Continuous, low-dose oral exposure to sodium chlorate reduces fecal generic Escherichia coli in sheep feces without inducing clinical chlorate toxicosis1,2

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ABSTRACT: Our objectives were to determine an effective, yet safe, daily dose of sodium chlorate for reducing fecal shedding of generic Escherichia coli in mature ewes. In a completely randomized experimental design, 25 Targhee ewes (age ~18 mo; BW = 62.5 ± 7.3 kg, mean ± SD) were assigned randomly to 1 of 5 sodium chlorate treatments, which were administered in the drinking water for 5 consecutive days. Treatments were control group (no sodium chlorate) and 4 targeted levels of daily sodium chlorate intake: 30, 60, 90, and 120 mg·kg−1·BW·d−1 for 5 d. Individual ewe ad libitum intake of water (with treatments) was measured daily, and BW was measured at the beginning of and 15 and 51 d after the 5-d treatment period. Serum chlorate, whole blood methemoglobin and packed-cell volume (PCV), and fecal generic E. coli and general Enterobacteriaceae coliforms were measured from corresponding samples collected at the end of the 5-d treatment period. Average daily intakes of sodium chlorate from drinking water treatments were 95%, 91%, 90%, and 83% of the target treatment intakes of 30, 60, 90, and 120 mg·kg−1·BW·d−1, respectively. Daily sodium chlorate intake remained constant for all treatment groups except for ewes offered 120 mg NaClO₃·kg−1·BW·d−1, which decreased (quadratic; P = 0.04) over the course of the 5-d treatment period. This decrease in sodium chlorate intake indicated that the 120-mg NaClO₃ level may have induced either toxicity and/or an aversion to the drinking water treatment. Serum chlorate concentrations increased (quadratic; P < 0.001) with increasing sodium chlorate intake. At the end of the 5-d treatment period, mean (least squares ± SEM) serum chlorate concentrations for ewes offered 30, 60, 90, and 120 mg NaClO₃·kg−1·BW·d−1 were 15.6 ± 14.1, 32.8 ± 15.8, 52.9 ± 14.1, and 90.3 ± 14.1 μg/mL, respectively. Whole blood methemoglobin and PCV were similar (P = 0.31 to 0.81) among the control group and ewes offered sodium chlorate. Likewise, BW was not affected by sodium chlorate (P > 0.27). Ewes consuming approximately 55 mg NaClO₃·kg−1·BW·d−1 or more (i.e., ewes offered 60, 90, and 120 mg) had a >1.4 log unit reduction in fecal E. coli and Enterobacteriaceae coliforms compared with control ewes. We suggest that for a short-term, 5-d dosing strategy, 55 to 81 mg NaClO₃·kg−1·BW·d−1 is an effective, yet safe, daily oral dose range for mature ewes to achieve a 97% to 99% reduction in fecal shedding of generic E. coli.

Key words: antibiotic resistance, chlorate, diarrhea, Escherichia coli, shedding, toxicity
**INTRODUCTION**

We are interested in sodium chlorate as a prophylactic tool to minimize problematic diarrhea induced by Enterobacteriaceae (EB; American Sheep Industry Association, 2002) in shed-lambing systems. The effects of oral sodium chlorate dosing to reduce fecal pathogenic and generic EB shedding from livestock and poultry immediately before slaughter have been extensively investigated (Anderson et al., 2005; Smith et al., 2012). In studies with sheep, dose durations of sodium chlorate did not exceed 24 h. Unfortunately, the bactericidal effects of sodium chlorate seem to be short-lived and may not persist beyond 24 h (Anderson et al., 2001a; Smith et al., 2013). Because lambs are more at risk of contracting EB-related diarrhea during the first 8 d of life (American Sheep Industry Association, 2002), prophylactic strategies must persist well beyond 24 h in newborn lambs.

