

Effect of water sprinkling on incidence of zoonotic pathogens in feedlot cattle¹

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ABSTRACT: Heat stress and dusty conditions are common challenges for cattle during the summer, and a typical method of alleviating these problems involves sprinkling cattle and pens with water. The effect of sprinkling water on the incidence of zoonotic pathogens has not been previously studied. Four pens of heifers (n = 41) were cooled using sprinklers, and four pens (n = 43) served as controls. Heifers were crossbred Charolais, with white and red hair coats. Sprinkling was initiated when cattle were on full concentrate feed (July). Fecal samples, hide swipes, and BW were collected on d 0, 28, 63, 95, and 98. Average daily gain, DMI, and G:F were calculated, and carcass traits were

collected 36 h after processing. Performance data were analyzed as a randomized complete block design, and zoonotic pathogen data were analyzed using χ^2 analysis. Sprinkling tended ($P = 0.054$) to increase the incidence of fecal *Salmonella* spp. populations on d 98, but simultaneously tended to decrease ($P = 0.058$) the *Escherichia coli* O157:H7 incidence on hides on d 98. The most prevalent *Salmonella* serovars in this study were Kentucky, Muenster, Meleagridis, and Cerro. Performance measures and carcass traits did not differ between treatments ($P > 0.10$). Under our conditions, sprinkling cattle with water did not affect the incidence of zoonotic pathogens in feces or on hides.

Key Words: Food Safety, Sprinkling, Zoonotic Pathogens

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J. Anim. Sci. 2005. 83:1959–1966

Introduction

Heat stress can dramatically decrease cattle productivity, primarily through decreased DMI and feed efficiency (West, 1999). Cattle performance can be improved during hot weather by introducing environmental modifications such as shade, water misting, and water sprinkling (Beede and Collier, 1986; Bucklin et al., 1991). Providing shade structures has not been considered to be cost effective (Mitloehner et al., 2001, 2002), but some feedlot managers have adopted the practice of sprinkling water on pens to cool the cattle.

In addition, sprinkling is often used in commercial feedlots to alleviate dust.

Although alleviating heat stress and dust generated in feedlots can improve animal performance and decrease environmental impacts, sprinkling water might affect food safety. Cattle intestinal tracts and hides can be reservoirs of food-borne pathogenic bacteria, including *Salmonella* spp. and *Escherichia coli* O157:H7 (Elder et al., 2000; Schlosser et al., 2000). When cattle are heat-stressed, their rectal temperatures increase (Brown-Brandl et al., 2003), potentially affecting the intestinal microbial ecology, and the incidence of some pathogenic bacteria has been correlated with an increase in ambient temperatures (Hancock et al., 1998). Thus, sprinkling water as a cooling or dust control method might alter environmental conditions in the pen and on the hide of the cattle, thereby affecting pathogenic bacterial populations in or on cattle (Bohm, 1993; Van Donkersgoed et al., 2001); however, this hypothesis has not been tested previously. Thus, the current study was designed to determine the effect of sprinkling feedlot cattle with water on the incidence of *E. coli* O157:H7 and *Salmonella* in the feces and on the hides.

¹Proprietary or brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product, and exclusion of others that may be suitable.

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Received April 19, 2004.

Accepted April 21, 2005.

Materials and Methods

General

This experiment was conducted at the Texas Tech University Burnett Center experimental feedlot in New Deal from June 25 through October 2, 2001. Heifers were housed and were used in accordance with the guidelines suggested by FASS (1988), and the protocol was approved by the Texas Tech University Animal Care and Use Committee.

The Burnett Center feedlot is situated in an area with a dry steppe climate, characterized by hot summers and mild winters. Mean annual precipitation is 465 mm, with most of the precipitation occurring from April through October. Weather data were recorded at the site by a weather-monitoring station (Campbell Scientific 21× Micro Logger, North Logan, UT) every 10 min and averaged hourly and daily.

