Summary

A series of in vitro and in vivo trials was conducted to determine if continuous monensin feeding for up to 56 d would reduce ruminal conversion of L-tryptophan (TRP) to 3-methylindole (3MI). Fourteen mature beef cows were adapted to a maintenance diet for 3 wk. In trial I, the sampling time to optimize 3MI production was determined. Trials II through IV were to determine the duration of efficacy of monensin on reducing 3MI concentrations in vitro and in vivo. During trials II, III and IV one-half of the cows were fed 200 mg monensin·head⁻¹·d⁻¹ for 21, 36 and 55 d, respectively, while the remaining cows served as controls. All cows were fed the control diet for 21 d between each trial. Volatile fatty acid (VFA) concentrations and in vitro conversion of TRP to 3MI were determined in ruminal fluid samples collected during trials I through IV. On d 28 of trial IV, all cows were given an oral dose of .35 g TRP/kg of body weight to induce acute bovine pulmonary edema and emphysema (ABPE). Ruminal concentrations of 3MI and indole were measured at intervals for 96 h. Results of trial I demonstrated that ruminal fluid collected 15 h postfeeding produced the highest in vitro conversion of TRP to 3MI. Therefore, ruminal fluid samples were collected at that time in trials II, III and IV. In vitro conversion of TRP to 3MI was lower (P<.01) in samples from monensin-treated cows (12.1%) compared with controls (25.6%). Monensin reduced 3MI production for 55 d, the longest time tested in these experiments. Conversion of TRP to indole, a secondary degradation product, also was lower (P<.05) in samples from monensin-treated cows (4.3%) vs controls (5.8%). The addition of 5 μg/ml of monensin to the in vitro incubations further depressed 3MI production, suggesting that ruminal microorganisms remain sensitive to monensin and amounts of dietary monensin above the 200 mg·head⁻¹·d⁻¹ may be beneficial to further reduce 3MI production. Monensin increased ruminal propionate and decreased acetate in all trials (P<.05). Total VFA production averaged 79 μmol/ml and was not different between groups. Monensin reduced in vivo 3MI production (P<.05) at 12, 18 and 24 h after TRP challenge. Five control cows died from ABPE while only one cow in the monensin-treated group died. These results demonstrate that monensin reduced acute clinical cases of ABPE when prefed for 28 d and was effective in reducing in vitro 3MI formation for 55 d.

(Key Words: 3-Methylindole, Indole, Tryptophan, Bovine Edema, Bovine Emphysema, Monensin.)

Introduction

Studies have shown that monensin can alter ruminal fermentation by selecting against organisms that produce H₂ and formate and for organisms that produce propionate (Dennis et al., 1981). Monensin sustains increases in propionate production for extended periods of time at the expense of less desirable end products, such as acetate, H₂, formate and methane. It has been shown that monensin will decrease 3-methylindole (3MI) production (Hammond et al., 1978), an undesirable end product of ruminal tryptophan catabolism that causes acute lung injury and death in ruminants. Carlson et
al. (1983) reported data indicating that inhibition of ruminal 3MI production was the greatest during the first 4 d after monensin administration, but this study did not determine the effect of prolonged feeding of monensin on 3MI production. A number of bacteria convert L-tryptophan (TRP) to indoleacetic acid (IAA, Lacoste, 1961; Scott et al., 1964), but a Lactobacillus sp. is the only known ruminal organism to decarboxylate IAA to form 3MI. Monensin has a bacteriostatic effect on this Lactobacillus microorganism (M. T. Yokoyama, personal communication). However, the effect of prolonged exposure to monensin on the capacity of ruminal microorganisms to produce 3MI is not known. Carlson et al. (1983) suggested the possibility of microbial adaptation to monensin in relationship to its effect on ruminal 3MI formation. Therefore, the objective of this study was to determine if continuous monensin feeding for up to 56 d would reduce its effectiveness in decreasing 3MI formation.

**Methods**

The research was conducted in four phases. Trial I was to determine the time after feeding when maximum conversion of TRP to 3MI occurred. Trials II and III were to determine whether monensin feeding would inhibit 3MI production for up to 56 d. Trial IV monitored in vitro conversion of TRP to 3MI, but it also included an in vivo evaluation of the effect of monensin on TRP-induced acute lung injury.

Mature Hereford (eight) and Angus (two) cows averaging 466 kg (range 348 to 571 kg) were preconditioned on the control diet for 21 d preceding each trial. Diets were fed at maintenance requirement (NRC, 1976). The diets were composed of 6.8 kg chopped forage and .5 kg ground barley with or without monensin (400 mg/kg). The forage was composed of grass-legume hay (IFN 1-00-321) and wheat straw (IFN 1-05-175) mixed to contain 6.5 to 7% crude protein. The crude protein content of the grass-legume hay was variable (5.4 to 12.2%); therefore, the ratio of hay to straw was adjusted to maintain the desired crude protein. Cows were fed once daily at preset times for each trial. Trace mineralized salt and water were offered ad libitum. Cows were individually fed monensin supplement. All cows received the control diet in trial I. In trial II, one-half of the cows were randomly assigned to the monensin group. Those cows receiving monensin in trial II were switched to control diets and vice versa in trial III. Four additional cows were randomly assigned to treatment in trial III for a total of seven cows/treatment. In trial IV, cows were completely randomized to experimental diets. After receiving monensin for 28 d, cows were challenged with an oral dose of .35 g TRP/kg body weight (BW) in gelatin capsules. The number of days of monensin prefeeding before the challenge was chosen based on the results of trials II and III, which indicated in vitro TRP conversion to 3MI was still inhibited by monensin.

