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Effects of experimental chlorate preparations as feed and water supplements on *Escherichia coli* colonization and contamination of beef cattle and carcasses

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Abstract

The effects of feed or water administration of experimental chlorate preparations on recovery of generic *Escherichia coli* and *E. coli* O157:H7 from the gut, hide and carcasses of feedlot cattle were tested. Sixty-four naturally colonized cattle were randomly assigned to one of four feed treatments with or without a 12 h chlorate-containing water treatment. An analysis of variance revealed a main effect of feed treatment ($P = 0.002$) on generic *E. coli* concentrations in feces collected before shipment to slaughter. Main effect means were 5.57, 4.75, 5.00 and 4.08 $\log_{10}$ cfu/g for animals fed an experimental chlorate product at 0, 0.01% in last meal, 0.01% for last 5 d and 0.05% of body weight in last meal, respectively. A main effect of feed treatment was observed ($P = 0.041$) on generic *E. coli* concentrations in feces collected at slaughter (means for the respective treatments were 4.92, 3.57, 3.98 and 3.20 $\log_{10}$ cfu/g) as well as on numbers of generic *E. coli* recovered ($P = 0.034$) from hide swabs collected at the rump (means for the respective treatments were 4.86, 3.92, 3.87 and 4.06 $\log_{10}$ cfu/swab). A main effect of water treatment was observed ($P < 0.016$) on generic *E. coli* concentrations in rumen contents (3.44 vs. 2.72 cfu $\log_{10}$ cfu/g for animals administered 0 or 2500 ppm active chlorate ion, respectively). Logistic regression analysis revealed a main effect of feed treatment ($P < 0.001$) on the incidence of *E. coli* O157:H7 recovered from feces collected at slaughter (75%, 33%, 20% and 25% for animals fed an experimental chlorate product at 0%, 0.01% in last meal, 0.01% for last 5 d and 0.05% of body weight in last meal, respectively). Animals exhibited no symptoms of chlorate toxicity and negative effects on feed or water intake or animal performance were not observed.

Keywords: Beef cattle; *Escherichia coli*; Experimental chlorate product; Feed additive; Water additive

1. Introduction

Enterohemorrhagic *Escherichia coli* strains such as *E. coli* O157:H7 are important foodborne pathogens. In the United States, an estimated 73,000 human infections caused by *E. coli* O157:H7 occur each year (Mead et al., 1999). Cattle are an important reservoir of *E. coli*

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O157:H7 (Orskov et al., 1987; Rasmussen et al., 1993), with practically all herds containing at least some colonized animals and, depending on the season, with incidences within herds $\geqslant$30% (Elder et al., 2000). Despite implementation of numerous post harvest interventions by beef processors (Bacon et al., 2000; Cutter and Rivera-Betanourt, 2000), costly product recalls (FSIS, 2002, 2003a, b; Thomsen and McKenzie, 2001) and disease outbreaks (Vugia et al., 2003) resulting from consumption of food contaminated with this pathogen continue to occur. Not surprisingly, there is considerable interest in the development of pathogen reduction strategies that can be applied to the live animal before slaughter, particularly since some risk assessments predict that such interventions would significantly reduce human exposures to this pathogen (Hynes and Wachsmuth, 2000).

Recently, we reported that oral administration of low levels of an experimental chlorate product selectively reduced concentrations of *E. coli* and *Salmonella* in the gut of pigs, cattle and sheep (Anderson et al., 2001a, b, 2002; Callaway et al., 2001, 2002, 2003; Edrington et al., 2003). Mechanistically, the chlorate is catalytically reduced to cytotoxic chlorite by the bacterium's respiratory nitrate reductase enzyme (Anderson et al., 2000; Stewart, 1988). A particularly attractive feature of this intervention is that it not only reduces gut concentrations of *E. coli* and *Salmonella* in the gut but it is harmless to most commensal and mutualistic anaerobes (competitive exclusion bacteria) that lack respiratory nitrate reductase (Anderson et al., 2000, 2001b). Whereas results from these earlier studies proved the concept of this technology (Anderson et al., 2002; Callaway et al., 2002), the present study was conducted to test the practicality and efficacy of administering experimental chlorate preparations to beef cattle as feed and water supplements under conditions more likely to be encountered during the finishing of beef cattle.

2. Materials and methods

2.1. Animals and experimental design

Sixty-four head of feedlot heifers of Mexican origin and averaging 402 ± 5.1 (SD) kg were sorted at random and without regard to *E. coli* O157:H7 culture status to one of eight treatments in a two $\times$ four factorial design (two drinking water treatments and four feed treatments; two heifers per treatment) that was replicated four times thus bringing the final number of animals to eight per treatment. Previous studies have demonstrated that an $n \leqslant 8$ animals per treatment was more than sufficient to detect differences in naturally colonized and experimentally infected animals (Anderson et al., 2001b, 2002; Callaway et al., 2002, 2003; Edrington et al., 2003). Each replicated trial was conducted at 2 week intervals. The two water treatment factors were a control treatment containing no chlorate (0X) or a specially prepared 1X solution containing 2500 ppm active ion which were provided to the animals during the last 12 h prior to being shipped to slaughter. The factors for feed treatment were the provision of an experimental chlorate product (EKA Chemicals, Inc., Marietta, GA) at 0, 0.01% of body weight for the last day on feed, 0.01% of body weight for the last 5 days on feed or 0.05% of body weight for the last day on feed. The experimental chlorate product, containing less than 40% active ion and 4% nitrate ion by weight (the latter to induce expression of respiratory nitrate reductase), had been prepared to possess rumen bypass characteristics to increase delivery of the active ion to the lower gut (Edrington et al., 2003).

Beginning 1 week prior to administration of the different treatments, each animal was weighed and moved to a separate pen where they were adapted to their pen and finishing diet (Table 1). Meals (equal to 1.25% of body weight on an as fed basis) were fed twice daily, at 08:00 and 16:00, except on the day before slaughter, at which time feed was offered during the morning feeding only. Feed intake was measured during the 5 days immediately preslaughter by recovering and weighing feed refusals immediately before the next meals offering, or in the case of the last meal, after the animals were shipped to slaughter. Homogenous portions of feed offered and recovered were dried at 100 °C until achieving a constant weight to determine dry matter percentages. Each pen was fitted with a tank-type watering trough capable of holding approximately 115 l so that each animal could individually be provided ad libitum access to drinking water or treatments and volume consumed during the 12 h water treatment period was measured by use of a precalibrated measuring gauge.

Four hour after offering of the last meal, the cattle were weighed and then withheld from water until 16:00 h

Table 1
Ingedient composition of feedlot diet provided to cattle

Ingredient (As fed basis)	%
Steam rolled corn	79.50
Cottonseed meal	7.39
Vitamin premix[a]	0.05
Trace mineral premix[a]	0.05
Urea	0.98
Cottonseed hull	6.49
Soy oil	3.61
Limestone	1.43
Salt	0.50

[a]Vitamin Premix 6905; Trace Mineral Premix 6962 (Producers Cooperative Association, Bryan, Texas, USA).