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Bactericidal Effect of Sodium Chlorate on *Escherichia coli* Concentrations in Bovine Ruminal and Fecal Contents *In Vivo*

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Enterohemorrhagic *Escherichia coli* causes a potentially fatal disease in humans. Since human infections often occur following consumption of contaminated meat, strategies are sought to rid these pathogens from food-producing animals. *E. coli*, like most members of the family *Enterobacteriaceae*, possess respiratory nitrate reductase, an enzyme that coincidentally reduces chlorate to toxic chlorite. Consequently, a study was performed to assess the effect of intraruminal chlorate administration on *E. coli* in the gut of fed and fasted cattle, the latter having been reported to harbor increased concentrations of enteric pathogens. As hypothesized, *E. coli* concentrations were lower ($p < 0.05$) 10 and 24 h post chlorate administration, respectively, in rumen contents and feces of chlorate-treated cows than in untreated cows. Fasting had little effect on gut *E. coli* concentrations and did not effect the bactericidal effect of chlorate against *E. coli*. Chlorate treatment had little or no effect on fermentation efficiency, as evidenced by pH, volatile fatty acid production and concentration of total culturable anaerobes, and had no observable adverse effects on any of the cows. These results suggest that chlorate may be useful in the pre-harvest control of *E. coli*. *Key words*: *Escherichia coli*, chlorate, food safety, ruminant.

INTRODUCTION

Enterohemorrhagic *Escherichia coli* strains such as *E. coli* O157:H7 are of public health and economic significance, causing an estimated 73 000 human infections in US each year (1). The association of disease outbreaks with the consumption of undercooked ground beef, along with other epidemiological evidence, has strongly implicated cattle as important animal reservoirs of *E. coli* O157:H7 (2, 3). Recently, Chilean pigs have also been identified as important carriers of certain enterohemorrhagic *E. coli* serotypes (4). Whereas numerous technologies have been developed to effectively reduce contamination of red meat products by enteropathogens after slaughter (post-harvest) (5, 6), none of these are infallible, as evidenced by continued outbreaks of food-borne disease (1). Consequently, there exists considerable interest in the development of pathogen reduction strategies that can be applied immediately prior to slaughter.

As members of the family *Enterobacteriaceae*, *E. coli* possess respiratory nitrate reductase activity (7) that coincidentally reduces chlorate intracellularly to the toxic chlorite ion (8, 9). Thus, chlorate is lethal to bacteria possessing respiratory nitrate reductases (i.e. *E. coli* and *Salmonella*) but not to bacteria lacking respiratory nitrate reductase activity (i.e. many commensal and mutualist bacteria). Since chlorate is only mildly toxic to most animals, with a lethal dose of 1 g or more chlorate per kg body weight, and since precedence exists for the use of chlorate salts in veterinary and human medicine (10), perhaps chlorate could be safely fed to ruminants to kill enterohemorrhagic *E. coli* in the gut just before slaughter. The objective of the present study was to test the hypothesis that chlorate may selectively kill *E. coli*, but not beneficial bacteria, within the rumen and hindgut of cattle. Since fasting of cattle, a condition that often occurs during transit to the abattoir, has been reported to increase *E. coli* populations throughout the bovine gastrointestinal tract (11, 12), our study included provisions to test the effects of chlorate in both fed and fasted cattle.

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Table I

Factorial arrangements of treatments within Latin square experimental design

Experimental schedule[a]	Cow number			
	0037	3020	2039	2040
Period 1	Untreated fed	Treated fasted	Untreated fasted	Treated fed
Period 2	Treated fasted	Untreated fed	Treated fed	Untreated fasted
Period 3	Untreated fasted	Treated fed	Untreated fed	Treated fasted
Period 4	Treated fed	Untreated fasted	Treated fasted	Untreated fed

[a] Cows were acclimated for at least one week to a 9:1 alfalfa hay:cracked corn diet prior to the start of each period.

