EFFECT OF HISTAMINE AND AMMONIA ON HYPOMAGNESEMA IN RUMINANTS¹,²

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SUMMARY

The effects of exogenous histamine and ammonia on hypomagnesemia were determined in a $2^2$ factorial arrangement of treatments with 16 ruminal fistulated lambs fed a semipurified low magnesium (.08%) ration and given intraruminal infusions of histamine, ammonia or histamine + ammonia and compared to a control receiving deionized water. Samples of blood and ruminal fluid were taken at the beginning and end of an 80-day trial and analyzed for histamine, electrolytes and ammonia, and ammonia and pH, respectively. One lamb in the histamine + ammonia group and two lambs in the ammonia group showed signs of hypomagnesemic tetany accompanied by low phosphorus levels. The two groups receiving ammonia had lower serum magnesium levels ($P<.05$) than the histamine or control groups. There was no treatment effect on serum calcium, potassium, phosphorus or ammonia levels. Blood histamine was higher ($P<.05$) in the group receiving histamine + ammonia. Ruminal ammonia levels were higher ($P<.01$) for the two groups receiving ammonia; however, no differences in ruminal pH were observed. These studies suggest that high levels of ammonia may be responsible for depression of serum Mg levels leading to tetany, but histamine has no effect.

In the second trial, analysis of serum from range cows indicated no correlation between serum histamine and magnesium levels.

(Key Words: Grass Tetany, Magnesium Requirements, Histamine, Ammonia.)

INTRODUCTION

Hypomagnesemia has been reported in ruminants managed under many feeding regimes, ranging from winter roughage feeding in drylot to the grazing of lush spring pastures (Allcroft and Burns, 1968). Winter hypomagnesemia is usually caused by a dietary magnesium deficiency, but this is not generally true in pasture situations where grass tetany has occurred. In the latter case, a defect in magnesium absorption or metabolism in the animal may assume a role of primary importance. Other factors such as weather, fertilization and soil type also may influence the availability of forage magnesium to the animal (Wilcox and Hoff, 1974). Nitrogen fertilization, high crude protein levels in forage and high ruminal ammonia levels have been associated with hypomagnesemia for many years (Sjollema, 1932; Bartlett et al., 1954; Head and Rook, 1955). Stillings et al. (1964) reported that magnesium utilization was lower for lambs consuming heavily fertilized forages in metabolism studies.

Blood histamine has been reported to increase in magnesium deficient rats due to liberation of endogenous histamine from mast cells (Bois et al., 1963). Fowler (1962, 1963) stated that histamine may be a factor in immature grass that results in reduced availability of magnesium to the animal. Antihistaminics have been effective in treating hypomagnesemia with and without supportive calcium-magnesium therapy (Hendricks, 1962). This study involved evaluation of the effect of exogenous ammonia and histamine on hypomagnesemia in sheep fed a semipurified diet and an examination of the relationship of endogenous histamine and magnesium in grazing cows.

EXPERIMENTAL PROCEDURE

Sheep Trial. Sixteen ruminal fistulated Finn x Rambouillet lambs were housed in individual pens with wooden slatted floors and galvanized metal screen sides. The lambs, approximately 6 months of age, were allowed a 7-day period to adapt from a commercial pelleted ration to the
semipurified experimental diet (table 1) and deionized water. *Ad libitum* intake of the diet averaged 1.40 kg per head daily during the experiment. Lambs were randomly assigned by sex to four treatment groups each consisting of two ewe and two wether lambs in a $2^2$ factorial arrangement involving two levels of histamine (0 or 3 mg histamine diphosphate per kg body weight) and two levels of ammonia (0 or 2.2 g 73% ammonium acetate-27% ammonium carbonate mixture per kilogram body weight daily). The following treatments were infused daily into the rumen via the fistula: (1) deionized water; (2) histamine; (3) ammonia; (4) histamine + ammonia. The ammonium acetate-ammonium carbonate mixture was administered daily in four doses at 4-hr intervals to avoid ammonia toxicity. When tetany-like signs were observed, blood samples were collected immediately and the animals treated intravenously with a calcium-magnesium solution.

**Collection and Analytical Procedures.** Twenty-milliliter samples of blood were collected via jugular puncture 2 hr after feeding and dosing at the beginning and end of the 80-day trial. Sodium fluoride-thymol was added to individual 5 ml samples of whole blood and subsequently frozen for later histamine determination (Anton and Sayre, 1969). The remaining blood was centrifuged at 37,000 × g for 30 min in a Sorvall RC2-B centrifuge. The serum was frozen and later analyzed by atomic absorption spectrophotometry for calcium, magnesium and potassium (Perkin-Elmer 303 spectrophotometer). Serum inorganic phosphorus was determined by the method of Harris and Popat (1954) and serum ammonia by the method of Chaney and Marbach (1962).