Animals and Diets

Charolais-crossbred heifers were received at a commercial feedlot in Lockney, TX, where each heifer underwent typical commercial processing. The heifers were then shipped (85 km) by commercial transport to the Burnett Center. Upon arrival at the Burnett Center, heifers were sorted randomly to soil-surfaced pens and were fed a 65% concentrate starter diet. Over the next month, the diet was gradually increased to contain 90% concentrate (Table 1). Heifers were implanted with Revalor H (Intervet, Millsboro, DE) approximately 30 d before the beginning of the study on June 25.

Eighty-nine heifers were sorted and blocked by BW into four weight blocks before being assigned to treatments in a randomized complete block design (44 treated (**SPRINKLED**) and 45 control (**CONTROL**); 11 heifers in each of the four treated pens and three of the control pens, with 12 heifers in the fourth control pen). Five heifers were removed from the study for health problems unrelated to treatments, resulting in 41 **SPRINKLED** and 43 **CONTROL** heifers completing the study. Heifers had ad libitum access to water and feed, with fresh feed offered once daily in the morning (Table 1).

Housing and Husbandry

The eight pens (4.9 × 30.2 m) used were predominantly soil-surfaced, with a concrete apron (4 m) at the front of the pens by the concrete feed bunk. Fences were of metal pipe and cable construction. Each pen was shaded over the feed bunk (3 m wide). One water trough with a float-activated water supply was shared between two pens.

Four unmodified pens were used as controls (**CONTROL**), whereas four pens (**SPRINKLED**) were equipped with a Rainbird S61S impact sprinkler (Azusa, CA) located 1 m outside the pen at a height of 4 m on top of the shade unit located over the feed bunk.

Table 1. Formulated ingredient and analyzed chemical composition of the diet fed to finishing heifers from June to October 2001

Item	% of DM
Ingredient composition	
Steam-flaked corn	74.72
Sorghum silage	10.09
Supplement premix ^a	2.5
Cottonseed meal	4.44
MGA premix ^b	0.25
Urea	0.89
Fat	2.92
Molasses	4.19
Chemical composition^c	
DM	63.69
CP	13.18
Ash	4.62
Ca	0.44
P	0.3
ADF	8.98

^aContained (DM basis) Rumensin (33 mg/kg), Tylan (8.8 mg/kg), vitamin A, vitamin E, and minerals.

^bProvided 0.044 mg/kg (DM basis) of melengestrol acetate (Pfizer Animal Health, New York, NY).

^cAnalyzed values, expressed on a DM basis.

Sprinklers had flow rates of 22.12 L/min, and a water throw radius of 13.4 m. Sprinkler head movement was limited to approximately 35°C to ensure only one pen was covered by each sprinkler. The sprinklers were turned on for 2-min periods every hour from 1100 through 1700 daily when ambient temperatures exceeded 30°C (a total of 64 d during the 98-d study). Pens were checked daily to verify that water was delivered to the **SPRINKLED** pens.

Cattle Performance and Carcass Traits

Cattle were weighed on d 0, 28, 63, 95, and 98 of the study. All weights were obtained before the morning feeding, and live weights without a pencil shrink are reported. Feed refusals were determined for each weigh period, and DMI was calculated on a pen basis. The ADG was calculated for heifers for each weigh period, and G:F was calculated as the ratio of pen ADG to pen DMI. The performance portion of the study ended on d 95 (a Friday), but we were unable to ship the cattle to slaughter until d 98 (a Monday). Hence, microbiological sampling was done on d 98 when the cattle were shipped to slaughter rather than d 95, and a BW also was measured on d 98 to be used for the calculation of dressing percent; however, this d-98 BW measurement was not used for calculation of performance responses.

Carcasses were chilled at 0°C for approximately 36 h after slaughter, at which time USDA quality and yield grades were obtained. The carcass measurements were as follows: actual and adjusted preliminary yield grades, fat thickness (mm), KPH (%), LM area (cm²), dressing percent, and HCW (kg).

