Determination of a sodium chlorate dose that results in safe concentrations of tissue residues in beef cattle

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Background

Contamination of beef carcasses with pathogens such as E. coli, and Listeria during slaughter and processing have led to the annual recall of over 1.8 million pounds of beef during the last decade; this average excludes a recall of 22 million pounds of beef in 2002. Food-animal products containing undetected pathogens continue to contribute to an un-quantified number of foodborne illnesses. In beef cattle, it has been established that hides are a major source of carcass contamination and that hide washing intervention steps effectively reduce subsequent pathogen loads on carcasses. Although post-harvest sanitation techniques are becoming increasingly efficient, they are in use because no practical methods exist for eliminating pathogens from live animals prior to slaughter. Recently, a new pre-harvest technology that greatly reduces, or eliminates, the numbers of Gram-negative pathogens inhabiting gastrointestinal tracts of cattle has been developed. The technology is based on the feeding of a sodium chlorate containing product (ECP) 24 to 72 h prior to an animal’s slaughter. During the chlorate exposure period, bacterial species containing intracellular respiratory nitrate reductase are thought to metabolize chlorate (ClO3 -) to the bacterial toxin chlorite (ClO2 -). Chlorate toxicity is specific to nitrate-reductase containing bacteria that have the ability to intracellularly convert chlorate to chlorite, but which lack chlorite dismutase enzymes capable of rapidly detoxifying chlorite to chloride. Use of chlorate does not adversely affect the commensal microflora of gastrointestinal tracts. Unlike many antibiotics, development of chlorate resistance seems to occur only in pure bacterial cultures and not in mixed bacterial cultures.

Our laboratory has previously determined that sodium [36Cl] chlorate administered to ruminating cattle is transformed only to the chloride (Cl-) ion, although residues of parent chlorate ion were present in edible tissues. Because chloride is a nutrient essential for life in mammalian and avian species, the presence of chloride residues in animal tissues does not represent a food safety risk. Chlorite (ClO2 -) ions, which could be of food safety concern, were absent from edible tissues and urine of chlorate-treated cattle. Chlorate residues present in skeletal muscle and kidney were greater than estimated safe tissue concentrations of chlorate. But because cattle were exposed to chlorate for three consecutive days, with a dose representing 150% of the target dose, and because the cattle were slaughtered with an 8-hour withdrawal period, high chlorate residues in skeletal muscle and kidney were not believed to represent insurmountable obstacles for further development of sodium chlorate as a feed additive. The purpose of this study was to determine the effect of sodium chlorate dose on chlorate residues in edible tissues of cattle slaughtered with a 24-hour withdrawal period, and to determine if the magnitude of chlorate residues in skeletal muscle and kidney would be prohibitive for further development of chlorate as a possible pre-harvest pathogen intervention strategy.

The stated objectives for this work were:

To identify a dose of sodium chlorate that results in acceptable chlorate residues in edible tissues of cattle.

Methodology

Radiochemical purity of stock sodium [36Cl] chlorate (94.2%) was assessed using thin layer chromatography (TLC) and anion chromatography. The radiochemical impurity consisted of [36Cl]chloride, which was the starting material for the [36Cl]chlorate synthesis. Test [36Cl] chlorate
was not purified further because chloride is a natural product common to known life forms. Under the TLC and HPLC chromatographic conditions employed at the initiation of the study a 0.5% radiochemical impurity was not detected. However, the presence of approximately 0.5% perchlorate in the stock material was subsequently verified. The specific activity of formulated sodium chlorate was 202 dpm/μg.

Three-quarter blood Loala (Angus/Loala x Loala) heifers (n=3; 103.5 ± 4.9 kg) and steers (n=3; 159.7 ± 23.0 kg) were purchased from Auction Effertz, Ltd. (Bismarck, ND) and transported to Fargo, ND. Animals were housed in concrete floored pens (3.5 x 3.1 m; room A112) covered with wood shavings to absorb urine and feces. All cattle were given ad libitum access to an alfalfa and grass hay mixture and water for the pre-study and study periods and were provided 454 g of cracked corn daily from 14 days after delivery to the USDA facilities until the end of the study. The cattle were adapted to the USDA barn facilities for 18 days prior to ruminal cannulation. Animals were trained to stand in head gates and then metabolism crates (1.0 x 2.1 x 2.7 m; W x H x L) during the 4 to 6-wk period after surgery. Cattle were ruminally cannulated (Appendix 2) using a 1-step cannulation procedure (Dr. Joel Caton, Animal and Range Sciences, North Dakota State University, Fargo). At the end of the surgery each animal was turned back to its pen and given access to food and water. Surgeries were uneventful.