We propose a prophylactic short-term strategy in which sodium chlorate is added to the drinking water of periparturient ewes for 5 d to reduce fecal shedding of pathogenic *Escherichia coli* in the lambing environment. Before the validity of this proposal can be evaluated, the safety of short-term dosing of ewes with sodium chlorate must be determined. Chlorate can be toxic under certain circumstances. For example, subclinical toxicity occurred when sheep consumed only 210 mg NaClO$_3$/kg BW as a single oral dose is safe and near the minimal amount necessary to achieve >90% reduction in fecal *E. coli* (Taylor et al., 2012; Smith et al., 2013). However, providing 150 mg NaClO$_3$/kg BW as a daily dose for 5 d may be toxic, but dividing the dose over a 5-d period may result in an insufficient daily dose (i.e., 30 mg NaClO$_3$/kg BW·d$^{-1}$) to effectively reduce fecal *E. coli*. Therefore, the objective of this investigation was to determine a minimal daily dose of sodium chlorate to be included in the drinking water of mature ewes for 5 d that is safe yet effectively reduces fecal shedding of generic *E. coli*.

**MATERIALS AND METHODS**

**Ewes and Husbandry**

The Institutional Animal Care and Use Committee (Range Sheep Production Efficiency Research Unit, Dubois, ID) reviewed and approved all experimental and husbandry procedures described herein. Twenty-five, Targhee ewes (age ~18 mo; BW = 62.5 ± 7.3 kg, mean ± SD) were used. Ewes were moved from sagebrush-steppe pasture to an indoor facility (continuous lighting), randomly assigned to individual pens (1 × 1.8 m), and acclimated to the environment for 7 d. Following acclimation, ewes were treated for 5 d according to the experimental design described below. Throughout the acclimation and experimental period, a 1:1 sugar beet pulp pellet and alfalfa hay pelleted diet was fed (2.1 kg, as-fed basis) daily; daily feed intake was monitored and scored as visual appraisals of “100% consumed”, “25% refused,” 50% refused,” or “>50% refused”; the same individual conducted daily appraisals. A metered amount of fresh water was provided for ad libitum intake each day in the morning and evening, and the amount consumed for each animal was recorded daily after feed refusals were scored. After the experimental period, ewes were moved to a common outdoor pen for 15 d and fed 1.9 kg (as fed) of a total mixed ration (55.5% alfalfa hay, 25.5% whole corn, 11.8% barley straw, and 7.3% sugar beet condensed separator byproduct; as-fed basis) each day. After 15 d, ewes were returned to sagebrush-steppe pasture.

**Experimental Design and Treatments**

Using a completely randomized experimental design, ewes were assigned randomly to 1 of 5 treatments, which were administered in the drinking water for 5 consecutive days (i.e., 120 h). Treatments were control group (no sodium chlorate) and 4 targeted levels of sodium chlorate intakes of 30, 60, 90, and 120 mg·kg$^{-1}$ BW·d$^{-1}$ that were offered over a 5-d period, which were equivalent to 5-d cumulative (sum total) doses of 150, 300, 450, and 600 mg/kg BW, respectively. Each day at 0800 and 1700 h, a stock solution of 20 g NaClO$_3$/kg H$_2$O was prepared and diluted into 3.60 ± 0.03 kg of drinking water for each ewe according to BW and treatment assignment. In the same fashion, sodium chloride (NaCl) was added to the drinking water of control ewes and ewes offered 30, 60, and 90 mg NaClO$_3$/kg BW·d$^{-1}$ to equalize molar concentrations of sodium and chloride across all treatment drinking waters. Daily subsamples (50 mL) of drinking water preparations were collected and pooled (stored at −20°C) for each ewe over the 5-d treatment period. The amount of drinking water offered was based on ad libitum daily intakes (6.890 ± 1.414 kg, mean ± SD) that were determined during acclimation. At 0700 h daily, refused drinking water treatment was weighed.

Throughout the text, treatment exposure is expressed in days. However, daily treatments and sampling measurements were conducted respective to a specific hour within each treatment day. Each treatment day began at 0800 h and concluded at 0759 h the following day. Therefore, treatments were provided during the first (i.e., 0800 h) and ninth (i.e., 1700 h) hour of each day. Likewise, treatment refusals were collected...
during the 23rd hour (i.e., 0700 h; see above description) of each day, or in other words, at the end of the day.