Table 2. Climatic measures in the Texas Tech University Burnett Center Feedlot, New Deal, from June 25 through October 2, 2001

Month (sampling day)	Air temperature, °C				Relative humidity, %				Sprinkling days
	Mean	SE	Max ^a	Min ^b	Mean	SE	Max ^a	Min ^b	
June (d 0)	27.5	0.45	39.9	19.8	37.2	1.31	70.6	15.10	5
July (d 28)	28.4	0.18	38.8	17.3	43.0	0.65	91.4	14.75	31
August (d 63)	25.2	0.18	35.5	16.2	58.6	0.81	97.0	15.90	21
September (d 95)	21.7	0.21	35.1	7.6	59.3	0.79	100.0	8.95	7
October (d 98)	22.8	0.51	26.0	11.0	38.2	5.23	61.9	19.3	0

^aMonthly maximum temperature or relative humidity.^bMonthly minimum temperature or relative humidity.

Bacterial Sample Collection

On each weigh day (except d 95 as noted previously), fecal samples were collected from each heifer during the morning weighing via rectal grab using a new individual palpation sleeve for each sample, and a hide swab sample was collected from a 100-cm² surface of the left hock of each animal by vigorous swabbing with a sterile 20-cm² gauze pad saturated with sterile PBS, using an area template sterilized by 70% ethanol immersion between each use. Samples were individually bagged in sealed Whirl-Pak (Nasco, Modesto, CA) bags immediately after collection and kept on ice for 24 h before further analysis.

Quantitative Enumeration of Fecal Bacterial Populations

Fecal samples were serially diluted (10-fold increments) in PBS (pH 7.0) for enumeration of total coliform bacteria and generic *E. coli*. Enumerative dilution series were plated on MacConkey's agar (to enumerate total coliforms), and M-Endo LES agar (for enumeration of *E. coli*; Callaway et al., 2002). Colonies were counted on plates after 24 h of incubation at 37°C (Callaway et al., 2002). Fecal samples from d-63 sampling (September 10, 2001) were not quantitatively enumerated because the samples were unavoidably delayed in shipment; however, qualitative enrichments for *Salmo-*

nella and *E. coli* O157:H7 were carried out on these samples.

Feces and Hide Swab Preparation and Pathogen Enrichments

Sterile brilliant green bile broth (BGB; Oxoid Ltd., Basingstoke, U.K.) was added to Whirl-Pak bags containing hide swabs to dilute material recovered from the hide, and bags and swipes were mechanically mixed (via Stomacher) for 1 min to thoroughly mix each swab sample and diluent. Fluid from this initial swab dilution was used for enrichments of hide swabs (Elder et al., 2000; Keen and Elder, 2002).

To qualitatively enrich for *E. coli* O157:H7 populations, 10 g of feces was added to 90 mL of GN Hajna-VCC broth (Difco Laboratories, Sparks, MD) supplemented with vancomycin (8 µg/mL; Sigma Chemical Co., St. Louis, MO), cefsulodin (10 µg/mL; Sigma), and cefixime (1.42 µg/mL; Wyeth Pharmaceuticals, St. Davids, PA). For hide swabs, 1 mL of hide swab diluent was added to a 10-mL volume of BGB. These enrichments were incubated at 37°C for 6 h and rapidly cooled to 4°C for overnight storage before immunomagnetic bead separation (Elder et al., 2000).

