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Effects of repeated-low level sodium chlorate administration on ruminal and fecal coliforms in sheep

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The objective of this study was to evaluate the efficacy of oral sodium chlorate administration on reducing total coliform populations in ewes. A 30% sodium chlorate product or a sodium chloride placebo was administered to twelve lactating Dorper X Blackbelly or Pelibuey crossbred ewes averaging 65 kg body weight. The ewes were adapted to diet and management. Ewes were randomly assigned (4/treatment) to one of three treatments which were administered twice daily by oral gavage for five consecutive days: a control (TC) consisting of 3 g sodium chloride/animal/d, a T3 treatment consisting of 1.8 g of sodium chlorate/animal/d, and a T9 treatment consisting of 5.4 g sodium chlorate/animal/d; the latter was intended to approximate a lowest known effective dose. Ruminal samples collected by stomach tube and freshly voided fecal samples were collected daily beginning 3 days before treatment initiation and for 6 days thereafter. Contents were cultured quantitatively to enumerate total coliforms. There were no significant differences in total coliform numbers (log10 cfu/g) in the feces between treatments (P = 0.832). There were differences (P < 0.02) in ruminal coliform counts (log10 cfu/mL) between treatments (4.1, 4.3 and 5.0 log10/mL contents in TC, T3 and T9 Treatments, respectively) which tended to increase from the beginning of treatment until the 5th day of treatment (P < 0.05). Overall, we did not obtain the expected results with oral administration of sodium chloride at the applied doses. By comparing the trends in coliform populations in the rumen contents in all treatments, there was an increase over the days. The opposite trend occurred in the feces, due mainly to differences among rumen contents and feces in ewes administered the T9 treatment (P = 0.06). These results suggest that the low chlorate doses used here were suboptimal for the control of coliforms in the gastrointestinal tract of ewes.

Keywords: Feed additive, Escherichia coli, Salmonella, pre-harvest control, sheep.

Introduction

Escherichia coli and Salmonella spp. are common microorganisms in the gastrointestinal microflora of all animals, including humans. Of these, E. coli O157:H7 produces hemorrhagic colitis, which is a potentially lethal disease in humans.1-4 In the USA, they first detected a case of poisoning from contaminated burgers in 1982. Approximately 73,000 people in the USA get sick from this strain annually.5 Several outbreaks of human disease have been linked to the consumption of meat and milk. Ruminants are a natural reservoir for this pathogenic bacterium and meat can become contaminated during the slaughter process by fecal contact6,7 Elder et al.8 reported that approximately 28% of cattle in the United States are infected with E. coli O157:H7. Escherichia coli and Salmonella spp. are also recognized as important causes of neonatal diarrhea in animals and are typically transmitted by the fecal-oral route.9-11 Because these pathogens are transmissible, morbidity is often greater when sheep are reared in close proximity to one another. For the sheep industry, this risk of horizontal transmission is very important because about half of the sheep in the United States are usually born in pens. In the last decade, several intervention strategies have been used to control these public health risks.12-15 In processing plants for instance, a variety of post-harvest interventions have been implemented to reduce bacterial contamination of carcasses. However, producers and processors are also interested in the

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development of complementary on-farm strategies to reduce the carriage and horizontal spread of zoonotic pathogens within their flocks so as to reduce the overall concentration of pathogens entering the processing plant. In this regard, feeding sodium chlorate may be an attractive antimicrobial against *E. coli* and *Salmonella* because unlike most gastrointestinal anaerobes, *E. coli* and *Salmonella* are capable of reducing nitrate to nitrite intracellularly by the reductase enzyme. Membrane bound respiratory nitrate reductase enzymes also reduce chlorate to form the final cytotoxic product chlorite and it was thus hypothesized and subsequently proven that chlorate can effectively kill *E. coli* and *Salmonella* in bovine ruminal fluid in vitro. Since then, a number of in vivo studies have demonstrated that chlorate is an effective intervention strategy to control coliforms in cattle and other domestic ruminants. Despite these earlier published works, however, the method of administering chlorate has not been sufficiently studied in sheep, as the lowest effective dose has not yet been established. Accordingly, the objective of this study was to evaluate the efficiency of repeated low sodium chlorate doses administered orally as a regulator of populations of total coliforms in ewes.

**Materials and methods**

**Location of research**

This research trial was carried on in the facilities of the College of Animal Science and Ecology of the Autonomous University of Chihuahua, in Chihuahua City, Mexico. These facilities are located along the Periférico Francisco R. Almada km 1, being its geographic coordinates 28° 38′ N and 106° 04′ W.