**Sample Collection, Processing, and Analyses**

Body weights (not fasted) were collected at 0700 h on the day before treatments began and again 15 and 51 d after treatments ended. At the end of the 5-d treatment period (i.e., 23rd hour of d 5), 2 venous jugular blood samples were collected (4.0 mL mixed with 95 USP units lithium heparin; 6 mL mixed with a clotting activator; BD Vacutainer, Becton, Dickinson and Co., Franklin Lakes, NJ). Heparinized samples were stored (~30 min) on ice until methemoglobin analysis and determination of packed-cell volume (PCV). Fecal samples (>5 g) were hand collected from the rectum at the end of the 5-d treatment period and 15 d after treatments ended. For each ewe, sterile gloves were donned, and feces were collected and placed in sterile polypropylene tubes. Contact outside the rectum and surrounding area was avoided while transferring the feces from the rectum to the tube. Tubes with contents were immediately sealed and placed on ice (~3 h) until bacterial enumeration.

Fecal *E. coli* cfu (at the end of and 15 d after the treatment period) and fecal EB cfu (only at the end of the treatment period) were quantified using 3M Petrifilm plates (3M Microbiology, 2008; 3M, St. Paul, MN). Briefly, ~0.5 g of wet fecal material was transferred in duplicate to sterile 50-mL polypropylene tubes. Forty milliliters of sterile PBS and 1 large glass bead (1.5 cm diam.) were added to each tube; tubes were sealed with a screw cap and shaken for 30 min on a horizontal shaker at 150 oscillations/min. In duplicate, contents were serially diluted (10-fold increments) in PBS. Dilutions (1 mL) were placed on duplicate plates to enumerate total cfu for EB (Petrifilm Enterobacteriaceae Count Plates; 3M) and *E. coli* (Petrifilm *E. coli*/Coliform Count Plates; 3M). Plates were incubated aerobically at 35°C for 24 h, and bacteria were counted per the manufacturer’s (3M) directions. If CV for tube or plate duplicates exceeded 10%, the enumeration procedure was repeated using the original samples, which were stored at ~1°C.

Whole-blood methemoglobin (%) was measured using the methods of Evelyn and Malloy (1938) and Hegesh et al. (1970) with the modifications of Smith and Taylor (2012). Samples were analyzed in triplicate; analysis was repeated if sample CV exceeded 10%. Assay was validated using fresh whole blood (5 mL; heparinized) that was collected from a nonexperimental ewe and treated with 10 mg of sodium nitrite for a 10-min period. Blood was rinsed with saline (5 mL) and centrifuged at 13,000 × *g* for 10 min at 4°C, the pellet was suspended and lysed in ice-cold water (q.s. to a 5-mL volume) and centrifuged at 13,000 × *g* for 10 min, and the supernatant was added back to fresh whole blood (heparinized) to achieve 2.5%, 5%, 10%, and 20% methemoglobin. Resulting data from the methemoglobin analysis were plotted against expected data, which yielded a slope of 0.94 and *R*² = 0.99.

Sodium chlorate levels in drinking water were measured by ion chromatography with conductivity detection. Pooled water samples were thawed and duplicate 250-, 200-, 100-, 75-, and 50-μL aliquots of the 0, 30, 60, 90, and 120 mg·kg⁻¹ BW·d⁻¹ treatments, respectively, were diluted with ultrapure water to 50 mL in volumetric flasks. Volumetric flasks were sealed, and contents were mixed by inversion 10 times. Chlorate analyses were performed using a Thermo-Fisher Scientific (Waltham, MA) ICS-2100 ion chromatograph. Standards consisting of 5, 25, 100, 500, 1,000, and 5,000 μg/L of sodium chlorate were prepared in nanopure water (*R*² > 0.999). Chlorate was not detectable in control samples (limit of detection [LOD] = 1 ng/mL) and was separated from interferences on a 4 × 250 mm Dionex (Thermo-Fisher Scientific) AC19HC column protected by a 4 × 50 mm AS19HC guard column with an isocratic mobile phase of 20-mM KOH. The mobile phase was prepared using a Thermo-Fisher eluent generator. Ions were detected using a DS6 conductivity detector (Thermo-Fisher Scientific) with external water suppression (50 mA; ASRS 300, Thermo-Fischer Scientific). Sodium chlorate standards were run at the beginning and end of each sample set. Blank samples were also concurrently run with each analysis. Concentrations of chlorate in unknowns were determined using least squares regression of peak areas of known standards. The limit of quantitation was 5 μg/L; the LOD of 1 μg/L was determined empirically.