For qualitative enrichment of *Salmonella*, feces (3 g) or 3 mL of hide swab diluent (in BGB) was added to tubes containing 27 mL of tetrathionate broth (Difco Laboratories) and incubated at 37°C for 24 h (Difco, 1998). After this incubation, 200 µL of the tetrathionate enrichments were added to 5 mL of Rappaport-Vassiliadis R10 broth (Difco Laboratories) and incubated an additional 24 h at 42°C before being streak-plated onto brilliant green agar (Oxoid, Ltd.) supplemented with novobiocin (25 µg/mL). These plates were incubated for 24 h at 37°C; colonies that exhibited typical *Salmonella* morphology were individually picked for further physiological characterization. Picked-putative *Salmonella* colonies were inoculated onto triple sugar iron agar (Difco Laboratories) slants and lysine iron agar slants (Difco Laboratories; Ferris et al., 1999; Dargatz et al., 2000; Wells et al., 2001). Each slant was incubated at 35°C for 24 h. *Salmonella*-positive samples were confirmed by slide agglutination using SM-O antiserum poly A-I and V-I, and group C1 factors (Difco Labora-

Table 3. Least squares means, pooled standard errors, and probability values of performance by finishing heifers either sprinkled or not sprinkled with water

Item	Control	Sprinkled	SE	P-value
No. of heifers	43	41	—	—
No. of pens	4	4	—	—
Initial BW, kg	383.9	388.5	14.21	0.32
BW at 95 d, kg ^a	539.8	545.6	17.52	0.69
BW at 98 d, kg	541.1	547.9	17.25	0.62
DMI, kg/d, d 0 to 95	9.74	9.77	0.309	0.94
ADG, kg/d, d 0 to 95	1.65	1.68	0.052	0.77
G:F, d 0 to 95	0.17	0.17	0.003	0.61

^aThe d-98 BW was taken immediately before the cattle were shipped to slaughter and was not used for calculation of ADG.

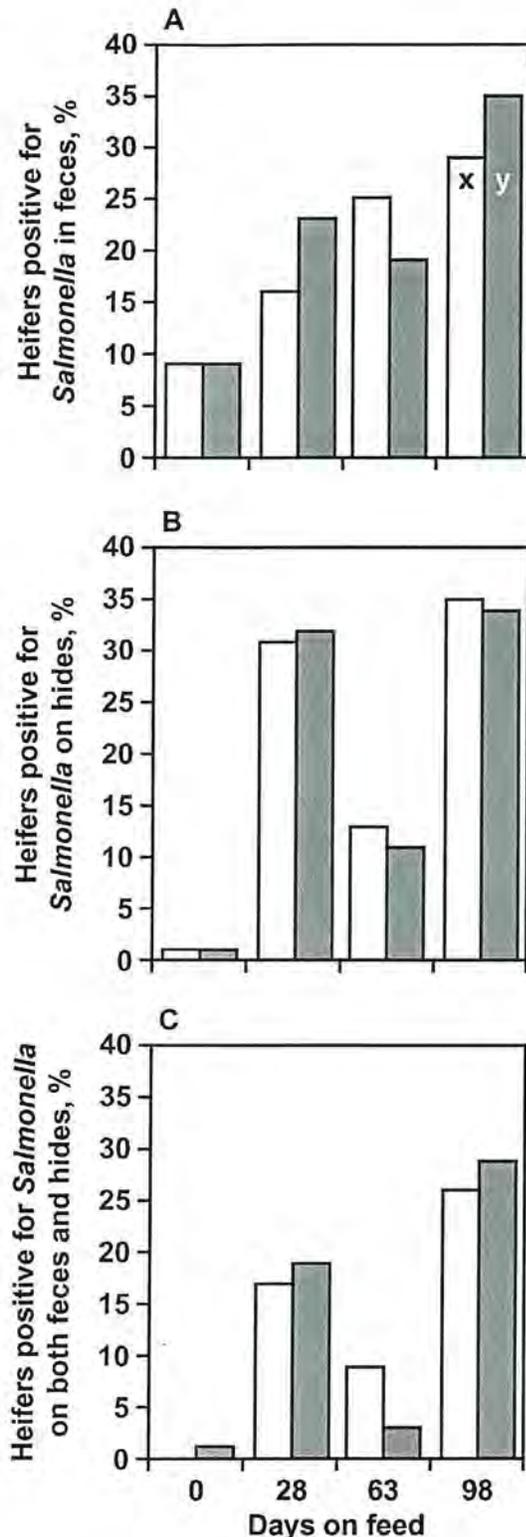


Figure 1. Percentage of heifers positive for *Salmonella* in feces (A), hides (B), and both feces and hides (C) on various sampling days. Open bars represent control (non-sprinkled) heifers, and shaded bars represent heifers that were sprinkled with water. Bars that do not have common letters differ, $P = 0.054$.