One to 1.5 hours before dosing, permanent jugular catheters (0.050 ID x 0.090 OD x 54 inch length) were placed using an 11-gauge needle. Catheters were secured with a single stitch 2 to 3 cm proximal to the catheter exit point. When not in use, catheters were filled with saline containing 100 U/mL of heparin. Steer no. 173 (high dose steer) was initially catheterized, but pulled its catheter before dosing. For safety reasons, no attempts were made to re-catheterize the jugular of Steer 173.

One steer and one heifer each were randomly selected to receive nominal daily doses of 21, 42, or 63 mg/kg body weight of sodium [36Cl] chlorate. Doses were selected to bracket the preferred use of 42 mg/kg. To prevent the radiochemical contamination of the study facilities, and to ensure quantitative dosing, sodium [36Cl] chlorate was administered through ruminal cannuulas. Total daily doses for each animal were divided into four equal aliquots that were administered at 0, 8, 16, and 24 hours. Sodium nitrate is hypothesized to render pathogenic bacteria more susceptible to sodium chlorate by inducing the expression of nitrate reductase. Therefore, a total dose of 14 mg/kg body weight sodium nitrate was intraruminally delivered to each animal in aliquots of 3.5 mg/kg body weight at 0, 8, 16, and 24 hours.

During the dosing and withdrawal periods, urine and feces were collected in 12-hour intervals and blood was collected through jugular catheters at regular intervals. Cattle were slaughtered 24 hours after the last administration of sodium chlorate and edible tissues were collected for the analysis of total residues and chlorate residues.

Urine and feces were collected in 12-hour intervals until slaughter at 48 hours, with time zero being defined as the time of the initial sodium [36Cl] chlorate administration. Modified
incontinent bags were fitted to steers to ensure that urine collection was quantitative and that the urine remained free from fecal contamination. Urine was collected into stainless steel basins, of known weights, nested in plastic basins. Urine from heifers was collected into stainless steel basins at various times by inducing micturition. Some urine excreted from heifers was collected into fecal trays; this urine was collected but kept separate from clean urine samples. At 12-h sampling intervals, all urine was weighed, clean urine was mixed thoroughly, and 150 to 400 g sub-samples were collected.

Animals were stunned using a captive bolt stun gun, elevated with a hoist, and exsanguinated. Cattle were eviscerated, and liver, kidney, skeletal muscle, and adipose tissue samples were removed, ground, and frozen. For skeletal muscle, aliquots of the ground sample were either frozen immediately after grinding or were stored for 2 weeks at 3.1 ± 0.3 °C and then frozen. After the collection of edible tissues, animals were skinned, boned, and boxed for disposal.

Figure 5 (page 8) illustrates the strategy used for the extraction and speciation of tissue radiochlorine. Tissues were analyzed in sets consisting of 5-g duplicates each of control, fortified control, and test-animal tissues. Control tissues were fortified with 25 μL of a standard solution containing 1 μg/μL each of Na36Cl and Na36ClO3 having specific activities of approximately 200 dpm/μg. Samples were extracted essentially as described by Smith et al. (2005) except that ice-cold acetonitrile was added to precipitate protein from the aqueous supernatant following the initial extraction and centrifugation. Acetonitrile was evaporated under N2 prior to C18 solid phase extraction; otherwise the procedure used was described previously. Ion chromatography is described below. For each chromatographic run, fractions were collected at 2 to 4 minute intervals and the presence of chloride or chlorate was determined by the presence of radiochlorine in trapped fractions. Smith et al. (2005) have described the chromatographic resolution of chlorite, chloride, and chlorate. Limits of quantitation of radioactive peaks were determined for each chromatographic run by multiplying the standard deviation of quadruplicate background determinations by three and adding the product to the mean background value.