Ruminal fluid samples were collected by esophageal tube at various times (table 1) for laboratory analysis. Indole and 3MI were analyzed by gas chromatography using the method of Carlson et al. (1983). Twenty-four milliliters of strained ruminal fluid and 1 ml of TRP were incubated anaerobically to evaluate in vitro formation of 3MI and indole (Hammond et al., 1978). In trials I and II, in vitro incubations were for 10 h, whereas in trials III and IV the incubations were for 24 h. Single samples were incubated from each cow in trial I.

**TABLE 1. NUMBER OF COWS AND SAMPLING TIMES FOR THE FOUR EXPERIMENTAL TRIALS**

<table>
<thead>
<tr>
<th>Item</th>
<th>Cows/treatment</th>
<th>Hours or days of sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial I&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10</td>
<td>3, 6, 9, 12, 15, 18, 21, 24 h</td>
</tr>
<tr>
<td>Trial II&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5 control, 5 monensin</td>
<td>0, 2, 4, 6, 8, 10, 12, 14, 18, 21 d</td>
</tr>
<tr>
<td>Trial III&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7 control, 7 monensin</td>
<td>4, 8&lt;sup&gt;b&lt;/sup&gt;, 12, 16&lt;sup&gt;b&lt;/sup&gt;, 24, 32&lt;sup&gt;b&lt;/sup&gt;, 36 d</td>
</tr>
<tr>
<td>Trial IV&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7 control, 7 monensin</td>
<td>14, 27, 42, 49&lt;sup&gt;b&lt;/sup&gt;, 55 d</td>
</tr>
<tr>
<td>In vitro</td>
<td></td>
<td>0, 6, 12, 18, 24, 36, 48, 72, 96 h</td>
</tr>
<tr>
<td>In vivo, after TRP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>All cows were adapted to the forage and control barley diet 21 d preceding each trial.

<sup>b</sup>Incubation flasks containing 5 µg/ml monensin were included along with the regular incubation.
and were run in triplicate for the other trials. Additional flasks containing 5 μg/ml monensin were incubated on d 8, 16 and 32 of trial III and d 49 of trial IV. Ruminal volatile fatty acids (VFA) were quantitated as described by Carlson et al. (1983).

Clinical signs and deaths resulting from acute bovine pulmonary edema and emphysema (ABPE) in trial IV were recorded. Cows that died were necropsied to verify the cause of death. Differences in mean 3MI, indole and VFA concentrations were tested using Student’s t-test (Steel and Torrie, 1960).

**Results and Discussion**

The highest in vitro conversion of TRP to 3MI occurred in ruminal samples taken 15 h postfeeding (figure 1) and the formation of indole remained low throughout the 24-h period. Because the conversion of TRP to 3MI was the highest 15 h after feeding, in vitro incubations in trials II, III and IV were conducted with ruminal fluid collected at this time.

The conversion of TRP to 3MI was consistently lower (P<.05) when ruminal inocula were from cows fed monensin (figure 2). Incubations containing an additional 5 μg/ml monensin further decreased 3MI formation; the concentrations of 3MI were 1.42 and .92% of TRP converted to 3MI when inocula came from control cows and cows fed monensin, respectively. This suggests that an increased intake of dietary monensin would further decrease 3MI formation and that this enhanced inhibitory effect of monensin on 3MI formation remains effective for at least 49 d. Although monensin reduces 3MI concentrations, complete inhibition has not been achieved at the dosages tested. Because 3MI formation is dose responsive to monensin in vitro (Hammond and Carlson, 1980), additional studies are warranted to test the effects of higher monensin levels on 3MI production in vivo. Monensin was also effective in reducing indole concentrations (figure 3). Although the quantity of TRP converted to indole was low, monensin further decreased (P<.01) its formation.

In vitro incubations conducted during trial IV confirmed the results in trials II and III. Ruminal 3MI concentrations after an oral dose of TRP on d 28 were lower in cows fed monensin. Monensin reduced ruminal 3MI concentrations (P<.05) at 12, 18 and 24 h post-dosing (figure 4). Formation of 3MI was reduced by monensin without increasing ruminal indole concentrations (figure 5). At all times the observed indole levels were not different between groups (P>.05). These results are in contrast to other studies that demonstrated that antibiotic treatment can shift microbial metabolism of TRP toward indole production (Hammond and...
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TRIAL II

TRIAL III

TRIAL IV

Figure 3. Percentage of tryptophan (TRP) dose converted in vitro to indole in trials II, III and IV: ○ = control, ● = monensin.