tories). *Salmonella* isolates were stored in glycerol and TSB at -80°C until confirmatory serotyping was performed by the National Veterinary Services Laboratory in Ames, IA.

Immunomagnetic Bead Separation of *E. coli* O157:H7

The GN-Hajna-VCC- and BGB-enriched samples were combined with immunomagnetic beads (Neogen Corp., East Lansing, MI) and analyzed according to the method of Keen and Elder (2002) using CHROMagar O157 plates (DRG Int., Mountainside, NJ), and serotypes were determined by standard ELISA methods (Keen and Elder, 2002).

Experimental Design and Statistical Analyses

A randomized complete block design was used in this study; with initial BW as the blocking factor. The experimental unit was the pen (two pens per block; four pens per treatment), with two treatments: CONTROL (pens with no sprinklers) vs. SPRINKLED (pens with sprinklers). Performance and carcass data were analyzed on a pen-basis using Proc Mixed in SAS (SAS Inst., Inc., Cary, NC) with a block (random effect), treatment, and treatment \times sampling day interaction included in the model. To quantitatively analyze coliform and generic *E. coli* bacterial populations, the GLM procedure in SAS was used, with block, treatment, animal, sampling day, and sampling day \times treatment interaction included in the model. The individual animal was used as the experimental unit. Differences in the proportion of animals positive for *Salmonella* and *E. coli* O157:H7 between treatments were analyzed by Pearson Exact χ^2 analysis of SAS, with treatment, animal, sampling day, and sampling day \times treatment interaction included in the model. When a sampling day \times treatment interaction occurred, each sampling day was examined individually by χ^2 to determine treatment effects.

Results

Climatic Measures

Ambient temperatures exceeded 30°C nearly every day of the study in June, July, and August (57 of 67 d), resulting in pens being sprinkled with water for 2 min every hour, but temperatures decreased during September (Table 2). The daily relative humidity fluctuated diurnally, between 30% at noon to nearly 80% in the evening.

Performance and Carcass Traits

Sprinkling with water did not affect BW, DMI, ADG, or G:F for any of the weigh periods (data not shown) or for the overall 95-d feeding period (Table 3). Hot carcass weight, fat thickness, LM area, KPH, dressing percent, and USDA yield and quality grades did not

Table 4. *Salmonella* serotypes pooled across treatments isolated from finishing heifers that were sprinkled or not sprinkled with water^{a,b}

Serotype	Fecal isolates		Hide isolates		Total isolates	
	No.	%	No.	%	No.	%
Kentucky	105	57.38	79	46.47	184	52.12
Muenster	46	25.14	50	29.41	96	27.2
Meleagridis	21	11.48	22	12.94	43	12.18
Cerro	2	1.09	12	7.06	14	3.97
Untypable	5	2.73	5	2.94	10	2.83
Gaminara	2	1.09	1	0.59	3	0.85
Anatum	0	0.00	1	0.59	1	0.28
Lille	1	0.55	0	0.00	1	0.28
8, 20:1 monophasic	1	0.55	0	0.00	1	0.28
Total <i>Salmonella</i>	183		170		353	

^aNumber of *Salmonella* isolates from sampling on d 0, 28, 63, and 95.

^b*Salmonella* serotypes did not differ between sprinkled and nonsprinkled treatments, $P = 0.35$.

differ ($P > 0.10$) either between treatments (data not shown). On average, carcasses graded Select.

