Vaccine

Efficacy of a vaccine and a direct-fed microbial against fecal shedding of *Escherichia coli* O157:H7 in a randomized pen-level field trial of commercial feedlot cattle

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ABSTRACT

Our primary objective was to determine the efficacy of a siderophore receptor and porin proteins-based vaccine (VAC) and a *Lactobacillus acidophilus*-based direct-fed microbial (DFM) against fecal shedding of *Escherichia coli* O157:H7 in commercial feedlot cattle fed a corn grain-based diet with 25% distiller's grains. Cattle projected to be on a finishing diet during the summer were randomly allocated into 40 study pens within ten blocks based on allocation dates. Blocks were complete; each of the four pens within a block was randomly assigned one treatment: control, VAC, DFM, or VAC + DFM. The DFM was fed (10^6 CFU/animal/day of *Lactobacillus*) throughout the study periods (84–88 days) and cattle were vaccinated at enrollment and again three weeks later. Fresh fecal samples (30/pen) from pen floors were collected weekly for four consecutive weeks (study days 52–77). Two concurrent culture procedures were used to enable estimates of *E. coli* O157:H7 shedding prevalence and prevalence of high shedders. From 4800 total samples, 1522 (31.7%) were positive for *E. coli* O157:H7 and 169 (3.5%) were considered high shedders. Pen-level linear mixed models were used for data analyses. There were no significant interactions among treatments and time of sampling. However, vaccinated pens had lower ($P < 0.01$) overall prevalence of *E. coli* O157:H7 (model-adjusted mean ±SEM = 17.4 ± 3.95%) and lower ($P < 0.01$) prevalence of high shedders (0.95 ± 0.26%) than unvaccinated pens (37.0 ± 6.32% and 4.19 ± 0.81%, respectively). There was no evidence of a DFM effect on either measure of *E. coli* O157:H7 shedding. Results indicate that a two-dose regimen of the vaccine significantly reduces fecal prevalence of *E. coli* O157:H7 (vaccine efficacy of 53.0%) and prevalence of *E. coli* O157:H7 high shedders (vaccine efficacy of 77.3%) in commercial feedlot cattle reared in the summer on a finishing diet with 25% distiller's grains.

1. Introduction

Escherichia coli O157:H7 is an important cause of food-borne illness [1]. In addition to public health concerns, the economic impact of *E. coli* O157:H7 has been severe [2]. Pre-harvest interventions that reduce fecal shedding of these bacteria in cattle have the potential to enhance food safety and reduce economic impacts of *E. coli* O157:H7. It has been proposed that beef processors extend their food safety plans to the pre-harvest phase by purchasing cattle from producers who implement *E. coli* O157:H7 control programs [3]. However, most pre-harvest interventions have not been validated for the diverse production settings in the beef industry [3–5]. Both prevalence and concentration of *E. coli* O157:H7 in cattle feces are associated with beef contamination; occasionally cattle shed *E. coli* O157:H7 at high concentrations (e.g., >10^4 CFU/g of feces; hereafter "high shedders") [6–8]. Although few factors associated with shedding have been consistently observed, cattle shed more *E. coli* O157:H7 in summer than winter months [4,9,10]. Dietary components also influence fecal shedding [4,9]. For instance, diets containing distillers grains (DG), a co-product of the ethanol industry, can increase *E. coli* O157:H7 fecal shedding [9,11,12]. Since efficacy of pre-harvest interventions is most important during periods of high fecal shedding [13], data from studies of cattle fed DG-supplemented diets in the summer months are important.

Two interventions that are commercially available in the United States and have demonstrated efficacy for reducing *E. coli* O157:H7 shedding in cattle are a siderophore receptor and porin (SRP) proteins-based vaccine and a *Lactobacillus acidophilus*-based direct-fed microbial (DFM) [5,14]. This DFM includes a strain of *L. acidophilus* (NP51) shown to have inhibitory effects on *E. coli* O157:H7 [10]. The vaccine uses SRP proteins as antigens so immunized animals produce anti-SRP antibodies that bind to outer membrane proteins of bacterial cells and block iron transport [15]. Although literature indicates potential benefits of these products, there is a need for additional data on efficacy in commercial settings [5,14]. Further, there are no data on concurrent use of these interventions. Therefore, our primary objective was to determine the efficacy of intervention programs including the SRP vaccine, the DFM, or both products against fecal shedding of *E. coli* O157:H7 in pens of commercial feedlot cattle fed a DG-supplemented finishing diet during the summer. A secondary objective was to evaluate impacts of intervention programs on cattle health and performance outcomes as compared to control cattle reared using standard practices.