MATERIALS AND METHODS

Animals and experimental design

Four ruminally cannulated Holstein–Friesian cows averaging 937 kg body weight were allocated to a 4×4 Latin square design, with a factorial arrangement of treatments (chlorate treatment vs no treatment) and feeding regimes (fed vs fasted) (Table I), to test for effects on gut concentrations of *E. coli*, total culturable anaerobes and on fermentation parameters. The Latin square design controlled for both period and animal effects. Beginning at least 1 week prior to each test period, all cows were acclimated to a free choice alfalfa hay:cracked corn (9:1) diet supplemented with a commercially available mineral mix. During each 48-h test period, two cows continued to receive this diet, whereas two other cows were fasted. Upon completion of the test period and until beginning of the next acclimation period, all cows were allowed to graze a predominantly rye grass pasture. Midway through (24 h after starting) each test period, two cows (one fasted and one fed) were administered a single sodium chlorate treatment (0.2 g per kg body weight) intraruminally. At no time was chlorate administered to an individual animal during two consecutive periods. The factorial arrangement of treatments within the Latin square (Table I) was used to test for differences due to the main effects of treatment (chlorate-treated vs untreated) and feeding regime (fed vs fasted) and for possible interactions between these main effects. Data were analyzed using the General Linear Model procedures of SAS (13). *E. coli* concentrations obtained using MacConkey and M-Endo media were compared using the correlation procedure in SAS to assess the strength of the relationship between the two *E. coli* measures.

Bacteriology

Ruminal contents and fecal specimens (1–2 g) collected immediately before and at intervals post-chlorate treatment were cultured quantitatively for *E. coli* via selective differentiation (24-h incubation) of serial 10-fold dilutions on MacConkey agar, as well as on M-Endo agar (Difco, Laboratories Inc, Detroit, MI, USA). Ruminal and fecal concentrations of total culturable anaerobes were estimated via a three-tube most probable number method (14) using anaerobically prepared Reinforced Clostridial medium (Difco) supplemented with 40% (v/v) clarified rumen fluid (15), 0.0001% resazurin (w/v) and with cellobiose and xylose (0.025% w/v each) as a general growth medium. Tubes were scored positive for growth based on visual inspection for turbidity following 1 week of incubation. All incubations were performed at 37°C.

Analytical

Ruminal and fecal fluids, obtained via straining of contents through a nylon mesh paint strainer (Reaves and Co, Durham, NC), were measured for pH and were analyzed for volatile fatty acid concentrations using gas chromatography (16).

RESULTS

Significant treatment (chlorate-treated vs untreated) $\times$ feeding regime (fed vs fasted) interactions were not observed ($p > 0.05$) at any time point for any of the variables tested (VFA concentration, pH or concentrations of total culturable anaerobes or wild-type *E. coli*) (data not shown). However, main effects observed due to treatment and to feeding regime were observed and these are presented below. For ease of presentation, treatment effects are presented relative to time post chlorate administration and effects of feeding regime are presented relative to initiation of the experimental feeding/fasting period. Note, however, that 0, 10 and 24 h post-chlorate administration correspond to 24, 34 or 48 h, respectively, post-initiation of the experimental feeding/fasting period. Comparisons of the two media used for enumerating *E. coli* revealed that MacConkey and M-Endo yielded almost identical recoveries of *E. coli* in fecal contents, with correlation values exceeding 0.90. Ruminal *E. coli* concentrations determined using the two media were considerably more variable and thus concentrations obtained using both media are presented, however, results obtained using either medium support the same conclusions.

Table II

Test for main effects of chlorate (ClO_3) treatment vs no treatment on concentrations of wild-type E. coli and total culturable anaerobes in ruminal and fecal contents[a]

Time[b]	Wildtype *Escherichia coli* concentration (log_{10} CFU/g)				Total culturable anaerobes (log_{10} cells per g)	
	Via MacConkey agar		Via M-Endo agar			
	Untreated	ClO_3-treated	Untreated	ClO_3-treated	Untreated	ClO_3-treated
Ruminal contents						
0 h	4.10 (0.43)	4.07 (0.38)	2.81 (0.15)	3.60 (0.47)	12.58 (0.27)	12.43 (0.20)
10 h	4.76 (0.37)	3.76* (0.73)	4.57 (0.29)	2.93* (0.67)	11.91 (0.22)	12.42* (0.24)
24 h	4.20 (0.33)	3.57 (0.55)	4.43 (0.22)	3.63 (0.49)	12.32 (0.17)	12.88 (0.24)
Fecal contents						
0 h	5.51 (0.33)	5.30 (0.45)	5.39 (0.43)	5.41 (0.46)	11.70 (0.26)	11.61 (0.23)
24 h	5.42 (0.38)	2.87* (0.61)	5.42 (0.40)	2.83* (0.54)	11.78 (0.33)	11.98 (0.38)

