THE PROPHYLACTIC EFFECT OF CORN SUPPLEMENTATION ON EXPERIMENTAL NITRATE INTOXICATION IN CATTLE

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ABSTRACT

Sodium nitrate was administered through rumen cannulae to produce NO\textsuperscript{−}\textsubscript{3} intoxication in four cows (382 to 445 kg body wt) fed prairie grass hay and a protein-mineral supplement. The cows were fed 0, 1.6 or 3.2 kg of dry rolled corn daily for 10 d prior to sodium nitrate administration. Sodium nitrate administration was followed by a marked increase in intraruminal NO\textsuperscript{−}\textsubscript{3} and NH\textsubscript{3} and blood NO\textsuperscript{−}\textsubscript{3} and methemoglobin. Six of eight cows fed 0 and 1.6 kg of corn were given methylene blue to treat severe methemoglobinemia, while none of the cows fed 3.2 kg corn required such therapy. Feeding of 3.2 kg of corn protected against nitrate poisoning by reducing intraruminal nitrite and blood methemoglobin (P<.05). (Key Words: Cattle, Nitrates, Nitrites, Maize, Poisoning, Methemoglobinemia.)

Introduction

Since the report by Mayo in 1895, nitrate (NO\textsubscript{3}) has been recognized as toxic to cattle. Nitrate is reduced to nitrite (NO\textsubscript{2}) in the rumen. When NO\textsubscript{2} is absorbed into blood (Lewis, 1951; Wang et al., 1961), it causes oxidation of ferrous hemoglobin (Hb) to ferric methemoglobin (MHb; Vertregt, 1977). Methemoglobin is incapable of oxygen transport; thus, disease occurs when sufficient MHb is formed to compromise tissue respiration.

Nitrate uptake by plants is an essential step in the incorporation of soil nitrogen into plant protein. Normally NO\textsubscript{3} is absorbed and rapidly assimilated with little hazard to foraging ruminants. However, under certain circumstances some plants, particularly the sorghum/sudan forages, may accumulate toxic concentrations of NO\textsubscript{3} (Clay et al., 1976; Haliburton and Edwards, 1978). Because of the continuing seriousness of the problem, many suggestions and recommendations have been promulgated to reduce the disease hazard. These recommendations usually relate to reducing NO\textsubscript{3} accumulation by plants, and procedures to reduce the hazard of feeding forages high in NO\textsubscript{3}. Among the latter, the use of high-energy feeds is often recommended (Hanway et al., 1963; Clay et al., 1976; Prewitt, 1978). However, in most cases the published recommendations are qualitative in nature rather than quantitative. For actual situations where high NO\textsubscript{3} concentrations are expected in feed there is little information available on which to base a recommendation of feeding a specific amount of high-energy supplement.

The purpose of these experiments was to quantify protective effects of increasing amounts of corn on NO\textsubscript{3} intoxication. Information from this study would aid in allowing for continued use of high NO\textsubscript{3} forages while minimizing disease and death losses.

Materials and Methods

Five Hereford cows approximately 3 yr of age ranging in weight from 382 to 445 kg were utilized. The cows were treated with fenbendazole prior to experimentation, and were maintained in pairs in large, outdoor, drylot pens. They were fed ad libitum prairie grass hay, which consisted primarily of Little Bluestem (Andropogon scoparius) and Switchgrass (Panicum virgatum) and contained .09% NO\textsubscript{3}, and .5 kg·head\textsuperscript{−1} of a protein-mineral supplement

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that contained the following (percent of dry matter): soybean meal, 93.1%; sugarcane molasses, 2.5%; dicalcium phosphate, 2.2%; trace-mineralized salt, 2.2%; and vitamin A, 26.219 IU/kg. In addition, the cows were fed 0, 1.6 or 3.2 kg of dry rolled corn daily at approximately 0730, beginning 10 d prior to challenge with .3 g NaNO₃/kg body weight.

