EFFECT OF THIOPEPTIN AND SODIUM BICARBONATE ON THE PREVENTION OF LACTIC ACIDOSIS INDUCED IN SHEEP\(^1,2\)

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Summary

Four crossbred wethers were utilized in a randomized block design to test Thiopeptin, an antibiotic, at .25% of the amount of wheat given, two levels of NaHCO\(_3\) (2 and 4%) expressed as a percentage of the wheat given, and a combination of the Thiopeptin (.25%) plus 2% NaHCO\(_3\). Acidosis was induced in the four sheep (controls) by feeding cracked soft white wheat at 50 g/kgBW divided among three feedings given over an 8-hr period on the third day of the experiment. Additional wheat was given depending on rumen pH values at 0800 of the fourth day. All control sheep received wheat until rumen pH was below 4.5. Ruminal pH, rumen lactic and volatile fatty acid levels in rumen fluid were monitored four times daily for 96 hr postengorgement.

The 2 and 4% NaHCO\(_3\) and the antibiotic plus 2% NaHCO\(_3\) treatments were all effective in maintaining a higher pH than the control treatment (P<.01) and these treatments maintained a higher pH than the antibiotic by itself (P<.05). Low concentrations of rumen lactic acid (< 30 mM) were detected on the antibiotic treatment. All treatments were effective in preventing acute acidosis and none of the animals went off feed. The 4% NaHCO\(_3\) treatment resulted in higher (P<.05) acetic acid concentrations than the treatments containing Thiopeptin. Treatments containing Thiopeptin resulted in increases in propionic acid (P<.01) and reduced acetic to propionic ratios compared to the buffer treatments. Differences in total volatile fatty acids or gross energy calculated from volatile fatty acids were not significant among the treatments. (Key Words: Acidosis, Antibiotic, Sodium Bicarbonate, pH, Sheep, Lactic Acid.)

Introduction

When cattle or sheep are abruptly changed from a roughage to a concentrate diet, lactic acidosis may result causing the animal to go off feed for several days and sometimes resulting in death (Ryan, 1964; Vestweber \textit{et al.}, 1974; Allison \textit{et al.}, 1975; Dougherty \textit{et al.}, 1975a; Slyter, 1976).

Klatte and Thomas (1967), Prins and Mulder (1969) and Streeter \textit{et al.} (1974) have suggested that selective antibiotics should be useful in preventing lactic acidosis. When using \textit{in vitro} methods, Beede and Farlin (1977) found four antibiotics to be effective in decreasing lactate production and three additional antibiotics were moderately effective.

Shelton \textit{et al.} (1969) showed improvement in the feedlot performance of lambs fed high concentrate rations when a 1:1 combination of sodium and potassium carbonates were fed as 2% of the ration. Several other workers (Embry \textit{et al.}, 1968; Brethour and Duitsman, 1972, 1973; Saville \textit{et al.}, 1973; Ralston and Patton, 1976) have shown the use of buffers to be beneficial when included in high concentrate rations. Herod \textit{et al.} (1977) tested the buffering ability of 23 combinations of compounds \textit{in vitro} and reported carbonates and bicarbonates, in proper combination, were the most promising buffers tested.

The purpose of this experiment was to test the effectiveness of NaHCO\(_3\) used at two levels. Thiopeptin, an antibiotic, and combination of the two in preventing lactic acidosis in sheep abruptly switched from an all roughage diet to one containing 75% cracked wheat.
Experimental Procedures

Animal and Engorgement Feeding. Four crossbred wether sheep ranging in weight from 41 to 60 kg were surgically prepared with rumen cannulas and then maintained on a grass-alfalfa hay diet. At the beginning of the experiment, the sheep were placed in wooden crates and given cracked white wheat (4-08-142) at a rate of 30 g/kgBW·75 divided between two feedings 8 hr apart. This feeding regimen was repeated on day two of the experiment. During the first 2 days, chopped alfalfa (1-00-063) was fed as 35% of the diet. On the third day of the experiment, each sheep was fed 50 g/kgBW·75 of cracked wheat divided between three feedings 4 hr apart. Beginning on the third day the amount of chopped alfalfa was reduced to 25% of the total diet. Feed not consumed within a 30-min period was added through the rumen cannula. The feeding regimen begun on day 3 was continued until each animal had a rumen pH between 4.0 and 4.5. When this pH was maintained for a few hours, the animal was given 50 g of NaHCO₃ in a 1-liter solution, added through the cannula. After 30 min, fluid was pumped out through the cannula until no more fluid could be obtained and an equal amount to that removed was added from a donor animal maintained on a grass hay (2-04-073) diet. Water and trace mineralized salt were available ad libitum.