2. Materials and methods

A commercial feedlot in Nebraska, USA was identified based on criteria that included: capacity to fill 40 pens with cattle on a finishing diet during summer, use of a finishing diet that included $\geq 25\%$ DG, ability to feed the DFM, willingness to vaccinate cattle according to protocol, and ability to perform research. Individual cattle were eligible for inclusion if projected to be on a finishing diet during summer; with this feedlot's management system, cattle had to be enrolled approximately 100 days prior to harvest of the first subset. Following a brief transition period, cattle were fed a finishing diet which included (dry matter basis): 46.4% high moisture corn, 25.0% wet DG, 17.0% corn gluten, 7.1% silage, 2.5% steep, and 2.0% micro/minerals mix (including 280 mg of monensin/animal/day and 90 mg of tylosin/animal/day (Elanco Animal Health, Greenfield, IN, USA)). The feedlot's standard operating procedures were followed for cattle care and management; sprinklers were used as needed to reduce heat stress risks. Kansas State University (KSU) Institutional Animal Care and Use Committee approved the study (#2723).

The study was designed as a randomized complete block with a 2×2 factorial treatment structure. *A priori* sample size estimates were generated by data simulation and power calculations; assumptions included: 40% mean control group prevalence of *E. coli* O157:H7 [16], 25% mean prevalence in pens receiving an intervention, and no interaction among interventions. Forty pens (10/treatment) and 120 samples (30/week for four weeks) per pen were considered sufficient for 80% statistical power to detect expected treatment differences with a 5% Type 1 error. Individual cattle were randomly allocated to 40 pens grouped in 10 blocks (defined based on allocation dates; March 31 through May 14, 2011). Within block, one pen each was randomly allocated to one treatment: control, administered vaccine (VAC), fed DFM (DFM), or both VAC and DFM (VAC + DFM). Cattle in VAC and VAC + DFM groups were administered a 2 mL dose of the vaccine subcutaneously (SC, 1½ in. needle) in the left lower neck on study day 0 and again three weeks later (*E. coli* SRP® vaccine, Pfizer Animal Health, New York, NY, USA; lot # 840-0006, expiration August 19, 2011). Cattle allocated to DFM or control groups never received a placebo and were not re-handled three weeks following enrollment. The DFM, labeled for 10^6 CFU/animal/day of *L. acidophilus* and 10^9 CFU/animal/day of *Propionibacterium freudenreichii*, was fed throughout the study periods (Bovamine®, Nutrition Physiology Corp., Guymon, OK, USA). On study day 0, all cattle received a herpes

virus vaccine (Pyramid IBR, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO, USA; 2 mL, SC) and a growth promoting implant (Synovex Choice, Pfizer Animal Health, New York, NY, USA; SC in the left ear).

The feedlot's computer system randomly allocated animals to treatment groups as they were handled on study day 0. For each block, four contiguous pens within the feedlot were identified and pen locations for treatment groups within blocks were then randomly allocated using the computer's randomization algorithm. The primary study outcome was within-pen *E. coli* O157:H7 prevalence, whereas within-pen prevalence of high shedding animals was considered a secondary outcome. Thus, each sample was classified twice (independently) as positive or negative to: (1) a culture procedure including immunomagnetic bead separation (IMS) to assess fecal shedding, and (2) a direct plating culture procedure to assess high shedding. Laboratory personnel were blinded to treatment: samples were tracked only by sequential numbers. Cumulative (study period) risks of cattle mortality and culling, and cattle performance measures also were considered secondary outcomes and were collected by feedlot staff blinded to treatment group; personnel administering treatments did not collect health or performance data. Average (mean) daily weight gain (ADG) and feed conversions (F:G; ratio of feed weight to gained weight of cattle) were calculated as:

$$ADG = \frac{\text{Total weight gain of cattle (as defined below)}}{\text{Total cattle days}}$$

$$F : G = \frac{\text{Total dry matter weight of feed}}{\text{Total weight gain of cattle (as defined below)}}$$

where total weight gain of cattle equals out-weight of cattle finishing the trial plus out-weight of cattle culled plus out-weight of dead cattle minus total enrollment weight of cattle.