[a] Values are reported as the mean and standard error (SE) from $n = 8$. Fed cows were fed an 9:1 alfalfa hay:cracked corn diet. For fasted cows, fasting commenced 24 h prior to and continued until 24 h after chlorate administration; untreated cows were fasted concurrently.
[b] Time relative to intraruminal administration of chlorate to treated cows, untreated cows were sampled concurrently.
* ClO_3(chlorate)-treated mean differs from untreated mean ($p < 0.05$).

Main effects of treatment regime (chlorate-treatment vs no treatment)

Ruminal concentrations of *E. coli* were lower ($p < 0.05$) in chlorate-treated animals 10 h but not 24 h after treatment when compared with concentrations in untreated cattle (Table II). For treated cattle, ruminal *E. coli* concentrations measured 10 h post treatment were reduced by 0.3–0.7 log_{10} U from 0 time concentrations (depending on which medium was used). In contrast, ruminal *E. coli* concentrations were increased 0.7–1.8 log_{10} U over 0 time concentrations during the same interval in untreated animals (Table II). Fecal *E. coli* concentrations were 2.6 log units lower ($p < 0.05$) in chlorate-treated animals 24 h after treatment than in untreated cattle (Table II), a difference of more than 99% in actual numbers of *E. coli*. When expressed as a change from 0 time, fecal *E. coli* concentrations measured 24 h post chlorate administration were reduced by 2.4–2.6 log_{10} U in chlorate-treated cattle but were reduced by less than 0.1 log_{10} U in untreated cattle (Table II). Fecal concentrations of total culturable anaerobes did not differ between chlorate-treated or untreated cattle (Table II). Chlorate treatment had no significant effect ($p > 0.05$) on ruminal or fecal volatile fatty acid concentrations or pH (Table III).

Main effects of feeding regime (fasting vs feeding)

Fed cattle had numerically higher ruminal acetate, propionate and butyrate concentrations than fasted cattle at all sampling times (Table IV). However, significance ($p < 0.05$) was detected only for comparisons to contents col-

Table III

Test for main effects of chlorate (ClO_3) treatment vs no treatment on volatile fatty acid concentrations and pH of ruminal and fecal contents[a]

Time[b]	Acetate (μmol/g)		Propionate (μmol/g)		Butyrate (μmol/g)		pH	
	Untreated	ClO_3-treated	Untreated	ClO_3-treated	Untreated	ClO_3-treated	Untreated	ClO_3-treated
Ruminal contents								
0 h	47.3 (7.1)	46.9 (9.5)	10.6 (2.3)	11.6 (3.5)	5.4 (1.6)	6.4 (2.2)	6.73 (0.2)	6.64 (0.2)
10 h	45.0 (14.4)	28.2 (4.8)	10.4 (3.9)	5.4 (1.1)	4.8 (1.8)	3.1 (0.6)	6.80 (0.2)	6.84 (0.3)
24 h	36.8 (10.3)	28.0 (5.4)	8.6 (3.0)	8.8 (3.2)	5.5 (2.5)	4.2 (1.3)	6.88 (0.3)	6.87 (0.3)
Fecal contents								
0 h	26.2 (2.3)	28.6 (3.6)	5.2 (0.8)	5.7 (1.0)	1.5 (0.3)	1.7 (0.4)	6.66 (0.1)	6.70 (0.1)
24 h	25.2 (2.2)	22.4 (4.2)	5.4 (0.6)	6.9 (1.7)	1.4 (0.2)	1.5 (0.5)	6.99 (0.1)	6.81 (0.1)

[a] Values are reported as the mean and standard error (SE) from $n = 8$. Fed cows were fed an 9:1 alfalfa hay:cracked corn diet. For fasted cows, fasting commenced 24 h prior to and continued until 24 h after chlorate administration; untreated cows were fasted concurrently.
[b] Time relative to intraruminal administration of chlorate to treated cows, untreated cows were sampled concurrently.