**Urine.** Urine was analyzed in sample sets corresponding the collection period. Sample sets consisted of duplicates of control (blank) urine, fortified, and incurred samples. Urine was thawed, 1-mL aliquots were removed for analysis, fortified samples were spiked with 25 μL of a mixture of [36Cl]chloride and [36Cl]chlorate, and 1 mL of nanopure water was added to each tube. Tubes were vortex mixed and their contents loaded onto preconditioned (5 mL of methanol followed by 7.5 mL of water) C18 SPE tubes (Bakerbond, 500 mg sorbent, 3-mL; J. T. Baker; Phillipsburg, NJ). The non-retained aqueous eluent, was collected into pre-weighed glass liquid scintillation vials. Each tube was subsequently rinsed with 1 mL of water and this “rinse” fraction was collected into the same vial as the “load” fraction. Vials were weighed and a 100-μL aliquot was removed, weighed, and subjected to liquid scintillation counting after the addition of LSC fluid. The entire “load/rinse” fraction, collected from the C-18 SPE tubes, was loaded onto preconditioned (5 mL methanol followed by 5 mL of water) SCX tubes (LC-SCX, 3-mL; Supelco; Bellfonte, CA) and the non-retained eluent passing through each tube was collected into a weighed glass vial. Cation exchange tubes were rinsed with 1.5 mL of water and the “rinse” was collected into the same vial as the “load” fraction. A 0.25 mL aliquot was removed from each tube, weighed, and radiochlorine was quantified after addition of LSC fluid. About 1 mL of each of each sample was filtered through a 0.45 μM PTFE syringe filter (17 mm; Alltech; Deerfield, IL) in preparation for ion chromatographic analysis. Aliquots of each sample were injected onto the HPLC system described for the tissue analysis and radiochlorine eluted using the gradient described below. Fractions of HPLC eluent were collected into LSC vials during each chromatographic run and radiochlorine in each fraction was quantified by liquid scintillation counting. Limits of quantitation were calculated as described for chromatographic analyses of the tissue extracts.
The ion chromatographic system consisted of a Waters 600 controller and pump equipped with a Dionex conductivity detector and ion suppressor. Tissue and urine extracts were injected onto Dionex AG-11 HC and AS-11 HC guard and analytical columns, respectively, and eluted with a sodium hydroxide gradient consisting of 10 and 100 mM NaOH. Each run was initiated with 10 mM NaOH being held isocratically for 10 min followed by a linear gradient to 50% 100 mM NaOH at 30 min; 50% 100 mM NaOH was held for 2 minutes, and then the gradient was linearly returned to starting conditions by 40 min. Flow rates were 0.5 mL/min for tissue analyses and 1 mL/min for urine analyses.

Figure 5. Diagram of tissue extraction technique for preparation of samples prior to ion chromatography.
Findings
Results of this study clearly indicate that from a residue chemistry point of view, the development of sodium chlorate as a feed additive should continue. Chlorate residues from all animals and tissues fell below provisional safe tissue concentrations established by the US Food and Drug Administration Center for Veterinary Medicine. In addition, chlorite residues were absent from tissues and excreta of dosed animals. A significant quantity of chlorate was rapidly absorbed and excreted as chloride and parent chlorate in urine. Absorbed radioactivity represents dosed chlorate that did not reach the lower gastrointestinal tract. Some chlorate was excreted in the feces, but only a fraction of the total dose “survived” absorption and metabolism and made it to the lower gastrointestinal tract. For example, during the 24-hour withdrawal period only 2.5% of the dosed chlorate was excreted unchanged in the feces.

Several facets of sodium chlorate make its development as a pre-harvest food safety tool appealing. First, oral chlorate clearly works to reduce pathogen loads. Second, chlorate is inexpensive and is easily formulated into premixes. Third, chlorate salts are palatable and oral delivery to cattle is feasible. Fourth, the US FDA Center for Veterinary Medicine (FDA-CVM) has provided provisional safe tissue concentrations (pSTC) for chlorate and chlorite residues in edible tissues of food animals. The availability of pSTCs provides “benchmark” residue concentrations that are extremely beneficial during the development of a practical product. Fifth, the environmental impact of chlorate feeding will likely be negligible because chlorate-metabolizing bacteria are surprisingly ubiquitous in the environment. And sixth, parent chlorate excreted from dosed animals could potentially reduce burdens of pathogens present in animal facilities and decrease the rates of pathogen transmission. Collectively, these factors and the fact that chlorate is transformed to a naturally occurring nutrient, strongly support the further development of sodium chlorate as a feed additive for cattle.

Implications
For all of the doses tested, chlorate residues in liver, kidney, muscle, and fat fell well below amounts that the FDA have estimated to be safe. The major metabolite of chlorate was chloride, a nutrient already present in almost all human food sources. Further research on the chlorate-based product is warranted because it could have a significant impact on lowering the incidence of harmful bacteria on meat products.

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