Sodium chlorate in serum samples was determined by liquid chromatography–mass spectrometry as described by Smith and Taylor (2011). Briefly, serum aliquots from test (15 to 200 μL) or control (200 μL) sheep were combined with 0 to 185 μL of chlorate-free serum from unexposed sheep. To each sample, 10 ng of Cl₁₈O₃⁻ (internal standard, equivalent to 50 ng/mL serum) was added, followed by the addition of 200 μL of ice-cold acetonitrile. Samples were mixed and placed into a laboratory freezer (<-20°C) for a 1-h period. Tubes were centrifuged (15,000 × *g*) for 20 min at 5°C, pellets were resuspended, and each tube was recentrifuged. Aliquots containing 200 μL of each supernatant were removed and combined with 2 mL of ultrapure water previously added to 5-mL volumetric flasks. Flasks were diluted to the 5.0-mL mark with nanopure water, capped, and mixed by inversion. Sample aliquots (1 mL) were syringe filtered (17 mm, 0.45 µm) into autoinjector vials for liquid chromatography–mass spectrometry analyses. Serum samples requiring dilution beyond the dilutions described above were diluted with blank sheep serum to
Table 1. Cumulative and average daily intake arithmetic means (±SD) and serum chloride, blood methemoglobin, blood packed-cell volume, and BW least squares means (±SEM) of ewes offered various doses of sodium chlorate, mixed in the drinking water, over a 5-d period1,2

<table>
<thead>
<tr>
<th>Item</th>
<th>Sodium chlorate offered, 3 mg·kg⁻¹ BW·d⁻¹</th>
<th>P-value4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Cumulative sodium chlorate intake, 5 mg·kg⁻¹ BW·5 d⁻¹</td>
<td>141.9 (10.1)</td>
<td>273.7 (15.9)</td>
</tr>
<tr>
<td>Average daily sodium chlorate intake, mg·kg⁻¹ BW·d⁻¹</td>
<td>28.4 (1.5)</td>
<td>54.7 (2.5)</td>
</tr>
<tr>
<td>Serum chloride, 6 μg/mL</td>
<td>15.6 (5.6)</td>
<td>32.8 (2.3)</td>
</tr>
<tr>
<td>Whole blood methemoglobin, %</td>
<td>0.31 (0.04)</td>
<td>0.30 (0.03)</td>
</tr>
<tr>
<td>Whole blood packed-cell volume, %</td>
<td>42.5 (1.3)</td>
<td>44.3 (2.6)</td>
</tr>
<tr>
<td>BW, 7 kg</td>
<td>66.2 (3.4)</td>
<td>60.4 (3.5)</td>
</tr>
</tbody>
</table>

1,2Unless superscript letters within a row indicate a difference (P < 0.10).
1Blood methemoglobin was determined using procedures described in Smith and Taylor (2012).
2Each day at 0800 and 1700 h, a stock solution of 20 g NaClO₃/kg H₂O was prepared and diluted into 3.60 ± 0.03 (SD) kg of drinking water for each ewe according to BW and treatment assignment.
3n = 5 per treatment group.
4F test results for the model analysis.
5Analysis of daily sodium chlorate intake is presented in Fig. 1.
6See Fig. 3 for regression analysis results for serum chlorate vs. actual intake.
7BW were measured at the beginning of and 15 and 51 d after the 5-d treatment period. Neither treatment nor treatment × day effects were detected (P = 0.27 to 0.72).

a volume of 200 μL; this dilution was then added to control sheep serum as described above.

A Waters Acquity UPLC system (Milford, MA) online with a Waters triple-quadrupole mass selective detector was used to construct summed ion chromatograms of chlorate and internal standards. Data were acquired and processed and quantification was effected using MassLynx 4.1 with TargetLynx (Waters; Milford, MA). Sample aliquots (20 μL) were injected from an autosampler, maintained at 4°C on a Waters Ion-Pak Anion HR column (4.6 × 75 mm) maintained at 40°C, and eluted with an isotropic mobile phase of acetonitrile:20 mM ammonium bicarbonate (1:1) at a flow rate of 0.75 mL/min. Ions were detected in the negative ion mode, and calibration curves were prepared as described by Smith and Taylor (2011). For each sample set, blanks and recovery standards were run. The most sensitive sodium chlorate limit of quantitation for the serum assay was 37 ng/mL when 200 μL of serum were assayed.