The prairie hay was sampled twice during the study. It contained 4.5% crude protein (dry matter basis) and had an in vitro dry matter digestibility (IVDMD) of 40.1%. Crude protein was determined by macro-Kjeldahl procedures (AOAC, 1975). IVDMD was determined by the Tilley and Terry (1963) procedure, with urea (.5 g/liter) added to one part strained ruminal fluid:one part McDougall's Buffer (McDougall, 1948), using a 24-h, acid-pepsin digestion phase. Residual dry matter (DM) was collected in a Buchner funnel fitted with pre-weighed, oven-dried Whatman No. 4 filter paper.

Nitrate challenge was accomplished by dissolving NaNO₃ in 500 ml distilled water and administering it through a 10-cm ruminal cannula. Nitrate was administered at approximately 0900 in all the experiments. The experiments were carried out using four animals, two per week (table 1) in sequence such that a 2-wk interval occurred between NaNO₃ challenges for each animal. There was a total of eight observations (two in each of four cows) at 1.6 and 3.2 kg of corn and 12 observations (three in each of four cows) at 0 kg of corn. Samples of ruminal fluid were obtained through the ruminal cannula prior to and at 3, 6, 8, 10, 12 and 24 h after NaNO₃ challenge, and were strained through cheesecloth. A 40-ml aliquot of strained fluid was added to 10 ml 30% (w/v) lead acetate (Kemp et al., 1977) for determination of nitrite and ammonia. Nitrite and pH were determined immediately, and ammonia was determined the following day using a subsample that had been immediately frozen. Jugular blood samples (heparinized) were collected along with ruminal samples for determination of nitrite, ammonia, hemoglobin and methemoglobin.

Animals that manifested pronounced incoordination following NaNO₃ administration were treated with a single intravenous bolus of 1 g methylene blue in 50 ml of distilled water administered into the jugular vein. However, despite this precaution, one animal died during the first series of experiments (wk 1) and was replaced by a fifth animal. Blood samples were not collected following treatment with methylene blue. This affected 4 of 12 observations with 0 kg of corn and 2 of 8 observations with 1.6 kg of corn.

Blood and ruminal fluid were analyzed for NO₂⁻ by a spectrophotometric method employing diazotization of sulfanilic acid and coupling with naphthylethlyenediamine to give an azodye (Schneider and Yeary, 1973). Ammonia was determined in ruminal fluid by the method of Chaney and Marbach (1962) and in blood by an ultraviolet method (Sigma Chemical Co., 1980) based on the reductive amination of α-ketoglutaric acid. Methemoglobin determination was carried out using a spectrophotometric method (Leahy and Smith, 1960) with MHb calculated as a percent of total Hb. Hemoglobin was also determined using the cyanomethemoglobin colorimetric method (Sigma Chemical Co., 1979).

The variables analyzed were obtained as the simple average, the maximum, or the weighted average, which reflects the integrated time response (i.e., "area under the curve"). These statistics were based only on samples obtained prior to treatment with methylene blue. Analysis involved fitting a linear model that included treatment, cow and sequence effects, as well as interactions of cow and treatment and time and treatment. In general, these interactions were negligible and were omitted from the final model. The residual mean square of this reduced model was used as the error term for making treatment comparisons. Treatment differences were evaluated in tests for linear and quadratic trends over amount of supplemental corn. Student's t-test was used to compare the 3.2-kg corn treatment to 1.6 and 0 kg corn when linear and(or) quadratic effects were significant.

**Results and Discussion**

Following administration of NaNO₃ there was a consistent increase in the pH of ruminal fluid during the 24-h observation period. The minimum at the time of NaNO₃ administration was 6.8 for all three experimental groups, and ranged to maximums of 7.5 ± .07, 7.2 ± .22 and 7.6 ± 1.0 for cows fed 0, 1.6 and 3.2 kg corn,
TABLE 1. SEQUENCE OF SUPPLEMENTATION TREATMENTS AND INTOXICATION RESPONSES OF COWS ADMINISTERED .3 g NaNO₃/kg BODY WEIGHT VIA RUMINAL CANNULAE