Treatments. After the feeding regimens were determined that would result in lactic acidosis for each sheep (controls) and the animals had received the grass-alfalfa hay diets for at least 30 days, the sheep were returned to the crates and one of the following treatments was tested (expressed as % of wheat): NaHCO₃ at a level of 2 or 4%; Thiopeptin, the antibiotic 5, at a level of .25%; and a combination of .25% Thiopeptin plus 2% NaHCO₃. The exact feeding schedule that produced acidosis was followed during each of these treatments. On days 4, 5 and 6, when the control animals had been off feed, the treated animals received wheat at the level of 50 g/kgBW·75 divided between three feedings over an 8-hr period. A 3-week period between each of the treatments was allowed so the animals could readapt to mixed grass-alfalfa hay. The sheep received various treatments in pairs and the sequence of the four treatments was varied between the two pairs in order to discount any possible effects due to the order in which the treatments were tested.

Sampling. Four rumen samples were taken each day during each treatment. Samples were collected prior to feeding at 0700 and 1500 hr and again at 1100 and 1900 hours. Samples were obtained through the rumen cannula with the aid of a suction pump. The pH of each sample was determined within 1 min of collection. A 5-ml sample was placed in a centrifuge tube containing 1 ml of 25% metaphosphoric acid and refrigerated for at least 4 hr before being centrifuged at 12,500 rpm for 20 minutes. The supernatant was decanted and frozen for future volatile fatty acid and lactic acid analysis. During the control treatment, lactic acid analysis was done the same day samples were collected to insure the sheep had developed lactic acidosis.

Analysis. Volatile fatty acids (VFA) and lactic acid were determined using a gas chromatograph following procedures outlined by Carlsson (1973). Gross energies produced from VFA were calculated using values reported by Blaxter (1962).

Statistical Analysis. It was desired to analyse means by least squares analysis of variance procedures under a randomized block design, with sheep acting as blocks. Due to the time-sequential nature of the observations on the same treatment, it was possible that each set of observations had a serial correlation pattern (Neter and Wasserman, 1974), thus a least squares analysis was not done. Observations closer together in time were suspected of being more correlated than observations further apart. Since each set of means was comprised of averages of varying numbers of observations, the individual variance of the averages became a function of both the number of observations and the particular value of the serial correlation coefficient between observations. As the exact correlation coefficients were not known, an analysis was done on each set of data for a range of correlation coefficients between zero.

5 Produced by Merck & Co., Inc., Rahway, NJ.
6 Antibiotic at .25% represents the premix weight. The premix contained 2% activity of the antibiotic.
### TABLE 1. AVERAGE AMOUNT OF WHEAT GIVEN PER DAY (g) \(^a\) AND MINIMUM MEAN pH VALUE OBTAINED

<table>
<thead>
<tr>
<th>Day</th>
<th>Control pH</th>
<th>Control Wheat</th>
<th>2% NaHCO(_3) pH</th>
<th>2% NaHCO(_3) Wheat</th>
<th>4% NaHCO(_3) pH</th>
<th>4% NaHCO(_3) Wheat</th>
<th>Thiopeptin pH</th>
<th>Thiopeptin pH</th>
<th>Thiopeptin + 2% NaHCO(_3) pH</th>
<th>Thiopeptin + 2% NaHCO(_3) Wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.12</td>
<td>564</td>
<td>6.16</td>
<td>564</td>
<td>6.27</td>
<td>564</td>
<td>5.75</td>
<td>564</td>
<td>5.78</td>
<td>564</td>
</tr>
<tr>
<td>2</td>
<td>5.61</td>
<td>564</td>
<td>6.05</td>
<td>564</td>
<td>5.95</td>
<td>564</td>
<td>5.78</td>
<td>564</td>
<td>5.88</td>
<td>564</td>
</tr>
<tr>
<td>3</td>
<td>4.97</td>
<td>940</td>
<td>5.57</td>
<td>940</td>
<td>5.68</td>
<td>940</td>
<td>5.43</td>
<td>940</td>
<td>5.41</td>
<td>940</td>
</tr>
<tr>
<td>4</td>
<td>4.37</td>
<td>313</td>
<td>5.75</td>
<td>940</td>
<td>5.55</td>
<td>940</td>
<td>5.38</td>
<td>940</td>
<td>5.44</td>
<td>940</td>
</tr>
<tr>
<td>5</td>
<td>4.61</td>
<td>0</td>
<td>5.76</td>
<td>940</td>
<td>5.87</td>
<td>940</td>
<td>5.25</td>
<td>940</td>
<td>5.54</td>
<td>940</td>
</tr>
<tr>
<td>6</td>
<td>...(^b)</td>
<td>0</td>
<td>5.56</td>
<td>940</td>
<td>5.39</td>
<td>940</td>
<td>5.22</td>
<td>940</td>
<td>5.64</td>
<td>940</td>
</tr>
</tbody>
</table>