Statistical Analyses

Statistical analyses were conducted using SAS software (version 9.2; SAS Inst. Inc., Cary, NC). For all analyses, ewe was considered the experimental unit. Mixed-model and regression analyses were performed as described below. Response variables were sodium chlorate and drinking water intakes, serum chloride, whole blood methemoglobin and PCV, BW, and E. coli and EB cfu. For all analyses, α was set at 0.1. When F tests for mixed-model analyses were significant, mean separations were performed between treatments using planned pairwise t tests. Arithmetic means (±SD) are presented for 5-d cumulative and corresponding average daily intakes of sodium chlorate; least squares means (±SEM) are presented for all other response variables.

Cumulative and average daily sodium chlorate intakes over the 5-d period were not analyzed but are presented in Table 1 as arithmetic (±SD) means. Daily sodium chlorate intake, water intake, and BW were modeled as repeated measures, with treatment group, measurement day, and the corresponding interaction as fixed effects and ewe assignment within treatment group as the random effect; for the repeated (i.e., day) term, autoregressive order 1 for unequal variances was specified for the covariance structure. Serum chloride, blood methemoglobin, and PCV were analyzed as end point measures, with treatment group as the fixed effect and ewe assignment within treatment group as the random effect. In addition, serum chloride was regressed against the actual d 5 sodium chloride intake. Previously, we estimated the elimination of half-life sodium chlorate following a single low dose (14 and 42 mg/kg BW) to be approximately 2.6 h (Smith and Taylor, 2012). Given that the majority of a single dose would be eliminated within a 24-h period, a regression model plotting the concentration of chlorate in serum at the end of d 5 as a function of the most recent dose (i.e., d 5 actual) was constructed. Enterobacteriaceae (only at the end of treatment) and E. coli (at end of and 15 d after treatment; separate analyses) enumeration data (i.e., cfu) were log transformed and analyzed as an end point measure, with treatment group as the fixed effect and ewe assignment within treatment group as the random effect. In addition, individual ewe EB and E. coli data on d 5 were regressed against the corresponding average daily sodium chlorate...
intake. From the mixed-model and regression analyses, an apparent outlier was identified, which was EB and E. coli data from ewe 21. Data from d 5 were reanalyzed with the outlier removed. Results from analyses with and without the outlier are presented.

**RESULTS**

**Sodium Chlorate Intake and Serum Chlorate**

Actual 5-d cumulative and average daily intakes (±SD) of sodium chloride are presented in Table 1. Mean (least squares) daily sodium chloride intake is presented in Fig. 1. Daily intake remained constant for all treatment groups except for ewes offered 120 mg NaClO₃·kg⁻¹ BW·d⁻¹, whose intake fluctuated daily and decreased (quadratic; \( P = 0.04 \)) over the course of the 5-d treatment period (treatment × day interaction; \( P = 0.004 \)). On d 5, sodium chloride intake was not different between ewes offered 90 and 120 mg NaClO₃·kg⁻¹ BW·d⁻¹ (\( P = 0.97 \)).

Daily sodium chloride intake was a function of ad libitum water intake, which is presented in Fig. 2. Water intake remained relatively constant for all treatment groups except for ewes offered 120 mg NaClO₃·kg⁻¹ BW·d⁻¹, whose intake also fluctuated daily (treatment × day interaction, \( P = 0.01 \)) and decreased (\( P = 0.03 \)) over the course of the 5-d treatment period. On d 2 and 5, ewes offered 0 (control), 30, 60, and 90 mg NaClO₃·kg⁻¹ BW·d⁻¹, consumed more water than ewes offered 120 mg NaClO₃·kg⁻¹ BW·d⁻¹ (\( P = 0.003 \) to 0.08). On d 4, control ewes consumed more water than ewes offered 120 mg NaClO₃·kg⁻¹ BW·d⁻¹ (\( P = 0.08 \)).

Serum chloride was detected in all ewes except for 1 ewe offered 60 mg·kg⁻¹ BW·d⁻¹ and the control (not offered sodium chloride) ewes. Serum chloride concentrations are presented in Table 1. Ewes offered 120 mg NaClO₃·kg⁻¹ BW·d⁻¹ had greater serum chloride than ewes offered lower doses (\( P < 0.07 \)). Ewes offered 90 mg NaClO₃·kg⁻¹ BW·d⁻¹ had greater serum chloride than ewes offered the lowest dose (\( P = 0.08 \)). When regressed against the d 5 sodium chloride intake (Fig. 3), serum chloride concentrations increased (quadratic; \( P < 0.001 \)) with increasing sodium chloride intake.