<table>
<thead>
<tr>
<th>Week</th>
<th>Animals</th>
<th>Supplemental corn, kg·head⁻¹·d⁻¹</th>
<th>Cows requiring methylene blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
<td>AB/CD</td>
<td>1.6</td>
<td>1 (died before treatment)</td>
</tr>
<tr>
<td>3/4</td>
<td>AE/CD</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>5/6</td>
<td>AE/CD</td>
<td>3.2</td>
<td>0</td>
</tr>
<tr>
<td>7/8</td>
<td>AE/CD</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>9/10</td>
<td>AE/CD</td>
<td>1.6</td>
<td>1</td>
</tr>
<tr>
<td>11/12</td>
<td>AE/CD</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13/14</td>
<td>AE/CD</td>
<td>3.2</td>
<td>0</td>
</tr>
</tbody>
</table>

a Two cows per week.
b Substituted fifth animal to replace death loss.

respectively. There were no differences (P > .05) among the groups. Similar increases in ruminal ammonia concentrations were seen in all three experimental groups, with maximum values occurring at 6 h after nitrate challenge. There were no consistent increases in blood ammonia concentrations.

Ruminal and blood NO₂⁻ concentrations increased to maximums at 6 h (figure 1) and 6 to 8 h (figure 2), respectively, in all three

Figure 1. Mean (± SE) ruminal nitrite concentration following intraruminal administration of .3 g NaNO₃·kg⁻¹ body weight in four cows fed 0, 1.6 or 3.2 kg dry-rolled corn with prairie grass hay and a protein-mineral supplement. Each point prior to 10 h with 0 kg of corn represents 12 observations. Due to stopping blood sample collection after methylene blue treatment, only 11, 10 and 8 observations are represented at 10, 12 and 24 h, respectively. Similarly, for 1.6 kg of corn, eight observations are represented except for a decrease to seven at 10 and 12 h and six at 24 h. All points for 3.2 kg of corn represent eight observations.
groups of cows. There was a linear decrease (table 2) in the means of all concentrations and mean maximal concentrations of ruminal NO$_2^-$ with increasing level of corn supplementation. Distinct attenuation of the increase in ruminal NO$_2^-$ with 3.2 kg corn is also shown by t-test comparisons of means, maximums and area under the concentration/time curve (figure 1, table 2). There was no detectable reduction of ruminal NO$_2^-$ with 1.6 kg corn by most of the methods of comparison (figure 1, table 2). Blood NO$_2^-$ concentrations of all groups increased initially and then declined rapidly after maximal values were obtained. The differences were significant (P<.05) at 6 h between 1.6 and 3.2 kg of corn and at 8 h between 0 kg of corn and both 1.6 and 3.2 kg of corn.