\(^a\) Grams of wheat added to the rumen in controls was based on pH of rumen fluid prior to the addition of the wheat. Wheat was added until the rumen pH was between 4.0 and 4.5.

\(^b\) The pH values were not obtained after rumen fluid from donor animal was added.

### TABLE 2. LOWEST RUMEN pH VALUES OBTAINED IN EACH TREATMENT AND CORRESPONDING LACTIC ACID CONCENTRATION (mM)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control pH</th>
<th>Control Lactic</th>
<th>2% NaHCO(_3) pH</th>
<th>2% NaHCO(_3) Lactic</th>
<th>4% NaHCO(_3) pH</th>
<th>4% NaHCO(_3) Lactic</th>
<th>Thiopeptin pH</th>
<th>Thiopeptin Lactic</th>
<th>Thiopeptin + 2% NaHCO(_3) pH</th>
<th>Thiopeptin + 2% NaHCO(_3) Lactic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep A</td>
<td>4.52(^ad)</td>
<td>128.72</td>
<td>5.46(^b)</td>
<td>...(^c)</td>
<td>5.42(^b)</td>
<td>...</td>
<td>5.24(^b)</td>
<td>30.18</td>
<td>5.13(^b)</td>
<td>...</td>
</tr>
<tr>
<td>Sheep B</td>
<td>4.34(^ae)</td>
<td>115.15</td>
<td>5.39(^b)</td>
<td>...(^c)</td>
<td>5.28(^b)</td>
<td>...</td>
<td>5.06(^b)</td>
<td>23.34</td>
<td>5.22(^b)</td>
<td>...</td>
</tr>
<tr>
<td>Sheep C</td>
<td>4.25(^ae)</td>
<td>121.60</td>
<td>5.48(^b)</td>
<td>...(^c)</td>
<td>5.63(^b)</td>
<td>...</td>
<td>5.03(^b)</td>
<td>23.02</td>
<td>5.36(^b)</td>
<td>...</td>
</tr>
<tr>
<td>Sheep D</td>
<td>4.36(^ae)</td>
<td>107.06</td>
<td>5.84(^b)</td>
<td>...(^c)</td>
<td>5.89(^b)</td>
<td>...</td>
<td>5.24(^b)</td>
<td>17.08</td>
<td>5.48(^b)</td>
<td>...</td>
</tr>
</tbody>
</table>

\(^a, b\) Values on the same line with different superscripts differ significantly (P < .01).

\(^c\) No detectable acid.

\(^d\) 1500 hours, day four.

\(^e\) 1900 hours, day four.
Results and Discussion

The effect of the treatments on mean pH values recorded each day are shown in table 1. The lowest pH values recorded for each sheep and corresponding lactic acid concentrations are shown in table 2. The 2 and 4% NaHCO₃ and antibiotic + 2% NaHCO₃ treatments were effective in maintaining higher pH levels (P <.01) in the rumen than for controls. The antibiotic treatment was also effective in maintaining higher pH values (P<.05) than controls, but to a lesser degree.