**Experimental animals and treatments**

Twelve crossed Pelibuey and Blackbelly-Dorper lactating grazing ewes with an average body weight of 65 ± 4 kg were adapted to a balanced diet and allowed *ad libitum* access to water. Composition of the diet is shown in Table 1. The animals (*n* = 12) were housed individually in indoors pens and randomly assigned to one of the following three treatments (4 ewes/treatment). Treatments were a control (TC) to which each ewe was administered 3 g sodium chloride as a placebo, equivalent to 90 mg per kg BW on average; a T3 treatment consisting of 1.8 g of sodium chlorate per ewe, equivalent to 28 mg per kg BW of sodium chlorate on average; and a T9 treatment of 5.4 g sodium chlorate to each ewe, equivalent to 84 mg per kg BW of sodium chlorate on average. The differential dose of NaCl in relation to chlorate treatments in terms of concentration was to administer near equivalent molar concentration of sodium and chloride ions (approximately 51 mmole each) into the rumen for the TC and T9 treatments, respectively. In each case, half of the corresponding sodium chlorate or sodium chloride dose was placed in a funnel attached to an esophageal tube every 12 h, using water to wash down the chemical. These substances were administered during 5 consecutive days. Effects of these treatments on ruminal and fecal microbial diversity have been reported elsewhere.

**Ruminal and fecal sampling**

Rumen contents obtained by stomach tube and freshly voided feces were collected every 24 h (0800) for 9 days, starting 3 days prior to administration of placebo and sodium chlorate such that sampling occurred over the following three stages: pre-treatment, treatment and post-treatment. Samples were kept in a refrigerator until the end of the study and then shipped on ice to the Southern Plains Agricultural Research Center, USDA in College Station, TX for bacterial enumeration via viable cell count on *E. coli*/Coliform Petrifilm (3M Inc., Minneapolis, MN, USA). Total coliforms were counted after 24 h incubation at 39°C as per manufacturer’s instructions.

**Statistical analysis**

Statistical analysis for bacterial counts was carried out with SAS Version 9.0 package 107 (SAS Inst. Inc., Cary, NC, USA). Data were expressed as colony forming units per mL or per g (cfu/mL or cfu/g) in ruminal or fecal contents, respectively. For analysis, bacterial counts were transformed to base 10 logarithms and fitted to a mixed model that included the fixed effects of treatment (TC, T3 and T9 treatments) and day, and the interaction of treatment x day. The effects of ewe within treatment were set as random and this was the experimental unit for the treatment factor. LSMEANS multiple comparisons were made without further adjustments.

### Table 1. Composition of diet.

<table>
<thead>
<tr>
<th>Feedstuff Percentage</th>
<th>Percentage&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn grain</td>
<td>30.86</td>
</tr>
<tr>
<td>Cotton seed meal</td>
<td>1.29</td>
</tr>
<tr>
<td>Dicalcium carbonate</td>
<td>0.46</td>
</tr>
<tr>
<td>Dry distillery grains</td>
<td>13.39</td>
</tr>
<tr>
<td>Sugar cane molasses</td>
<td>1.00</td>
</tr>
<tr>
<td>Salt</td>
<td>0.46</td>
</tr>
<tr>
<td>Soy bean meal</td>
<td>1.80</td>
</tr>
<tr>
<td>Vitamin and mineral premix</td>
<td>0.73</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>50.00</td>
</tr>
</tbody>
</table>

<sup>1</sup> As fed basis.
Results and discussion

Bacterial counts in ruminal contents

The microbial count data (cfu) in rumen contents are presented in Table 2 and Figure 1. A significant difference ($P < 0.001$) was observed among treatments, but the mean of the T9 treatment registered the highest numbers (log$_{10}$ cfu/mL) of total coliforms when compared to the TC and T3 treatments (4.96, 4.08 and 4.25 log$_{10}$ cfu/mL, respectively). Overall, there were no marked decreases in cfu attributable to sodium chlorate. When assessing variation trends of cfu (Table 2), a linear trend ($P = 0.0082$) attributable to effect of chlorate but not due to the effect of day was observed. These results conflict with those reported earlier by Anderson et al.[3] who reported that administration of 2500 ppm chlorate in drinking water, equivalent to a daily consumption of approximately 124 mg sodium chlorate/d, achieved significant reductions in ruminal E. coli concentrations of about 0.7 log$_{10}$ cfu/mL in bovine rumen contents. These discrepancies could be attributed to the low doses of chlorate used in the present study. For instance, in the present case, there is an effect of treatment (chlorate), but not in the expected direction, i.e., a higher dose showed a 1.28 log$_{10}$ cfu/mL increase in total coliforms. There is no explanation for this result. The generalized mean coliform counts from ruminal contents across all treatments were 1.30, 1.55 and 2.80 log$_{10}$ cfu/mL for the 3 days of sampling during the pre-treatment period, were 3.46, 6.17, 5.88, 5.57 and 5.58 log$_{10}$ cfu/mL for the 5 days of sampling during the treatment period and were log$_{10}$ cfu/mL for the last day sampled post-treatment. As seen in Fig. 1, there was considerable variability in coliform counts measured throughout the study but an increase in total coliforms was obvious in all treatments after the initial dose, reaching maximum values 3–4 days after initiation of treatment. Since we could find no record of similar work involving the administration of sequential doses of chlorate to small ruminants and its effects on total coliforms meaningful comparisons cannot be made. Besides the description of the behavior of microbial populations coliform through the days of pre-treatment, treatment and post-treatment, it was not possible to give a causal explanation of this behavior.