**Methemoglobin Formation, Packed-Cell Volume, and Body Weight**

Methemoglobin concentrations, PCV, and BW are presented in Table 1. Methemoglobin concentrations and PCV were similar among control ewes and ewes
Sodium chlorate toxicity in sheep

When individual ewe fecal E. coli cfu were regressed against corresponding actual sodium chlorate intakes, fecal E. coli decreased (quadratic; \( P < 0.0001 \)) as the intake of sodium chlorate increased (indicated by the solid curve in Fig. 4). When analyzed (mixed model) within treatment group classification, ewes consuming at least 54.7 ± 2.5 mg NaClO₃·kg⁻¹·d⁻¹ or more (i.e., ewes offered 60, 90, and 120 mg/d) for 5 d had a reduction of 1.4 to 2 log units in fecal EB cfu compared with control ewes (\( P < 0.1 \)). As with the E. coli, a possible outlier (ewe 21) was observed in the group offered 120 mg·kg⁻¹·d⁻¹. Data were reanalyzed with the outlier removed. Removal of the outlier resulted in a quadratic response (\( P < 0.0001 \); dashed curve in Fig. 5) with a larger amount of variation accounted for in the model: \( R^2 = 0.47 \) vs. 0.28 for data without vs. with the outlier, respectively. Likewise, the resulting least squares mean (open square in Fig. 5) from the mixed-model analysis was 3 log units less than the control mean and less (\( P < 0.07 \)) than other ewe groups offered sodium chlorate in the drinking water.

Fecal EB cfu results are presented in Fig. 5. When individual ewe fecal EB cfu were regressed against corresponding actual sodium chlorate intakes, fecal EB decreased (quadratic; \( P < 0.0001 \)) as the intake of sodium chlorate increased (indicated by the solid curve in Fig. 5). When analyzed (mixed model) within treatment group classification, ewes consuming at least 54.7 ± 2.5 mg NaClO₃·kg⁻¹·d⁻¹ or more (i.e., ewes offered 60, 90, and 120 mg/d) for 5 d had a reduction of 1.4 to 2 log units in fecal EB cfu compared with control ewes (\( P < 0.1 \)). As with the E. coli, a possible outlier (ewe 21) was observed in the group offered 120 mg·kg⁻¹·d⁻¹. Data were reanalyzed with the outlier removed. Removal of the outlier resulted in a quadratic response (\( P < 0.0001 \); dashed curve in Fig. 5) with a larger amount of variation accounted for in the model: \( R^2 = 0.47 \) vs. 0.28 for data without vs. with the outlier, respectively. Likewise, the resulting least squares mean (open square in Fig. 5) from the mixed-model analysis was 3 log units less than the control mean and
Voluntarily Consumption of Drinking Water Treated with Sodium Chlorate

Actual sodium chlorate intake was less than targeted, ranging from 82% to 94% of the targeted 5-d cumulative (sum total) dose. Failure to achieve target-

DISCUSSION

Voluntarily Consumption of Drinking Water Treated with Sodium Chlorate

Less (P = 0.03) than that of control and ewes offered 30 mg·kg$^{-1}$·BW$^{-1}$·d$^{-1}$ but not different (P > 0.15) from that of ewes offered 60 and 90 mg·kg$^{-1}$·BW$^{-1}$·d$^{-1}$.

Posttreatment fecal E. coli cfu results from 15 d after the 5-d treatment period ended are presented in Fig. 6. In contrast to the results for control ewes, the reduction in fecal E. coli cfu still persisted (P = 0.08 and 0.03, respectively) in ewes offered 60 and 120 mg NaClO$_3$·kg$^{-1}$·BW$^{-1}$·d$^{-1}$ and tended (P = 0.13) to persist in ewes offered 90 mg NaClO$_3$·kg$^{-1}$·BW$^{-1}$·d$^{-1}$. More noticeable was the variation about the means; the variation in ewes exposed to any level of sodium chlorate was greater than that observed for control ewes.
Sodium chlorate concentrations in the daily drinking water offered to ewes that were assigned to the 120-mg treatment level ranged from 0.7 to 1.1 g/L, which was similar to and, in most cases, much less than that tested in comparable experiments. For example, turkeys (15 wk old) offered 1 to 4 g NaClO₃/L in drinking water for 38 h and turkeys offered water without sodium chlorate consumed similar amounts of water (Moore et al., 2006). Weaned (~26 d old) and finished pigs offered 0.6 and 0.9 g NaClO₃/L, respectively, in the drinking water for up to 36 h and respective pigs offered water without sodium chlorate consumed similar amounts of water (Callaway et al., 2003). One difference in the current experiment is that the duration of exposure is 3- to 5-fold longer. Nevertheless, on the basis of this evidence, we suggest that inclusion of sodium chlorate in the drinking water for ewes in the current study did not induce any aversion. Rather, the negative effect on ad libitum consumption of drinking water seems to indicate some degree of sodium chlorate toxicity.