Samples of ruminal fluid were collected via rumen fistula 2 hr after feeding and dosing at the beginning and end of the trial. The pH was determined immediately and an aliquot frozen for ammonia determination by the Conway (1957) microdiffusion method.

All data were analyzed by analysis of variance and means separated by Newman-Keuls test (Steel and Torrie, 1960).

**Cattle Trial.** Serum samples from five Angus

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**TABLE 1. INGREDIENT AND CHEMICAL COMPOSITION OF SEMIPURIFIED DIET – SHEEP TRIAL**

<table>
<thead>
<tr>
<th>Item</th>
<th>Internat'l. Ref. No.</th>
<th>Percenta</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ingredient composition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn, cob, grnd (1)</td>
<td>1-02-782</td>
<td>40.00</td>
</tr>
<tr>
<td>Peanut, kernels (mech-ext, grnd) mx 7% fiber, (5)</td>
<td>5-03-649</td>
<td>15.00</td>
</tr>
<tr>
<td>Wheyb</td>
<td>1-02-783</td>
<td>10.00</td>
</tr>
<tr>
<td>Glucose monohydratec</td>
<td></td>
<td>32.90</td>
</tr>
<tr>
<td>Limestone, grnd, mn 33% calcium (6)</td>
<td>6-02-632</td>
<td>.10</td>
</tr>
<tr>
<td>Salt, trace mineralizedd</td>
<td></td>
<td>.70</td>
</tr>
<tr>
<td>Calcium phosphate, dibasic, comm (6)</td>
<td>6-01-080</td>
<td>.40</td>
</tr>
<tr>
<td>Potassium carbonate</td>
<td></td>
<td>.85</td>
</tr>
<tr>
<td>Vitamins A and Dc</td>
<td></td>
<td>.05</td>
</tr>
<tr>
<td><strong>Chemical composition</strong></td>
<td></td>
<td>100.00</td>
</tr>
<tr>
<td>Crude protein</td>
<td></td>
<td>10.42</td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
<td>.15</td>
</tr>
<tr>
<td>Phosphorus</td>
<td></td>
<td>.20</td>
</tr>
<tr>
<td>Magnesium</td>
<td></td>
<td>.08</td>
</tr>
<tr>
<td>Sulfur</td>
<td></td>
<td>.03</td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td>.84</td>
</tr>
<tr>
<td>Gross energy, kcal/kg</td>
<td></td>
<td>1730</td>
</tr>
</tbody>
</table>

aDry matter basis.

bDried, demineralized; Melbrew, Inc., Milwaukee, WI.

cCerelose dextrose, granular No. 2025; CPC International, Inc., Argo, IL.

dContained (%): Zn, .110; Mn, .288; Fe, .160; Cu, .104; Co, .012; I, .007; NaCl, 98.5.

ejLevels were 4,400 U.S.P. units vitamin A palmitate and 466 U.S.P. units vitamin D (ergocalciferol) per kilogram of ration.
and three Shorthorn cows, 5- to 8-years-old, fed
fescue (*Festuca arundinacea*) as hay and pasture
were analyzed for histamine following the
above procedure. Animals were selected from a
herd of 56 cows on the basis of their serum
magnesium values for a 4-month interval. The
mean serum magnesium level was initially 2.17
mg/100 ml during the nonlactating pregnant
period. At the final bleeding, 2 to 4 weeks
post-partum, the mean serum magnesium level
was .60 mg/100 ml. Correlation coefficients
were derived by regression analysis.

RESULTS AND DISCUSSION

**Sheep Trial.** Signs of tetany were observed
four times and involved three different lambs
(table 2). On day 36 of the trial, a ewe lamb
(No. 723) in Group 4 (histamine + ammonia)
displayed uncoordinated limb movements im-
mediately following the am feeding and dosing.
This lamb did not collapse; however, 3 days
later similar signs developed and progressed to
the convulsive stage. Recovery occurred upon
intravenous treatment with 50 ml of CGP, a
commercial electrolyte solution (Haver-Lock-
hart Laboratories, Shawnee, Kansas). On day
61, another ewe lamb (No. 721) in Group 3
(ammonia) showed similar but slightly more
severe signs and also recovered following treat-
ment. A wether (No. 725) in Group 3 exhibited
staggering on the 65th day but without progres-
sion into convulsions. All animals showing
incoordination and convulsions had low serum
magnesium (.907 to 1.478 mg/100 ml) and
phosphorus levels (2.245 to 5.844 mg/100 ml)
but relatively normal calcium and potassium
levels.