Bacterial Populations

Fecal coliform and generic *E. coli* populations were approximately 10^7 cfu/g of wet feces, and there were no differences ($P > 0.10$) in generic *E. coli* or total coliform populations between CONTROL and SPRINKLED heifers on any of the sampling days (data not shown). There was no sampling day \times treatment interaction for *E. coli* or total coliform populations. The proportion of heifers shedding *Salmonella* in their feces differed ($P < 0.10$) over time, with an apparent linear increase over time resulting in the greatest percentage of animals shedding on the day of shipment to slaughter (d 98; Figure 1A). Because of the effect of time on the proportion of animals shedding *Salmonella*, each sampling day was examined separately. There were no differences between CONTROL and SPRINKLED treatments on the first three sampling dates, although on d 98, more heifers in the SPRINKLED group tended ($P = 0.054$) to be positive for fecal *Salmonella* than in the CONTROL group (Figure 1A). Proportion of heifers positive for *Salmonella* on hides varied by sampling day and was greatest on d 98 (Figure 1B); however, again, there was no difference between treatments. Proportion of heifers positive for *Salmonella* in both the feces and on the hide also varied by sampling day, but treatments did not differ significantly (Figure 1C).

The most common *Salmonella* serotypes isolated from these heifers were Kentucky, Muenster, Meleagridis, and Cerro (Table 4). The percentages of each serotype varied based on source (feces or hide), but the relative frequency (number of serotypes) and relative order of serotypes was consistent between feces and hide. There were no differences in the frequency of each *Salmonella* serotype between CONTROL and SPRINKLED heifers when data were analyzed by χ^2 analysis (data not shown).

Results for *Escherichia coli* O157:H7 isolated from cattle feces and hides during this study are shown in Figure 2. The proportion of heifers shedding *E. coli* O157:H7 varied by sampling day, but there was no sampling day \times treatment interaction. There were no differences in the proportion of heifers positive for *E. coli* O157:H7 in the feces between CONTROL and SPRINKLED treatments (Figure 2A); however, on d 98, there was a tendency ($P = 0.058$) for a greater proportion of CONTROL cattle to have *E. coli* O157:H7 on their hides than SPRINKLED cattle (Figure 2B). Several heifers tested positive for *E. coli* O157:H7 and *Salmonella* on the same sampling date, and the percentage of heifers shedding both pathogens varied from 5 to 17% (Figure 3). There was a tendency ($P = 0.079$) for more CONTROL than SPRINKLED heifers to simultaneously test positive for both pathogens on d 98.

Discussion

Heat stress affects cattle health and profitability (West, 1999). To alleviate the negative effects of heat stress on cattle, it has been suggested that producers physically modify the animals' environment. Therefore, it was thought that misting "performing" cattle (e.g., growing feedlot or high-producing dairy cows) could improve their productivity or efficiency (Mitloehner et al., 2001); however, misting did not alter the performance of heat-stressed cattle (Mitloehner et al., 2001). Therefore, we used a sprinkler treatment with larger water droplets to reach the skin and potentially lead to evaporative cooling. In early studies, sprinkling increased DMI and increased milk yield by dairy cows (Seath and Miller, 1948; Lofgreen et al., 1973; Bucklin et al., 1991). In the present study, daily high temperatures exceeded 30°C (the temperature threshold for sprinkler activation) on 57 of the first 67 d and on 64 of the 98-d period; however, the use of sprinklers during the hottest portion of the day (1100 through 1700 daily; 2-min periods every hour) did not affect heifer performance or carcass