For example, in the treatment group offered 120 mg NaClO₃·kg⁻¹·BW·d⁻¹, 4 of the 5 ewes refused 25% to 100% of the daily feed ration from d 3 through 5; this response was not observed in any other treatment group. Because DMI directly influences water intake, decreased feed consumption could be the cause of decreased water intake. Lack of desire to consume feed could indicate some minor toxicity from subacute sodium chlorate exposure. Heywood et al. (1972) reported appetite loss in dogs exposed (oral) to 200 to 300 mg NaClO₃·kg⁻¹·BW·d⁻¹ for 5 consecutive days; dogs exposed to >310 mg NaClO₃·kg⁻¹·BW·d⁻¹ for 5 consecutive days suffered from anorexia and vomiting, followed by death. Regardless of the effect on DMI and water intake in the current study, BW (not fasted) of ewes at 15 and 51 d after the treatment period were similar among all treatment groups. To the extent that BW is an indicator of general health, maintenance of BW indicated that the effect of sodium chlorate on DMI was short-lived and was alleviated once ewes stopped consuming sodium chlorate.

**Serum Chlorate and Indicators of Sodium Chlorate Toxicity in Whole Blood**

Serum chlorate was well within ranges that we have previously reported from single oral doses of sodium chlorate in sheep (Smith and Taylor, 2012; Smith et al., 2013). Variation in serum chlorate within treatment groups partly reflected the variation in voluntary intake of sodium chlorate each day, which was most pronounced in ewes offered 120 mg NaClO₃·kg⁻¹·BW·d⁻¹ for 5 d. Such variation is best demonstrated in Fig. 3, where individual ewe serum chlorate (determined at the end of d 5) was regressed against the corresponding d 5 sodium chlorate intake. Two ewes in the 120-mg group failed to consume the targeted amount, which resulted in these ewes only consuming amounts similar to ewes offered 90 mg NaClO₃·kg⁻¹·BW·d⁻¹ for 5 d. Variation in serum chlorate within treatment groups was also influenced by the time of day that ewes consumed drinking water. Because of the rapid clearance of chlorate (Smith and Taylor, 2012), ewes that consume the majority of the drinking water within the first 12 h will have lower serum chlorate at 24 h compared with ewes that consume the majority of the drinking water within the last 12 h. Therefore, serum chlorate data from the current study may vary in comparison with data from our oral, single-dose experiments in which we collected blood at specific intervals respective to a definite dosing time point.

Whole blood methemoglobin and PCV of ewes offered sodium chlorate did not differ from control ewes at the end of the 5-d treatment period. We have reported similar findings in sheep provided single sodium chlorate doses ranging from 14 to 150 mg/kg BW (Smith and Taylor, 2012; Smith et al., 2013). Likewise, Anderson et al. (2001b) reported no changes in blood methemoglobin or hematocrit in weaned pigs dosed with 45 to 90 mg/kg BW, in 3 separate doses, within a 24-h period. Acute chlorate salt toxicity in sheep most commonly results in early development of methemoglobinemia followed by hemolysis, organ lesions, and death (Steyn, 1933; McCulloch and Murer, 1939; Holzer and Stöhr, 1950), which is common in other species as well (Smith et al., 2012). Given that most ewes that were offered 120 mg NaClO₃·kg⁻¹·BW·d⁻¹ began refusing feed and water by d 3 of the experiment, we assumed that some degree of methemoglobin accumulation would be detected in blood samples collected on d 5. These data convincingly demonstrated that neither the dose level nor the 5-d exposure duration that was tested in the current experiment altered blood methemoglobin levels or PCV. On the basis of the literature (Smith and Taylor, 2012; Smith et al., 2012), these endpoints are negatively impacted during acute episodes of chlorate toxicity, which ultimately result in a number of apparent clinical symptoms in affected animals.