There was a marked decrease in methemoglobin provided by 3.2 kg corn compared with both 0 kg and 1.6 kg corn (figure 3). Methemoglobin concentrations often exceeded 50% by 8 h after NaNO$_3$ administration in cows given 0 or 1.6 kg of corn. These cows were usually in sternal recumbency but would stand when approached. Incoordination was distinct, and some apprehension (and occasional belligerence) was noted in cows not otherwise displaying such demeanor. Clear signs of incoordination were observed in six out of eight situations when MHb exceeded 60%. Treatment with methylene blue rapidly alleviated the signs. In the first experiment, one cow was found dead at 12 h after showing only moderate ataxia 10 h following NaNO$_3$ administration. The MHb concentration at 10 h was found to be 69%. This cow was replaced, and thereafter all cows were immediately treated with methylene blue when signs of incoordination were observed. No other deaths occurred, but 4 of 12 observations of cows receiving 0 kg corn and 2 of 8 observations of cows receiving 1.6 kg corn subsequently required treatment with methylene blue. Because blood samples were not collected after methylene blue treatment, the means for the
<table>
<thead>
<tr>
<th>Variable</th>
<th>Supplemental cow, kg·head⁻¹·d⁻¹</th>
<th>Analysis of variance comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1.6</td>
</tr>
<tr>
<td>Blood ammonia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean of all values, µg/ml</td>
<td>1.71</td>
<td>.70</td>
</tr>
<tr>
<td>Rumen ammonia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean of all values, µg/ml</td>
<td>8.1</td>
<td>9.8</td>
</tr>
<tr>
<td>Mean of maximums, µg/ml</td>
<td>13.7</td>
<td>15.9</td>
</tr>
<tr>
<td>Relative AUCb, µg·ml⁻¹·h⁻¹</td>
<td>183.0</td>
<td>213.0</td>
</tr>
<tr>
<td>Blood nitrite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean of all values, µg/ml</td>
<td>.07</td>
<td>.07</td>
</tr>
<tr>
<td>Mean of maximums, µg/ml</td>
<td>.21</td>
<td>.23</td>
</tr>
<tr>
<td>Relative AUC, µg·ml⁻¹·h⁻¹</td>
<td>1.52</td>
<td>1.63</td>
</tr>
<tr>
<td>Rumen nitrite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean of all values, µg/ml</td>
<td>1.89</td>
<td>1.45</td>
</tr>
<tr>
<td>Mean of maximums, µg/ml</td>
<td>4.96</td>
<td>4.25</td>
</tr>
<tr>
<td>Relative AUC, µg·ml⁻¹·h⁻¹</td>
<td>42.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Methemoglobin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean of all values, %Hb</td>
<td>25.3</td>
<td>23.7</td>
</tr>
<tr>
<td>Mean of maximums, %Hb</td>
<td>53.1</td>
<td>52.4</td>
</tr>
<tr>
<td>Relative AUC, %Hb/h</td>
<td>571.0</td>
<td>498.0</td>
</tr>
</tbody>
</table>

aComparisons are for the 24 h immediately following NaNO₃ administration.

bArea under the curve.

cLess than 0 and 1.6 kg corn (P < .05).

dLess than 0 kg corn (P < .05).
CORN SUPPLEMENTATION IN NITRATE INTOXICATION

Figure 3. Mean (+ SE) blood methemoglobin concentration following intraruminal administration of .3 g NaNO₃·kg⁻¹ body weight in four cows fed 0, 1.6 or 3.2 kg dry-rolled corn with prairie grass hay and a protein-mineral supplement. Each point prior to 10 h with 0 kg of corn represents 12 observations. Due to stopping blood sample collection after methylene blue treatment, only 11, 10 and 8 observations are represented at 10, 12 and 24 h, respectively. Similarly, for 1.6 kg of corn, eight observations are represented except for a decrease to seven at 10 and 12 h and six at 24 h. All points for 3.2 kg of corn represent eight observations.

The sequence of changes seen following NaNO₃ administration is consistent with gradual ruminal conversion of NO₃⁻ to NO₂⁻ and NH₃, absorption of NO₂⁻ into blood and subsequent oxidation of Hb to MHb. Increased concentrations of blood NH₃ were not observed, as would be expected because of the very small increases in ruminal NH₃ concentrations. In addition, ruminal pH was consistently below the physiological pH of blood, which would favor ruminal retention of the basic NH₃ by a slight ion-trapping effect.

The influence of fermentable substrates on the rate of ruminal metabolism of NO₃⁻ and NO₂⁻ was first shown by Sapiro et al. (1949). This increase in rate of reduction of NO₃⁻ and NO₂⁻ by supplemental concentrates was subsequently confirmed (Holtenius, 1957; Nakamura et al., 1979; Takahashi et al., 1980). In the present study there was clear indication that the highest level of corn supplementation provided protection against NO₃⁻ intoxication.

For persons dealing with NO₃⁻ intoxication in cattle, a note of caution should be followed. In other species, reports indicate a much greater disparity between MHb concentrations producing clinical signs (40 to 60% MHb) and those causing death (85 to 90% MHb; Bodansky, 1951; Kiese, 1974). With cattle, and probably sheep, clinical signs of intoxication may not be apparent until MHb exceeds 60%, which is close to the lethal concentration of approximately 75% MHb, as shown here and by previous research (Ashbury and Rhode, 1964; Burrows, 1979). Therefore, if indications of incoordination are observed with possible NO₃⁻ ingestion, immediate treatment with methylene blue is imperative.

various characteristics in cows given 0 or 1.6 kg of corn are based on a smaller number of observations at the latter times as indicated in figures 1, 2 and 3. Baseline hemoglobin concentrations were not affected by corn supplementation.