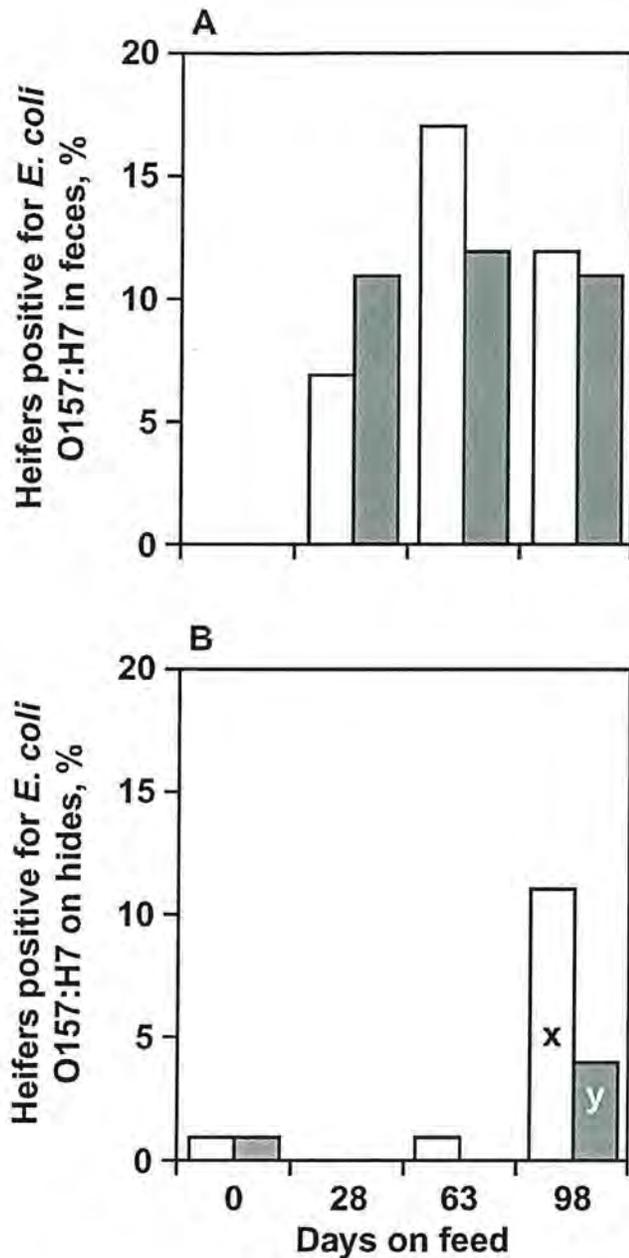


Figure 2. Percentage of heifers positive for *Escherichia coli* O157:H7 in feces (A) and hides (B) on various sampling days. Open bars represent control (nonsprinkled) heifers, and shaded bars represent heifers that were sprinkled with water. Bars that do not have common letters differ, $P = 0.058$.

traits compared with control heifers. It is likely that the shade provided over the feed bunk in the present study may have decreased the potential for sprinkling to positively affect feedlot performance and carcass characteristics. The incidence of zoonotic pathogens, not performance response, was the primary focus of the present study; however, performance data suggest that effects of sprinkling were not negative, and that the