**Low-Dose Sodium Chlorate Consumption and Generic Fecal E. coli and Enterobacteriaceae**

In sheep, efficacious single doses of sodium chlorate for reducing pathogenic and generic fecal E. coli have been established for neonatal lambs (Taylor et
Taylor and Smith

Concluding Remarks

The purpose of our recent interest in sodium chlorate is to find a potential prophylactic tool to reduce pathogenic Enterobacteriaceae in shed-lambing systems (high-animal density environments), where neonatal diarrhea (i.e., scours) is problematic. As far as we are aware, these data are the first to described short-term, continuous dosing of sodium chlorate in a ruminant livestock production system. Sodium chlorate can be toxic under certain exposure conditions. However, daily doses ranging from 55 to 81 mg/kg BW for a 5-d period did not elevate clinical end points commonly associated with acute chlorate toxicity, and this dose range was effective at reducing generic Enterobacteriaceae organisms shed in feces of mature ewes.

LITERATURE CITED


enterica 

T yphimurium concentrations in the weaned pig gut. J. Food Prot. 64:255–258.


Enterichia coli O157:H7


enterica 

serovar Typhimurium colonization in 

O157:H7


Escherichia coli O157:H7

populations in sheep can be reduced by chlorate supplementation. J. Food Prot. 66:194–199.


E. coli O157:H7


al., 2012), preslaughter lambs (Callaway et al., 2003; Edrington et al., 2003), mature wethers (Smith et al., 2013), and mature periparturient and lactating ewes (Taylor et al., 2012). However, in all these studies, dose durations did not exceed 24 h. A unique feature of the current study is that sodium chlorate doses were tested over a 5-d exposure period.

On the basis of the results, the minimal effective dose of sodium chlorate to achieve a >97% reduction in generic 

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and general Enterobacteriaceae coliforms in feces was approximately 55 mg·kg⁻¹ BW·d⁻¹ for 5 d. When considering intakes up to approximately 81 mg NaClO₃·kg⁻¹ BW·d⁻¹ for 5 d, a 99% reduction in coliforms was observed. Although 150 mg/kg BW was previously found to be the effective minimal single dose (i.e., one-time bolus) at reducing fecal 

coli

in mature ewes (Taylor et al., 2012), the bactericidal effects were lost in the current study when the dose was distributed over a 5-d period at 30 mg·kg⁻¹ BW·d⁻¹.

Sodium chlorate intakes >81 mg·kg⁻¹ BW·d⁻¹ for 5 d were much more effective at reducing fecal coliforms, but risks of toxicity seemed more probable. For example, ewes that consumed approximately 99 mg·kg⁻¹ BW·d⁻¹ refused feed and water around d 3 of the 5-d treatment period. Furthermore, the >3 log reduction observed at greater doses may not be necessary. As stated earlier, our overall goal is to minimize possible shedding of pathogenic Enterobacteriaceae in shed-lambing systems when ewe and lamb density in the lambing sheds is high. Ewes and lambs are in this environment for 2 to 6 d before returning to low-density pens or pastures. Therefore, only a moderate suppression of fecal coliforms may be needed during this time, which was accomplished with the minimal effective dose of approximately 55 mg·kg⁻¹ BW·d⁻¹ for 5 d.

The sustained effect of sodium chlorate treatments on suppression of fecal 
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beyond the treatment period and out to 15 d later was surprising. Fecal concentrations of 
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in ewes offered 60, 90, and 120 mg·kg⁻¹ BW·d⁻¹ remained at least 1 log less than control ewes, with greater variation about the mean concentrations relative to control ewes. Patchanee et al. (2007) also reported a sustained suppression effect of chlorate on fecal shedding of 

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in pigs for as long as 14 d following a 5-d chlorate treatment exposure. At this time, we do not know whether sustained suppression of 
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is a negative, positive, or neutral effect. As discussed, BW was similar among all treatments 51 d after the treatment period ended, which indicated no sustained negative effects occurred in ewes following daily consumption of sodium chlorate for 5 d.


