ABSTRACT

Ruminant animals are natural reservoirs for *Salmonella*. These bacteria can reduce nitrate to nitrite through the membrane bound enzyme nitrate reductase which also has the ability to reduce chlorate to the cytotoxic end-product chlorite. An experimental product containing sodium chlorate (ECP) has been investigated in recent years as a pre-harvest food safety strategy to reduce *Salmonella*. The addition of nitroethane has been shown to enhance the effectiveness of ECP. The objective of this research was to determine if feeding ECP, with and without nitroethane, is effective in reducing naturally occurring populations of *Salmonella* in cull dairy cattle on a commercial dairy prior to slaughter. Twelve cull dairy cows, dosed for two consecutive days with either 140 mg of ECP containing 30% sodium chlorate /kg BW/d or with 70 mg of the ECP plus 160 mg nitroethane /kg BW/d, were sampled 48 h post initial dose at 12 h intervals for *Salmonella* via fecal samples. Upon completion of the 48 h sampling animals were necropsied and gastrointestinal tissue and luminal content samples taken for bacterial enumeration. The data presented herein support the use of chlorate as a pre-harvest intervention strategy for reducing *Salmonella* in cull dairy cows prior to entering the food chain can serve as an effective means of reducing these bacteria.

Keywords: Sodium Chlorate product, *Salmonella*, nitroethane, cull dairy cattle

INTRODUCTION

According to the Centers for Disease Control and Prevention, approximately 48 million people get sick each year from foodborne diseases in the United States (CDC 2010). The bovine gastrointestinal tract is a well recognized reservoir for bacterial pathogens like *Escherichia coli* O157:H7, *Salmonella* and *Campylobacter*. In the United States these bacterial...
pathogens are responsible for more than 3.5 million human infections annually at an estimated annual cost of more than $3.5 billion a year (ERS/USDA, 2009). According to Wells and others (Wells et al., 2001), as many as 66% of the cull dairy cows have detectable amounts of Salmonella shedding, and these cull cows contribute substantially to the beef supply, especially ground beef. Thus, pre-harvest intervention strategies that reduce the shedding of food-borne pathogens in cull dairy cattle are essential to reducing the amount of pathogenic bacteria entering slaughter facilities and contributing to contamination of food products and potentially human infections.

Certain bacteria, such as Salmonella have the ability to reduce nitrate to nitrite through the intracellular enzymes nitrate reductases (NarA and NarZ) (Alaboudi 1982; Moreno-Vivia et al., 1999). It has been suggested that the NarA enzyme, which is expressed under anaerobic conditions, is primary a contributor to the reduction of nitrate to nitrite (Anderson et al., 2006a). However, the NarZ enzyme can account for approximately 10% the nitrate reductase activity (Anderson et al., 2006a). Furthermore, these reductase enzymes have the ability to also reduce chlorate to the cytotoxic end-product chlorite (Stewart 1988, Fox et al., 2005 and Moreno-Vivia et al., 1999). In recent years, chlorate supplementation has been investigated as a pre-harvest food safety strategy to reduce Salmonella and E. coli O157:H7 in vitro and in food producing animals (Anderson et al., 2000). Research also has demonstrated effects against Salmonella in swine and poultry (Anderson et al., 2001; Anderson et al., 2004; Burkey et al., 2004) but to date the effect on Salmonella in cattle has not been evaluated. Research has shown that the addition of short chained nitro compounds like nitroethane can enhance the ability of sodium chlorate to reduce Salmonella as much as ten-fold in vitro and in vivo (Anderson et al., 2006a & 2006b). The objectives of the current research was to determine if feeding an experimental sodium chlorate product, with and without nitroethane, is effective in reducing populations of Salmonella, in cull dairy cattle on a commercial dairy prior to slaughter.

**MATERIALS AND METHODS**

All cattle were obtained from a conventional commercial dairy in the Southern High Plains of the United States and were cared for according to guidelines pre-approved for by the Southern Plains Agriculture Research Center’s Animal Care and Use Committee (ACUC no. 2010005). Dairy cows that were sent to the hospital pen per the dairy’s standard operating procedures were prescreened for Salmonella. The dairy’s hospital pens are used to house animals that were truly “sick” animals as well as animals that were needing to be separated from the rest of the herd, due to significant loss in milk production, laminitis, mobility issues, etc., which classified them as potential candidates to be culled from the herd per the discretion of the herd manager and standard operating procedures. Cows were restrained in self-locking head stalls and approximately 30 g of fecal material was obtained via rectal palpation. Fecal samples were shipped on ice to the Food and Feed Safety Research Unit in College Station, TX, USA (FFSRU) for culture of Salmonella the following day. Five days post-pre screen sampling, animals confirmed as Salmonella positive were enrolled. Twelve lactating Holstein dairy cows (average BW 545 kg) testing positive for Salmonella were purchased from the dairy and six animals were randomly assigned to each treatment (chlorate or chlorate + nitroethane). All experimental animals remained on the dairy and were housed in a pen separate from the rest of the herd; otherwise all feeding and management schemes were as normal for the dairy. As all experimental animals were housed together in the same pen, cross contamination among animals was a possibility. However, in the production setting, culled animals are exposed to other potential carriers throughout their time up to slaughter. Therefore we felt that co-mingling in a pen would give a more “real-world” test of the experimental treatment.