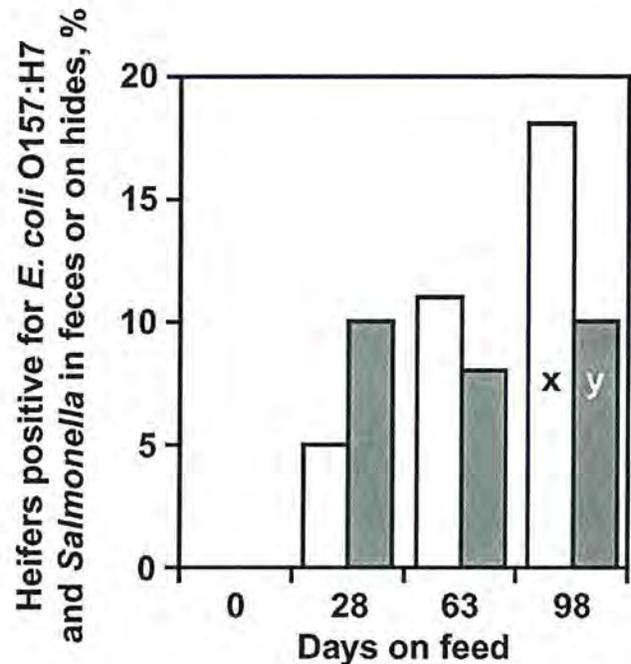


Figure 3. Percentage of heifers positive for both *Escherichia coli* O157:H7 and *Salmonella* in feces or hides on various sampling days. Open bars represent control (nonsprinkled) heifers, and shaded bars represent heifers that were sprinkled with water. Bars that do not have common letters differ, $P = 0.079$.

cattle gained and consumed feed within ranges expected under commercial conditions. Moreover, commercial facilities often use sprinkling as a dust control measure without regard to effects on performance.

Escherichia coli and other coliforms, as well as pathogenic *Salmonella* and *E. coli* O157:H7 are common members of the gastrointestinal ecosystem of cattle (Hungate, 1966; Wang et al., 1996; Losinger et al., 1997). Pathogenic bacteria also have been isolated from the hides of cattle at the time of slaughter (Elder et al., 2000; Keen and Elder, 2002). Pathogens on the hide and within the intestinal tract pose a potentially serious risk to the health and well being of beef consumers (Castillo et al., 1998; Hancock et al., 1998; Schloser et al., 2000), and these reservoirs been suggested to be an important source of contamination, particularly for *E. coli* O157:H7 (Gill and McGinnis, 1993).

It has been reported that the terminal rectum is a primary site of *E. coli* O157:H7 colonization of cattle (Naylor et al., 2003). Fecal shedding by cattle of this important pathogen reaches its peak during the hot summer months (Hancock et al., 1998). When cattle undergo heat stress or are reared under hot conditions, rectal temperatures are increased (Brown-Brandl et al., 2003; Gaughan et al., 2004). This increase in rectal temperature could potentially affect the colonization of the terminal rectum by *E. coli* O157:H7 and other pathogenic bacteria, but this hypothesis has yet to be

tested. Sprinkling has been shown to decrease rectal temperatures in heifers (Gaughan et al., 2004), but this study used very small numbers of heifers and did not examine the effect of sprinkling or rectal temperature on fecal populations of bacteria. In our study, sprinkling did not affect fecal shedding or hide contamination with *E. coli* O157:H7 or *Salmonella* except on d 98. At the end of the study period (d 98), ambient temperatures were cooler, and heat stress would not likely have been a factor. In fact, the sprinklers were not activated during the final 10 d of this study.

Feedlots sometimes produce large dust plumes in the evenings as cattle eat and display agonistic behaviors (Mitloehner et al., 1999; Morrow-Tesch, 2001). In the Texas Panhandle, these dust plumes from feedlots are occasionally visible for miles, especially when wind speeds are low and the air is cooling from the afternoon high. In the largest livestock-feeding countries of the world, including the United States and Canada, concerns have been raised about the health implications of these plumes in surrounding communities (B. Auvermann, Texas A&M Univ., Amarillo, TX; personal communication). In response to community concerns, some feedlots sprinkle cattle pens during hot or dry periods to alleviate dust plumes. Based on our results, sprinkling would not be expected to increase shedding or hide carriage of the tested pathogens; however, effects of water sprinkling on the feedlot dust, soil, and runoff remain to be elucidated.

Numerous methods have been proposed to decrease heat stress and to improve growth efficiency of cattle, including the use of shade, fans, misting, and sprinkling with water. The use of water to cool cattle has an added benefit of allaying the dust raised by feedlot cattle and subsequent dust plumes that might reach the surrounding community. In the present study, sprinkling heifers with water did not affect BW gain, gain efficiency, or carcass characteristics. Under our conditions, sprinkling cattle with water and providing access to shade did not change the incidence of *Salmonella* or *E. coli* O157:H7 in the feces or on hides compared with heifers provided shade without sprinkling.

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