For persons dealing with NO₃⁻ intoxication in cattle, a note of caution should be followed. In other species, reports indicate a much greater disparity between MHb concentrations producing clinical signs (40 to 60% MHb) and those causing death (85 to 90% MHb; Bodansky, 1951; Kiese, 1974). With cattle, and probably sheep, clinical signs of intoxication may not be apparent until MHb exceeds 60%, which is close to the lethal concentration of approximately 75% MHb, as shown here and by previous research (Ashbury and Rhode, 1964; Burrows, 1979). Therefore, if indications of incoordination are observed with possible NO₃⁻ ingestion, immediate treatment with methylene blue is imperative.
The ruminal and blood \( \text{NO}_2^- \) concentrations and MHB levels were all lower than respective values observed in cows fed 0 and 1.6 kg of corn. If the cows fed 0 and 1.6 kg of corn had gone without treatment with methylene blue, the mean MHB percentage at 8, 10 and 12 h (as shown in figure 3) would likely have been even higher.

The feedstuff and dosage of the \( \text{NO}_3^- \) challenge are important factors in application of these results. Nitrate salts are more toxic when administered as a drench than when fed with hay (Crawford, 1966), and \( \text{NO}_3^- \) in hay is more readily available for microbial reduction than that in grazed grasses (Geurink et al., 1979). While in our research 3.6 kg of corn per day protected the cows against poisoning from a fixed amount of nitrate, this amount of corn may be insufficient to protect against poisoning in actual field situations where variable or even higher amounts of nitrate may be ingested.

In addition to feedstuff and amount of \( \text{NO}_3^- \), the immediate past experience of the animal to dietary \( \text{NO}_3^- \) is an important factor in determining rate of formation of \( \text{NO}_2^- \). Adaptive increases in \( \text{NO}_3^- \) reductase activity occur within several days exposure to moderate dietary \( \text{NO}_3^- \) concentrations (Kemp et al., 1977; Allison and Reddy, 1984; Alaboudi and Jones, 1985; Farra and Satter, 1971). The adaptive increases in \( \text{NO}_3^- \) reductase activity are usually accompanied by increased \( \text{NO}_2^- \) reductase activity as well. By gradually increasing the amount of \( \text{NO}_3^- \), a very large amount can eventually be tolerated in the diet. However, under conditions where animals are adjusted to lower but significant amounts of \( \text{NO}_3^- \) in the diet, a very sudden increase in dietary \( \text{NO}_3^- \) may be associated with a more rapid reduction of \( \text{NO}_2^- \) than otherwise, without prior exposure (Kemp et al., 1977). If this rapid reduction of \( \text{NO}_3^- \) markedly exceeds \( \text{NO}_2^- \) reduction rates, there may be greater risk of intoxication. This may partially account for the reported increased hazard (Clay et al., 1976) associated with certain hay baling systems (in addition to the tendency to concentrate areas of high \( \text{NO}_3^- \) in the hay field). In the present experiments, the minimal exposure to \( \text{NO}_3^- \) in the feed may have limited the ability of the animals to respond to high \( \text{NO}_3^- \) challenge. Nevertheless, \( \text{NO}_3^- \) intoxication frequently occurs in animals that have not had prior exposure to nitrates.

An additional factor to consider is the time differential between concentrate feeding and \( \text{NO}_3^- \) challenge. In the present experiments this relationship was maintained at 1.5 h. However, a marked increase in this time interval may result in a reduction in the supply of readily fermentable substrate at the time of \( \text{NO}_3^- \) exposure, with a possible reduction in the protective effect of supplemental concentrates.

**Literature Cited**


Sigma Chemical Co. 1979. Kit No. 525-2, Hemoglobin by the method of Drabkin and Austin at 540 nm. St. Louis, MO.