Salmonella positive animals received either 140 mg of an experimental sodium chlorate product (ECP)/kg BW/d or 70mg ECP plus 160 mg nitroethane/kg BW/d. Based on previous research (Anderson et al., 2006a), a sub optimal dose of ECP with added
nitroethane enhanced the bactericidal effects of the ECP against *Salmonella*. The ECP was a proprietary product provided by EKA Chemicals Inc. (Marrietta, GA) and contained 30% sodium chlorate by weight. Nitroethane was administered in the form of nitroethane salt (Majak et al., 1986). Treatments were administered 4 times at 12 h intervals via stomach tube. Animals were restrained in headstalls in the A.M. according to the dairy’s standard operation, and for the P.M. dose animals were moved to a chute for proper head restraint. Treatments were mixed with approximately 100 mL of water just prior to dosing to allow for adequate volume and fluidity for passage through the stomach tube. Each treatment was then followed with approximately 100 mL of water, prior to removal of the stomach tube, to remove any treatment that might have adhered to the stomach tube during dosing. Fecal grab samples were collected immediately prior to first dosing and subsequently every 12 h for the next 48 h post initial dosing. Following the last fecal collection animals were euthanized according to the American Veterinary Medical Association guidelines on euthanasia. Luminal contents and tissues from the rumen, small intestine, cecum, spiral colon and rectum were aseptically collected upon necropsy. All samples were shipped daily on ice to the FFSRU for quantitative and qualitative bacterial culture.

Fecal and luminal contents were processed upon arrival for qualitative and quantitative analysis of *Salmonella* (Edrington et al., 2009). Serogrouping of *Salmonella*-positive samples was conducted using slide agglutination with *Salmonella* antiserum (Becton, Dickinson, Sparks, ND). Sample *Salmonella* populations were quantified by direct plating of the TSB phosphate/sample mixture (10 g sample + 90 mL TSB; prior to enrichment) onto XLD agar (Oxoid, LTD, Basingstoke, Hampshire, England) using a commercially available spiral plater (Spiral Biotech Autoplate 4000; Advanced Instruments, Inc., Norwood, MA), with a limit of detection of 1.99X10² CFU/g. Plates were incubated overnight at 37°C. Colonies were counted and concentrations calculated. Morphologically typical (black) colonies were confirmed as *Sal-

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<th>Table 1. Effect of ECP on <em>Salmonella</em> presence in enriched and direct plated feces over time and tissues at necropsy. A number represents the results of the direct plating (quantitative culture) expressed as cfu (log₁₀/g feces), whereas a positive or negative symbol indicates a negative result from spiral plating and either a positive or negative result following enrichment and culture of the sample (qualitative culture).</th>
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monella as described above and counted.

RESULTS AND DISCUSSION

The scope of this experiment was to evaluate the effects of ECP with and without nitroethane on fecal shedding of Salmonella in cull dairy cows. The experiment was performed on the farm to ensure that daily farm practices and feeding regimes were employed in an effort to accurately simulate on-farm application of the ECP and ECP + nitroethane products. Two animals in the ECP treatment group were shedding Salmonella at high concentrations at the time of pre-screening (Table 1). Figure 1 demonstrates the ability of ECP to effectively reduce (5 log_{10} CFU/g feces) Salmonella fecal shedding concentration in animals colonized with high concentrations of Salmonella. The remaining animals were positive for Salmonella following enrichment at pre-screening (Tables 1 and 2). Forty-eight hours post initial treatment with ECP all animals were negative for Salmonella via spiral plating and only 50% of the animals had any detectable amount of Salmonella from enriched samples (Table 2). Luminal contents yielded no detectable Salmonella via direct plating for all animals regardless of treatment except animal 4630, which had 4.88 log_{10} CFU’s present in the small intestine. All other sites for this animal were negative via spiral plating. One colony from each positive sample was serogrouped, of which 32, 25, 18, 17, 5, and 3 % were K, E1, C1, poly A-I, C2, and I respectively.

We recognize that there are potential criticisms associated with the current research. The lack of control animals in this study can beg the question as to whether there was an effect due to the ECP or a natural response in cattle that have been shedding for several days. As with any experimental research product, federal approval must be obtained before animals can enter the food chain or rendering process, and since ECP is still awaiting federal approval all animals were required to be purchased from the dairy. Upon termination of the study, animals were composted on the dairy to prevent entrance to the food or rendering chain. Additionally, due to the associated cost and welfare issues we did not utilize control animals. The authors recognize this is a weakness of the study but did not have funding required to euthanize six perfectly healthy animals that could otherwise be sold by the dairy. By selecting animals that were persistently and consistently shedding Salmonella for five days prior to initial dosing with the

Figure 1. Effect of ECP supplementation on Salmonella concentrations in feces over time through direct plating.
experimental treatments, there was a high likelihood that the response seen once experimental treatments were imposed would be due to the effects of the experimental treatments. Granted, in hindsight it would have been beneficial to have control animals that provided fecal samples even if they were not necropsied. However, extensive research by the authors has repeatedly demonstrated the sporadic nature of fecal shedding of *Salmonella* in dairy cattle. Therefore we felt that luminal populations throughout the digestive tract would be a better indication of the actual “*Salmonella* status” of the animal and be a better determinant of the effectiveness of the treatments examined in this research. While we recognize that further experimentation is needed, the data presented herein while not conclusive does support the idea of using chlorate as a pre-harvest intervention in cull dairy cattle.

**CONCLUSIONS**

Administration of ECP on farm immediately following the decision to cull and prior to shipping should allow adequate time for the chlorate to exert its lethal effect on *Salmonella* prior to the animal entering the abattoir. While the ECP did not kill 100% of the cultured *Salmonella*, it did appear to reduce populations in the high shedders to levels that are effectively controlled by modern processing intervention strategies. It is unknown why the nitroethane did not enhance the bactericidal effect of chlorate in this research as has been observed previously. Further investigation is needed and research should examine the effectiveness of on-farm ECP administration by following cull animals through the harvest process.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