All control treatments resulted in acute lactic acidosis with typical symptoms of lethargy, anorexia and moderate diarrhea. Increased respiration rates were noted in all animals when the rumen pH fell below 5. Maximum lactic acid concentrations ranged from 107.1 to 128.7 mM for the four animals on the control treatment. These concentrations are in agreement with values reported by Allison et al. (1964), Uhart and Carroll (1967) and Dunlop (1970). While the increased wheat feeding began on day 3, the lowest pH values and maximum lactic acid concentrations for the control animals occur-fed during the fourth day. Individual susceptibility to acidosis was evidenced by the fact that the total amount of wheat required on days 3 and 4 to induce acidosis ranged from 59.3 to 82.2 g/kgBW.⁷⁵

A rumen pH of approximately 5 seems critical in that none of the sheep went off feed until the pH was near or below 5. Lactic acid concentration increased rapidly when rumen pH fell below 4.8. Dougherty et al. (1975b) showed that a pH of 5 is the general value where the contractions of the reticulo-rumen decrease in magnitude and frequency and the rumen becomes static. At pH <5 the rumen ingesta changed markedly, becoming yellowish-green in color and very fluid in consistency, indicating that the ingesta had become hypertonic to the plasma with a resulting flow of body fluids into the rumen or that more than normal amounts of water were retained in the rumen.

Control animals were off feed for 28 to 40 hours. This period represents the time lapse until moderate feed intake was resumed. Because the animals were treated to raise the rumen pH, the time the animals were off feed is probably not indicative of what one might observe under field conditions.

The quantities of wheat used to induce lactic acidosis in this study were less than that used in the study by Beede and Farlin (1977). Levels of lactate observed in their study were less than in the present study despite the feeding of additional wheat. Data on the effects of different types of wheat and the incidence of lactic acidosis are lacking and might provide some insight to dissimilarities observed between various reports. Some of our unpublished data would indicate that time is a factor as well as amount fed. Pellets containing 75% cracked wheat fed at 40 g/kgBW.⁷⁵, divided equally between two feedings 8 hr apart, caused no problems when fed for a 10-day period, but acidosis developed when the 40 g/kgBW.⁷⁵ was fed at a single feeding on the 11th day. Additional animals developed acidosis when given a single feeding of the pellets at the 40 g/kgBW.⁷⁵ level after being maintained on a grass hay-alfalfa diet.

It was assumed in this study that there was no permanent damage to the animals during the control treatments in which lactic acidosis was induced or that any tissue damage would not

<table>
<thead>
<tr>
<th>Treatments</th>
<th>2% NaHCO₃</th>
<th>4% NaHCO₃</th>
<th>Thiopeptin</th>
<th>Thiopeptin + 2% NaHCO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2.34</td>
<td>2.09</td>
<td>2.22</td>
<td>.99</td>
</tr>
<tr>
<td>B</td>
<td>6.76a</td>
<td>1.97</td>
<td>2.38</td>
<td>1.28</td>
</tr>
<tr>
<td>C</td>
<td>4.46b</td>
<td>2.83</td>
<td>1.81</td>
<td>.82</td>
</tr>
<tr>
<td>D</td>
<td>3.17</td>
<td>2.09</td>
<td>2.07</td>
<td>.86</td>
</tr>
</tbody>
</table>

a,b High ratios are due largely to low concentrations of propionic acid during lactic acidosis.
affect subsequent experiments. The damaging effect upon the epithelial surface of the rumen and other parts of the gastro-intestinal tract has been reported by Ahrens (1967), Kay et al. (1969), Thomson (1967) and Dunlop (1967). To substantiate this assumption, acute acidosis was induced in six crossbred lambs by administering a sucrose solution via a stomach tube. The initial dose of sucrose was 15 g/kgBW. The rumen pH was kept <5 for varying lengths of time by additional doses of sucrose solution. When the sheep were slaughtered and the tissues examined, no gross tissue damage was found in any animal until the pH had been < 5 for more than 48 hours. The animals in this experiment were treated so that no animal had a rumen pH <5 for more than 28 hours.

While all treatments were effective in preventing acute lactic acidosis from developing, the antibiotic treatment did result in the presence of rumen lactic acid >20 mM in three of the four sheep (table 2). No adverse effects were observed and the animals continued to eat all feed that was offered. Giesecke et al. (1977) reported that lactic acid concentrations of 10 mM were tolerated by sheep but the present study would indicate higher levels can exist for short periods of time without any deleterious effects.

