *in vitro* when untreated (U) or ammoniated (A) rice straw was the fermentation substrate

| Antimicrobial compounds | Concentration (ppm) | Substrate U | Substrate A |
|---|---|---|---|
| Control | 0 | $16.52^{a}$ | $27.48^{af}$ |
| Monensin | 15 | $12.23^{d}$ | $22.36^{df}$ |
| | 30 | $12.21^{d}$ | $23.34^{df}$ |
| Virginiamycin | 15 | $7.23^{e}$ | $23.51^{edf}$ |
| | 30 | $12.73^{ed}$ | $26.97^{bf}$ |
| Monensin+ Virginiamycin | 15+15 | $13.99^{bc}$ | $24.30^{edf}$ |
| | 30+30 | $14.90^{e}$ | $26.06^{bcf}$ |
| SEM[g] | | 0.90 | 2.22 |

[a,b,c,d,e] Mean values with different superscripts within the same column are significantly different ($p<0.05$).

[f] Means differ from corresponding mean of untreated rice straw (U) ($p<0.01$).

[g] Standard error of the mean.

A variety of *in vitro* studies have indicated that monensin (Schelling et al., 1977; Van Nevel and Demeyer, 1977) and VM (Van Nevel et al., 1984) significantly reduce the ruminal degradation of dietary protein. Ruminal ammonia decreases (Dinius et al., 1976) are consistent with the depression of deamination or proteolysis, or both. Thus, the lower $NH_3$-N concentrations observed in antimicrobial compound treatments may be mainly due to the inhibition of microbial growth. Apart from the addition of antimicrobial compounds $NH_3$-N concentrations of A rice straw were significantly ($p<0.01$) higher than those of U rice straw probably due to the added N. This result is consistent with the result of Vagoni et al. (1995) who studied ammoniated hay using steers. Both monensin and VM decreased the DM digestibility of U and A rice straw (table 6), but the magnitude of the decrease was greater ($p<0.05$) for monensin and monensin and VM combination than for VM alone. Generally the decrease in fiber digestion was proportional to the decrease in acetate observed in table 1. A reduction in DM and ADF digestibility was also observed by Poos et al. (1979) when lambs were fed monensin for only 10 d, but after an additional 29 d of adaptation there was no significant difference. Thus the DM digestibility reduction with monensin and/or VM seems to be the lack of adaptation period for rumen juice donating animals in this experiment.

The potential impact of nitrogen-sparing in the rumen was also supported by the observation that monensin and VM always decreased $NH_3$-N concentrations.

Results suggest that monensin and VM combination might be beneficial to ruminants fed A rice straw by increasing ruminal pH and propionate production, and reducing lactate accumulation. However, more research is needed to examine the effects of monensin and VM combination on ruminant performance.

**Table 6.** Effects of monensin and virginiamycin on dry matter digestibility (%) *in vitro* when untreated (U) or ammoniated (A) rice straw was the fermentation substrate

| Antimicrobial compounds | Concentration (ppm) | Substrate | |
|---|---|---|---|
| | | U | A |
| Control | 0 | 39.34[a] | 48.75[a] |
| Monensin | 15 | 20.25[cd] | 18.86[b] |
| | 30 | 21.87[bc] | 21.05[b] |
| Virginiamycin | 15 | 29.29[b] | 46.23[ac] |
| | 30 | 27.22[bc] | 45.07[ac] |
| Monensin+ | 15+15 | 24.83[bc] | 19.23[b] |
| Virginiamycin | 30+30 | 13.22[d] | 18.21[bc] |
| SEM[f] | | 3.06 | 4.43 |

[a,b,c,d] Mean values with different superscripts within the same column are significantly different ($p<0.05$).

[e] Means differ from corresponding mean of untreated rice straw (U) ($p<0.05$).

[f] Standard error of the mean.

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