Carlson, 1980). Although hemolytic indole toxicity can be produced experimentally (Hammond et al., 1980b), it has not been reported under field conditions.

Dietary monensin reduced both the number of animals showing clinical signs and the number of animals that died from ABPE (table 2). Necropsy verified that ABPE was the cause of death in all cases. Clinical signs of ABPE have been reported elsewhere (Hammond et al., 1980a). The mean time of death for all cows was 134 h, with a range of 96 to 191 h after TRP administration. The reduction in 3MI concentrations and in animals showing clinical signs and deaths demonstrated that monensin reduced the onset and severity of TRP-induced ABPE when cows were fed monensin for 28 d before TRP challenge. Results from the in vitro experiments are predictive of in vivo conditions at least to 28 d of monensin supplementation.

A comparison of in vivo ruminal 3MI levels in individual cows (data not shown) suggests that some cows are more resistant to this disease than others. Because the 3MI concentration to cause death differs, other factors such as genetics, nutritional status or metabolic dif-

Figure 4. Ruminal 3-methylindole (3MI) concentrations after an oral dose of tryptophan (TRP) in trial IV.

Figure 5. Ruminal indole concentrations after an oral dose of tryptophan in trial IV.

TABLE 2. EFFECT OF MONENSIN SUPPLEMENTATION FOR 28 DAYS ON TRYPTOPHAN-INDUCED ACUTE LUNG DISEASE

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Clinical signs(a)</th>
<th>Deaths from ABPE(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6/7</td>
<td>5/7</td>
</tr>
<tr>
<td>Monensin</td>
<td>3/7</td>
<td>1/7</td>
</tr>
</tbody>
</table>

\(a\) Data are listed as the number of animals affected/number of animals in the treatment group.
Differences in xenobiotic detoxication (Nocerini et al., 1983) may be involved.

Differences in ruminal VFA (table 3) showed a response similar to that previously reported for monensin (Bartley et al., 1979; Fuller et al., 1982). Throughout all trials, ruminal propionate concentrations were higher (P<.05) in cows fed monensin, which verified that the monensin was actively altering ruminal metabolism. Total VFA concentrations were not different (P>.05) between groups and remained constant throughout this experiment.

Conversion of TRP to 3MI and VFA production becomes much more variable as forage quality varies. Crude protein content is an indicator of forage maturity and feeding value. We experienced a wide variation in crude protein (5.4 to 12.2%) of the hay. To normalize crude protein to 6.5 to 7%, hay(s) and straws were blended. It is known that grazing sparse, poor quality forage precedes the onset of ABPE (Carlson and Dickinson, 1978). However, the component(s) of the poor quality forage that predispose the ruminal microorganism to produce 3MI from TRP are not known. Therefore, we controlled total forage and crude protein intake. As a result, the variation observed in VFA and 3MI concentrations was minimized.

Monensin reduced the onset and severity of ABPE when cows were fed 200 mg monensin·head⁻¹·d⁻¹ for 28 d before a TRP challenge. This research also suggests that additional monensin would further decrease 3MI production and, based on in vitro results, monensin remains effective for at least 8 wk.

### Literature Cited


Fuller, J. B. and D. E. Johnson. 1982. Monensin and lasalocid effects on fermentation in vitro. J.

### Table 3: Ruminal Volatile Fatty Acids of Cows Supplemented with and Without Monensin

<table>
<thead>
<tr>
<th>Item</th>
<th>Monensin</th>
<th>Control</th>
<th>SE</th>
<th>Monensin</th>
<th>Control</th>
<th>SE</th>
<th>Monensin</th>
<th>Control</th>
<th>SE</th>
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<th>SE</th>
<th>Monensin</th>
<th>Control</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate, %</td>
<td>65.15</td>
<td>69.17</td>
<td>.37</td>
<td>66.59</td>
<td>71.43</td>
<td>.85</td>
<td>67.71</td>
<td>76.93</td>
<td>1.78</td>
<td></td>
<td></td>
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<tr>
<td>Propionate</td>
<td>21.19</td>
<td>16.39</td>
<td>.82</td>
<td>19.77</td>
<td>15.36</td>
<td>.82</td>
<td>21.77</td>
<td>18.36</td>
<td>.82</td>
<td></td>
<td></td>
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<tr>
<td>Butyrate, %</td>
<td>10.64</td>
<td>11.11</td>
<td>.94</td>
<td>12.82</td>
<td>11.92</td>
<td>.94</td>
<td>13.11</td>
<td>12.22</td>
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<tr>
<td>Total, mM</td>
<td>86.77</td>
<td>76.92</td>
<td>2.35</td>
<td>86.82</td>
<td>77.14</td>
<td>2.35</td>
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*Significantly different from control (P<.05). Mean values are reported from ruminal samples collected for in vitro incubations, excluding 0 in trial II.
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