Fecal bacterial counts

Data for bacterial counts (log$_{10}$ cfu/g) in the feces are presented in Table 2 and Fig. 2. There were no significant decreases fecal coliforms due to the effect of sodium chloride treatment ($P = 0.83$). These results disagree with those reported by Taylor et al.[24] who observed 3 log$_{10}$ reductions in generic E. coli populations in sheep orally treated once with 150 mg sodium chlorate per kg of BW although no further reductions were observed in sheep similarly treated with 300 and 450 mg sodium chlorate per kg BW. The choice of the doses used in this study, which are low in relation to those used by Taylor et al.[24] was due to the assumption that the continuous administration of sodium chlorate for 5 days would have a residual effect upon the reduction of coliform populations. Based on our results, it appears this was not the case. Perhaps one explanation for this is that sheep have a high capacity for absorption in the

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Table 2. Least square means$^1$ ± SE of total coliforms (log$_{10}$ cfu/mL) in ruminal contents and feces.

<table>
<thead>
<tr>
<th>Treatment$^2$</th>
<th>Rumen (R) (Mean ± SE)</th>
<th>Fecal (F) (Mean ± SE)</th>
<th>Difference (R – F) (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (TC)</td>
<td>4.07 ± 0.211$^a$</td>
<td>2.90 ± 0.345</td>
<td>2.99 ± 0.284$^a$</td>
</tr>
<tr>
<td>T3</td>
<td>4.26 ± 0.211$^a$</td>
<td>2.82 ± 0.345</td>
<td>2.99 ± 0.284$^a$</td>
</tr>
<tr>
<td>T9</td>
<td>4.96 ± 0.204$^b$</td>
<td>2.61 ± 0.345</td>
<td>3.81 ± 0.277$^b$</td>
</tr>
<tr>
<td>Linear</td>
<td>$P = 0.0082$</td>
<td>NS$^3$</td>
<td></td>
</tr>
<tr>
<td>Quadratic</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

$^1$Average of 3-9 day.

$^2$Treatments were control (TC), 90 mg sodium chloride/kg body weight per day; T3 = 28 mg sodium chlorate/kg body weight per day; and T9 = 84 mg sodium chlorate/kg body weight per day.

$^3$NS = Non-significant.

$^a,b$Within a column, means without a common superscript differ ($P = 0.06$).

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Fig. 1. Ruminal content least square means (log$_{10}$ cfu/mL) over time. Treatments were control (TC), 90 mg sodium chloride/kg of body weight per day; T3 = 28 mg sodium chlorate/kg of body weight per day; and T9 = 84 mg sodium chlorate/kg of body weight per day.
colostum, due to its spiral shape. This causes increased water absorption in sheep, so one would expect a greater absorption of sodium chlorate (R.C. Anderson, USDA/ARS, College Station, TX, personal communication). Data from a kinetics study for the disposition of sodium chlorate in sheep seems to corroborate this situation.[27] As in the rumen contents, there were differences ($P < 0.01$) between the means of the day (Fig. 2). The cfu generalized means of all treatments was $3.61, 5.00, 3.45, 2.05, 2.14, 1.28$ and $1.88 \log_{10} \text{cfu/mL}$ for the $3$ days of pre-treatment, $1-5$ days (treatment) and $1$ day (post-treatment), respectively. As shown in Figure 2, there is considerable variation in coliform counts with the highest value for each treatment achieved after the initial dose and a steady decline thereafter. As in the case of ruminal contents, we could not find records of similar work (sequential doses of chlorate and its effects on cfu), so no meaningful comparisons can be made. Again, outside the description of the behavior of microbial populations coliform through the days of pre-treatment, treatment and post-treatment, it was not possible to give a causal explanation of this behavior.

**Differences in cfu between ruminal and fecal contents**

When comparing the changes in cfu populations through the days, a general trend for an increase in these populations in the rumen contents with all treatments, while the opposite occurred in the feces (Figs. 1 and 2). Table 2 presents the average differences in cfu between rumen contents and feces. These differences were greatest for the T9 treatment relative to TC and T3 treatments ($P = 0.06$) which may suggest an effect of chlorate upon the diminution of coliform populations in the posterior digestive tract relative to populations observed in rumen. However, we found no earlier references to works that considered the differences between rumen contents and feces. In the light of the present findings, we conclude that no reductions in coliform populations derived from the use of the actual doses of sodium chlorate administered through oral gavage were obtained. It is highly recommended to continue this line of research, using higher concentrations of sodium chlorate.

**References**


