

ANTIBACTERIAL EFFECT OF SODIUM CAPRYLATE ON *ESCHERICHIA COLI* O157:H7 IN CATTLE DRINKING WATER

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Introduction

E.coli O157:H7 is a major food-borne pathogen in the United States. Cattle serve as the principal reservoir of *E.coli* O157:H7, excreting the pathogen in feces, thereby contaminating food, water, and the environment (Chapman *et al* 1993, Laegreid *et al.*, 1999, Shere *et al.*, 1998; Zhao *et al.*, 1998). Environmental persistence of *E.coli* O157:H7 is critical in its epidemiology on farms (LeJeune *et al.*, 2001). Several researchers have isolated *E.coli* O157:H7 from cattle water troughs, indicating that water troughs on farms could serve as a potential long-term reservoir of the pathogen. Persistence of *E.coli* O157:H7 in cattle water troughs can potentially act as a source of re-infection of cattle, birds, flies, and rodents, which, in turn can act as vectors of the pathogen (McGee *et al.*, 2002). Thus, there is a need for an effective and practical method for killing *E.coli* O157:H7 in cattle water troughs. Inactivation of *E.coli* O157:H7 in water at farm will potentially shut down one source of infection to cattle, thereby leading to a reduced carriage of *E.coli* O157:H7 in cattle. This in turn will translate into improved farm and animal hygiene, and a reduced contamination of beef products with *E.coli* O157:H7. Finally, a safe supply of beef products is critical for the economic viability of the beef industry.

Caprylic acid is a natural, eight-carbon fatty acid present in breast milk, bovine milk (Jensen *et al.*, 2002), and coconut oil (Jensen *et al.*, 1990, Sprong *et al.*, 2001). Caprylic acid is a food-grade chemical approved by the FDA as GRAS (CFR 184.1025). Previous research conducted in our laboratory indicated that caprylic acid was highly effective in killing *E.coli* O157:H7 in bovine rumen fluid (Annamalai *et al.*, 2004).

Objectives

To determine the antibacterial effect of sodium caprylate to kill *E.coli* O157:H7 in cattle drinking water.

Materials and Methods

Bacterial strains and media

Four strains of green fluorescent protein (GFP)-labeled *E.coli* O157:H7 were used in the study. The four strains of GFP-labeled *E.coli* O157:H7 were individually

cultured in 10 ml of Tryptic soy broth (TSB, Difco) containing 100 µg/ml of ampicillin (Sigma-Aldrich Chemical) at 37°C for 24 h with agitation (150 rpm). Following incubation, the cultures were sedimented by centrifugation (3600 X g for 15 min), washed twice, and resuspended in 10 ml of sterile deionized water. Equal portions from each of the four cultures were combined, and 100 µl (approximately 10⁸ CFU) of the four-strain mixture was used as the inoculum.

Sample inoculation and treatments

The efficacy of sodium caprylate for killing *E.coli* O157:H7 was determined in water with and without bovine feces or feed. Water was obtained from a local dairy farm, and aliquots of 100 ml each of water were dispensed into 250 ml sterile containers. Appropriate quantities of sodium caprylate (Sigma-Aldrich Chemical) were added to each water sample to obtain a final concentration of 75, 100 or 120 mM. Samples without sodium caprylate (0 mM) were used as controls for the study. In addition, a set of water samples containing bovine feces (1% w/v) (McGee et al., 2002) or feed (1% total mixed ration, TMR) were also included to determine the effect of feces/feed on the antibacterial property of caprylate. Each treatment and control water sample was inoculated with the four-strain mixture of *E.coli* O157:H7 to obtain an inoculation level 10⁶CFU/ml of water. The containers were loosely covered with plastic lids to enable free passage of air. The samples were incubated at 21°C, 10°C or 4°C. Triplicate samples of each treatment and control were included at each of the specified temperatures, and the entire study was duplicated.

Enumeration of E.coli O157:H7

The population of surviving *E.coli* O157:H7 in each water sample was determined by plating 0.1-ml portions of the samples directly or after serial dilutions (1:10 in phosphate buffered saline, PBS, pH 7.4) on duplicate Tryptic soy agar (TSA) plates containing 100 µg/ml of ampicillin. The plates were incubated at 37°C for 24 h and viewed under ultra violet light to enumerate *E.coli* O157:H7 (Vialette et al., 2004). At each sampling time, 1 ml of water from each container was also transferred to separate 250-ml flasks containing 100 ml of sterile TSB for enrichment at 37°C for 24 h. When growth was observed in TSB, the culture was streaked on TSA containing 100 µg/ml of ampicillin. The pH of each treatment and control sample was determined using an Accumet pH meter (Fisher Scientific, Pittsburgh, PA).

Statistical analysis

For each treatment and control, the data from independent replicate trials were pooled, and analyzed using a split-plot design with repeated sampling over time. The model included the treatment, concentrations, storage temperature and days. Significant differences ($P < 0.0001$) in bacterial counts due to treatment, concentrations, storage temperature and days were determined.

Results & Discussion

The magnitude of *E.coli* O157:H7 inactivation in water significantly ($P < 0.0001$) increased with increase in caprylate concentration and storage temperature. At 120 mM, sodium caprylate completely inactivated *E.coli* O157:H7 in all samples,

excepting those containing feces at 4°C. Feces or feed also had a significant effect ($P < 0.0001$) on the antibacterial property of caprylate. At all the storage temperatures, bovine feces substantially reduced the killing of *E.coli* O157:H7 by caprylate, whereas inactivation of the pathogen was rapid in presence of TMR.

Conclusions

This study indicated that sodium caprylate is effective in killing *E.coli* O157:H7 in cattle drinking water especially at higher environmental temperatures. This is important since fecal excretion of *E.coli* O157:H7 by cattle has been reported to be higher in summer months than in winter (Heuvelink et al., 1998; Jackson et al., 1998). Our future studies will focus on the palatability of water containing sodium caprylate to cattle.

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