The effect of the treatments on the formation of volatile fatty acids (VFA) was quite marked. Concentrations of acetic (C2) and propionic (C3) acids are shown in figures 1 and 2, respectively. The addition of either 2 or 4% NaHCO3 resulted in an increase in C2 (P<.01) compared to the control animals. Treatments containing the antibiotic (antibiotic or antibiotic + 2% NaHCO3) did not show any significant increase in C2. These results are explained by the fact that the C2 levels in the control animals decreased due to the occurrence of acidosis while the treatments containing the antibiotic (A) also showed a decrease in C2 concentrations. The 4% NaHCO3 resulted in more C2 (P<.05) than did the A or A + 2% NaHCO3 treatments. All treatments showed increases (P<.01) in C3 when compared to control values. These differences are largely due to the fact that C3 and butyric (C4) acids decreased rapidly as the lactic acid concentration increased. Similar results were reported by Reid et al. (1957). Treatments containing the antibiotic resulted in increases (P<.01) in C3 when compared to the 2 or 4% NaHCO3 treatments. The combination of A + 2% resulted in still a further increase in C3 (P<.05) when compared to the antibiotic used alone. It appears that the treatments containing the antibiotic are altering the microbial population.
of the rumen towards one capable of producing increased amounts of C3 and lesser amounts of C2 (table 3). Such a shift is recognized as beneficial under feedlot situations. No statistical differences were noted for any treatments concerning the production of butyric acid in the rumen.

Total volatile fatty acids (TVFA) and calculated gross energy (GE) from volatile fatty acids are presented in table 4. While all treatments were higher (P<.01) in TVFA when compared to the control, these differences are due to the decrease in VFA in the control values as acidosis progressed. No statistical differences for TVFA were observed between the NaHCO3 or antibiotic treatments. This observation illustrates the point that while the buffer treatments were higher in C2, the antibiotic treatments allowed higher levels of C3 with the result that total concentrations of VFA were not altered.

There were no statistical differences between any of the buffer or antibiotic treatments for GE content of TVFA. The fact that C3 acid is more efficiently produced in the rumen than C2 is not accounted for in these data; thus while GE data look very similar, the A and A + S% treatments which result in the production of more C3 may be more beneficial in a fattening ration that would be indicated by this experiment. The fact that Thiopeptin was successful in preventing acidosis as well as increasing the proportion of propionic acid produced, certainly would make it a potentially useful feed additive for protection against acidosis. The additive for protection against acidosis, while GE data look very similar, the A and A + 2% treatments which result in the production of more C3 may be more beneficial in a fattening ration that would be indicated by this experiment. Thiopeptin results illustrated the point that while the buffer treatments were higher in C3, the antibiotic treatments allowed higher levels of C3 with the result that total concentrations of TVFA were not altered.

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TABLE 4. TOTAL RUMEN VOLATILE FATTY ACIDS (TVFA) mM AND GROSS ENERGY (GE) FROM VFA (cal/ml) AS AFFECTED BY TREATMENTS

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>2% NaHCO3</th>
<th>4% NaHCO3</th>
<th>Thiopeptin</th>
<th>Thiopeptin + 2% NaHCO3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>TVFA</td>
<td>GE</td>
<td>TVFA</td>
<td>GE</td>
<td>TVFA</td>
</tr>
<tr>
<td>A</td>
<td>39.32a</td>
<td>12.81c</td>
<td>73.04b</td>
<td>22.07d</td>
<td>95.55b</td>
</tr>
<tr>
<td>B</td>
<td>16.26a</td>
<td>4.07c</td>
<td>95.71b</td>
<td>29.71d</td>
<td>104.86b</td>
</tr>
<tr>
<td>C</td>
<td>35.87a</td>
<td>9.51c</td>
<td>65.78b</td>
<td>18.84d</td>
<td>77.11b</td>
</tr>
<tr>
<td>D</td>
<td>41.64a</td>
<td>12.87c</td>
<td>78.15b</td>
<td>23.18d</td>
<td>78.87b</td>
</tr>
</tbody>
</table>

a,b Values on the same line with different superscripts differ significantly (P < .01).

c,d Values on the same line with different superscripts differ significantly (P < .01).

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