Table IV

Test for main effects of fasting vs feeding on volatile fatty acid concentrations and pH of ruminal and fecal contents[a]

Time[b]	Acetate (μmol/g)		Propionate (μmol/g)		Butyrate (μmol/g)		pH	
	Fed	Fasted	Fed	Fasted	Fed	Fasted	Fed	Fasted
Ruminal contents								
24 h	57.4 (9.9)	36.7 (3.5)	14.4 (3.6)	7.8 (1.2)	8.1 (2.4)	3.8 (0.7)	6.34 (0.2)	7.04* (0.0)
34 h	50.8 (13.8)	22.5 (1.7)	11.4 (3.8)	4.4 (0.7)	5.9 (1.6)	2.0* (0.4)	6.24 (0.1)	7.40* (0.0)
48 h	41.5 (10.1)	23.3* (4.0)	13.1 (3.6)	4.4* (0.8)	6.9 (2.5)	2.8 (0.7)	6.24 (0.2)	7.51* (0.1)
Fecal contents								
24 h	23.9 (1.0)	30.9* (3.7)	4.5 (0.6)	6.3* (1.0)	1.3 (0.3)	1.9* (0.4)	6.74 (0.1)	6.63 (0.1)
48 h	24.2 (2.9)	23.4 (3.8)	6.3 (1.5)	6.0 (1.1)	1.5 (0.4)	1.4 (0.3)	6.83 (0.2)	6.96 (0.1)

[a] Values are reported as the mean and standard error (SE) from $n = 8$ for all except 0 time pH, for which $n = 6$.
[b] Time relative to initiation of fasting regime; fed cows were sampled concurrently and were fed an 9:1 alfalfa hay:cracked corn diet.
* Fasted mean differs from fed mean ($p < 0.05$).

Table V

Test for main effects of fasting vs feeding on concentrations of wildtype E. coli and total culturable anaerobes in ruminal and fecal contents[a]

Time[b]	Wildtype *E. coli* concentration ($\log_{10}$ CFU/g)				Total culturable anaerobes ($\log_{10}$ cells per g)	
	Via MacConkey agar		Via M-Endo agar			
	Fed	Fasted	Fed	Fasted	Fed	Fasted
Ruminal contents						
24 h	4.45 (0.25)	3.73 (0.49)	3.46 (0.32)	2.95 (0.42)	12.14 (0.19)	12.87* (0.20)
34 h	4.15 (0.67)	4.37 (0.53)	3.85 (0.57)	3.65 (0.63)	12.27 (0.24)	12.07 (0.25)
48 h	3.79 (0.52)	3.98 (0.40)	4.28 (0.43)	3.78 (0.35)	12.92 (0.22)	12.28* (0.17)
Fecal contents						
24 h	5.16 (0.33)	5.65 (0.44)	5.09 (0.39)	5.71 (0.46)	11.65 (0.20)	11.66 (0.28)
48 h	3.81 (0.74)	4.48 (0.63)	3.77 (0.69)	4.48 (0.64)	11.87 (0.37)	11.89 (0.35)

[a] Values are reported as the mean and standard error (SE) from $n = 8$.
[b] Time relative to initiation of fasting regime; fed cows were sampled concurrently and were fed an 9:1 alfalfa hay:cracked corn diet.
* Fasted mean differs from fed mean ($p < 0.05$).

lected from cattle that had been fasted for 34 h (for butyrate) or 48 h (for acetate and propionate) (Table IV). Fed cattle had lower ($p < 0.05$) fecal acetate, propionate and butyrate concentrations than cattle fasted 24 but not 48 h (Table IV). Fasted cattle had higher ($p < 0.05$) ruminal pH values than fed cattle at all sampling times (Table IV). Fecal pH was unaffected by fasting (Table IV) as were ruminal and fecal concentrations of *E. coli* (Table V). Cattle fasted for 24 h had higher ($p < 0.05$) ruminal concentrations of total culturable anaerobes than fed cattle but cattle fasted for 48 h had lower ($p < 0.05$) ruminal concentrations of total culturable anaerobes than fed cattle (Table V). Fecal concentrations of total culturable anaerobes were unaffected by fasting (Table V).