Serum electrolyte values are presented in
table 3. Serum magnesium decreased (P<.05) in
the lambs receiving ammonia compared to the
histamine and control groups. The lowest final
mean was observed in Group 3 (1.400 mg/100
ml) but was not different from Group 4 (1.503
mg/100 ml). These findings agree with those of
Head and Rook (1955) when cows were dosed
intraruminally with ammonium salt solutions.

The magnesium content of the experimental
diet was .08% (table 1). Although 200 ppm
greater than the requirement established by
McAleese *et al.* (1959), this level might allow a
magnesium deficiency to develop when accom-
panied with high levels of nonprotein nitrogen
(NPN). Serum magnesium levels in Groups 1
and 2 remained normal which might suggest an
increased magnesium requirement or decreased
availability with higher nitrogen levels, as re-
flexed in Groups 3 and 4.

Although serum calcium levels of individual
lambs displaying signs of tetany were some-
times below normal in this trial, there were no
significant (P>.05) differences between treat-
ment groups (table 3). This contrasts with
observations of Ashton and Sinclair (1965) who
found decreased serum calcium with sheep
dosed daily with 200 g ammonium acetate. The
sub-optimal dietary calcium level (.15%) had no
apparent adverse effect upon serum calcium
levels.

The serum inorganic phosphorus decrease
was greater for Group 3 than any other
treatment group, but this was only statistically
significant at the 10% level (table 3). Animals
observed in tetany had low serum phosphorus
levels (2.245 to 5.844 mg/100 ml) which agree
with findings of Smith *et al.* (1975) for cows
that developed tetany on pasture. Serum potas-
sium values (table 3) were not different be-
tween treatment groups.

Oral administration of histamine resulted in
a significant increase in blood levels of acetyl-
histamine, the major metabolite of histamine,
but only a slight increase in blood histamine
(Dickinson and Huber, 1972). The mean blood
histamine level for Group 4, .035 μg/ml (table
4) agrees with values obtained for sheep (Dick-
inson and Huber, 1972), but the means of
Groups 1, 2 and 3 are lower and approximate

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Treatment</th>
<th>Magnesium</th>
<th>Calcium</th>
<th>Phosphorus</th>
<th>Potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td>721</td>
<td>Ammonia</td>
<td>1.478</td>
<td>6.869</td>
<td>2.245</td>
<td>16.6</td>
</tr>
<tr>
<td>723</td>
<td>Histamine + ammonia</td>
<td>.848</td>
<td>8.443</td>
<td>2.356</td>
<td>13.6</td>
</tr>
<tr>
<td>725</td>
<td>Ammonia</td>
<td>.907</td>
<td>10.129</td>
<td>5.844</td>
<td>16.8</td>
</tr>
</tbody>
</table>
values observed for calves (Code and Hester, 1939). No obvious explanation exists for the significant ($P<.05$) interaction between histamine and ammonia on blood histamine levels. If ammonia caused endogenous histamine release, possibly by displacing histamine from its intracellular storage sites or inducing histidine decarboxylase, this phenomenon should have resulted in Group 3 values averaging higher than .007 $\mu$g/ml. No significant ($P>.05$) correlation between magnesium and serum histamine levels was observed ($r = -.149$).

The serum ammonia values obtained 2 hr after dosing in table 4 indicate no differences ($P>.05$) between treatment groups, indicating that levels of serum ammonia did not exceed the capability of the liver to convert excess ammonia to urea.

The addition of ammonium salts and histamine did not alter ($P>.05$) pH of ruminal fluid (table 5). Ashton and Sinclair (1965) did not observe a notable change in ruminal pH upon addition of 200 g ammonium acetate to the diet of sheep. The slight depression in all treatments observed in the final values was possibly due to the nature of the experimental diet which contained a high level of readily fermentable carbohydrate.

Ammonium salts were administered intraruminally to simulate the amount of ammonia that would be found in a grazing situation. Head and Rook (1957) reported ruminal ammonia values of 13.2 and 41.3 mg/100 ml for hay-concentrate diets and pasture grazing, respectively. Table 5 lists mean ruminal ammonia levels of animals on the respective experimental treatments. Animals receiving the additional NPN had significantly higher ($P<.01$) ruminal ammonia levels than the lambs in Groups 1 and 2. These values are similar to those of Christian and Williams (1960) and Wilson (1963) who reported ruminal ammonia values of 75 to 80 mg/100 ml for lambs receiving additional NPN as urea. This magnitude of increase was similar to values observed in this study.