DISCUSSION

Results presented herein are the first to demonstrate that chlorate treatment reduced ruminal *E. coli* concentrations thus supporting the hypothesis that chlorate supplementation may be a practical way to reduce concentrations of pathogens such as *E. coli* O157:H7 in cattle before slaughter. While no attempts were made during the present study to evaluate the effect of chlorate against *E. coli* O157:H7 specifically, earlier work had shown that supplemental chlorate was bactericidal to *E. coli* O157:H7, as well as *Salmonella* serovar Typhimurium, *in vitro* and in experimentally infected swine (17–19). It was expected that chlorate concentrations would be rapidly diminished due to the highly reductive capacity of the anaerobic bacterial population and to ruminal passage. However, *E. coli* con-

centrations remained lower (although not significantly so) within the rumens of chlorate-treated cattle than in untreated cattle even at 24 h post-treatment. The finding that chlorate treatment reduced fecal concentrations of *E. coli*, but not concentrations of total culturable anaerobes, 24 h post-treatment further indicates that effective quantities of chlorate persisted long enough to reach the lower gut. This latter finding is particularly encouraging since a direct correlation between fecal *E. coli* O157:H7 concentrations and carcass contamination has been reported (3). Thus, it is reasonable to expect that optimal levels of chlorate supplementation at least 1 day before slaughter may reduce the risk of carcass contamination. Concentrations of total culturable anaerobes were not reduced at any time by chlorate treatment, which thus supports earlier evidence that the bactericidal activity of chlorate is selective against bacteria possessing respiratory nitrate reductase (17–19). The fact that chlorate treatment had no effect on amounts of volatile fatty acids produced supports the concept that chlorate did not inhibit the fermentative efficiency within the gut.

In contrast to earlier studies (11, 12), fasting of cattle in the present study had little effect on ruminal or fecal concentrations of *E. coli* despite having the expected effect on pH and volatile fatty acid concentrations. Lower volatile fatty acid concentrations and a near neutral pH associated with fasting are generally considered more favorable for growth of *E. coli* (20–22), which may place animals in transit to the abattoir at an increased risk for harboring high *E. coli* concentrations (11, 12, 22). Results obtained in this present study do show, however, that concentrations of total culturable anaerobes were decreased due to fasting thus suggesting a limitation in the availability of nutrients for microbial growth. Under such conditions, it is possible that the *E. coli* population was no more capable of competing for limiting nutrients than other indigenous anaerobes. In support of this latter contention, Harmon et al. found no increase in ruminal or fecal *E. coli* concentrations in similarly fasted cattle (23). The primary reason for including a fasting regime in the present study was to test for its possible effect on chlorate treatment. The fact that no treatment (chlorate-treated vs untreated) × feeding regime (fed vs fasted) interactions were observed suggests that chlorate supplementation would be effective if administered immediately before transit to or upon arrival at the slaughter plant, provided of course that sufficient time is allowed for the chlorate to pass to the lower gut. Work with rats has shown that 3 or 48 mM sodium chlorate supplied in drinking water did not adversely effect water consumption (24) but more work needs to be done to see if chlorate could be provided in feed. Supplementation of chlorate salts in feed or water 1 or 2 days before slaughter would certainly be practical and easily amendable to various production practices likely to be encountered in the food animal industry. For such a strategy to be practical; however, concerns regarding the effect of chlorate on meat quality and safety, as well as environmental considerations need to be addressed. Existing evidence indicates that the ultimate fate of ingested chlorate in animals and biological systems is the reduced chloride ion (25, 26), which suggests that concerns of residual chlorate in tissues or the environment may be unwarranted, particularly if chlorate could be substituted for dietary sodium chloride.

CONCLUSIONS

The results presented here show that intraruminal chlorate administration reduced *E. coli* concentrations *in vivo* and suggest that chlorate supplementation may be a practical and effective pre-harvest pathogen control strategy. Further research is needed to determine optimal feeding protocols that can deliver and maintain the most effective concentrations of chlorate to both the rumen and hindgut. For instance, while significant reductions in *E. coli* concentrations were obtained here with the single administration of chlorate, it is reasonable to expect that even more effective reductions could be achieved with longer duration or more numerous administration protocols. Further research is clearly needed; however, to ensure that chlorate feeding strategies are compatible with the production of high quality and safe meat products.

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