Based on the results of this trial, it is probable that high levels of NPN do increase the incidence of hypomagnesemia, possibly due to a change in ionic balance. Changes in the balance of monovalent ($K^+, NH_4^+, Na^+$) and divalent ($Ca^{++}, Mg^{++}$, diamines) ions could alter membrane potentials and, consequently, change nervous excitability. The additional ammonia in the diet could alter the amount of the diamines, putrescine and cadaverine pro-

### Table 3: Effect of Ammonia and Histamine on Serum Magnesium, Calcium, Inorganic Phosphorus, and Potassium (mg/100 ml - Sheep Trial)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Magnesium</th>
<th>Calcium</th>
<th>Potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>2.06</td>
<td>2.41</td>
<td>1.50</td>
</tr>
<tr>
<td>Final</td>
<td>2.01c ± 0.02</td>
<td>2.04c ± 0.13</td>
<td>1.50b ± 0.32</td>
</tr>
</tbody>
</table>

- Mean of four observations ± standard error of mean.
- Means with different superscripts differ significantly ($P<.05$).
- a,b,c = Means with different superscripts differ significantly ($P<.05$).
TABLE 4. EFFECT OF HISTAMINE AND AMMONIA ON SERUM AMMONIA (µg/100 ml) AND BLOOD HISTAMINE (µg/ml) - SHEEP TRIAL

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ammonia Initial</th>
<th>Ammonia Final</th>
<th>Histamine Initial</th>
<th>Histamine Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>.69</td>
<td>.63 ± .06</td>
<td>.004</td>
<td>.016 ± .007</td>
</tr>
<tr>
<td>Histamine</td>
<td>.52</td>
<td>.47 ± .06</td>
<td>.006</td>
<td>.009 ± .001</td>
</tr>
<tr>
<td>Ammonia</td>
<td>.64</td>
<td>.62 ± .09</td>
<td>.006</td>
<td>.007 ± .001</td>
</tr>
<tr>
<td>Histamine + ammonia</td>
<td>.62</td>
<td>1.19 ± .65</td>
<td>.019</td>
<td>.035 ± .012</td>
</tr>
</tbody>
</table>

*Mean of four observations ± standard error of mean.

A mean of four observations ± standard error of mean.

B Means with different superscripts differ significantly (P<.05).

duced by rumen microbes, thus increasing the divalent and monovalent ions which would have a similar effect.

Cattle Trial. No statistically significant correlation between serum magnesium and serum histamine levels was observed. The correlation coefficient for the initial mean serum magnesium value (2.17 mg/100 ml) and serum histamine value (1.234 µg/ml) was r = -.474. The correlation coefficient for the final mean serum magnesium value (.60 mg/100 ml) and serum histamine value (.008 µg/ml) was r = -.388. Histamine values generally agree with those of Code and Hester (1939) and Code and Jensen (1941).

The high initial histamine value was probably related to stresses of handling during the initial blood sampling procedure.

From these data it can be concluded that a magnesium deficiency, as indicated by the serum magnesium values, will not cause release of endogenous histamine in the bovine. No significant absorption of histamine occurs from the rumen wall (Dickinson and Huber, 1972) and rumen microbes are not responsible for any substantial histamine metabolism (Sjaastad and Stormorken, 1963) although some histamine is metabolized in the rumen wall. Histaminase (diamine oxidase) activity is greatest in the liver and kidney. Acetylation appears to be an important pathway of histamine inactivation in ruminants (Code, 1965). It is probable that dietary histamine has little effect on hypomagnesemic tetany.

LITERATURE CITED


TABLE 5. EFFECT OF HISTAMINE AND AMMONIA ON RUMINAL pH AND RUMINAL AMMONIA (mg NH₃-N/100 ml) - SHEEP TRIAL

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ruminal pH Initial</th>
<th>Ruminal pH Final</th>
<th>Ruminal ammonia Initial</th>
<th>Ruminal ammonia Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.30</td>
<td>6.03 ± .10</td>
<td>26.88</td>
<td>7.37c ± 1.88</td>
</tr>
<tr>
<td>Histamine</td>
<td>6.45</td>
<td>5.98 ± .21</td>
<td>20.42</td>
<td>11.28c ± .62</td>
</tr>
<tr>
<td>Ammonia</td>
<td>6.15</td>
<td>5.83 ± .22</td>
<td>18.94</td>
<td>58.04b ± 4.91</td>
</tr>
<tr>
<td>Histamine + ammonia</td>
<td>6.25</td>
<td>5.78 ± .05</td>
<td>33.54</td>
<td>72.66b ± 19.14</td>
</tr>
</tbody>
</table>

*Mean of four observations ± standard error of mean.

b,c Means with different superscripts differ significantly (P<.01).