Feedlot personnel performed daily health monitoring following standardized procedures. Animals were weighed individually at the beginning and end of the study. Fresh fecal samples (30/pen) from animals observed defecating were collected from separate pats in multiple areas throughout the pen. Care was taken to avoid ground contamination. Pens were sampled weekly for four consecutive weeks prior to study end-dates for each block. Samples (approximately 30 g) were placed in sterile bags, stored in coolers, and transported to KSU for refrigeration (4 °C) until the following morning. Samples were cultured for *E. coli* O157:H7 using IMS and direct plating methods previously described [7,8]. Confirmation included a multiplex PCR for identifying the *rfb*E (O157), *eae* (intimin), *stx*1 (Shiga toxin 1), *stx*2 (Shiga toxin 2), *hly*A (hemolysin), and *fli*C (H7) genes [17].

Pen-level general and generalized linear mixed models (LMM and GLMM, respectively) were used to assess potential treatment effects. For response variables recorded as pen-level proportions, data were fit using a GLMM with a binomial distribution and a logit link. Prevalence outcomes were the proportion of samples positive of the total samples collected within the pen at each sampling. Mortality and culling risks were proportions based on the number of animals that died or were culled, respectively, during the study period out of the total number of animals enrolled within the pen. Data on ADG and F:G were modeled using LMM that assume a Gaussian distribution. For all models, random effects were fitted to recognize block as the clustering factor and pen as the experimental unit for treatment. For *E. coli* data, additional random effects were used to account for pen-specific repeated measures over time. Independent variables included treatments (VAC, DFM, VAC x DFM interaction), and for *E. coli* data, effects of time and time-by-treatment interaction. Model diagnostics were based on studentized residuals (LMM) and functions of the
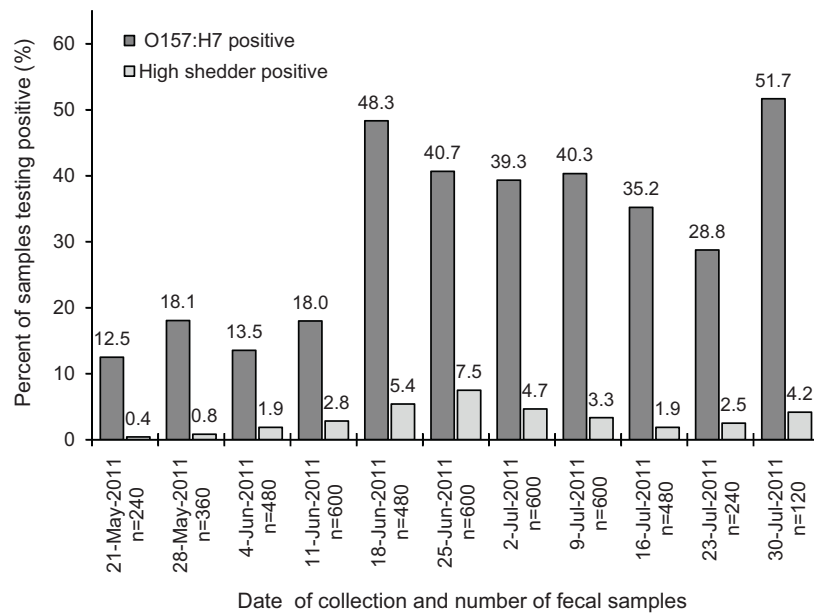
Fig. 1. Descriptive data on fecal samples from feedlot cattle that tested positive for *E. coli* O157:H7 shedding and *E. coli* O157:H7 high shedding for each week during the sampling period. Data are summarized across all pens (treatments and blocks) sampled for each week.

Pearson χ^2 statistic (GLMM). *P* values <0.05 were considered significant. Model-adjusted means (lsmeans back transformed to original scale) and SE were reported, and used to estimate vaccine efficacy using standard formula [18].

3. Results

Study pens were filled with 17,148 steers. Pen sizes ranged between 398 and 464 steers (mean = 430.0). Mean weight at enrollment was 378.4 kg with no significant difference among treatment groups. Projected finishing days were re-assessed by feedlot personnel during the study and determined to be 14 days earlier than expected. Resulting end-dates for study blocks ranged between June 20 and August 3, 2011; thus, days on study ranged between 84 and 88 (mean = 86.6 days) across blocks. Sampling began approximately five weeks prior to projected study-end for each block, resulting in samples collected (for four consecutive weeks) between study days 52–56 (week one), 59–63 (week two), 66–70 (week three), and 73–77 (week four).

From 4800 total samples, 1522 (31.7%) were positive for *E. coli* O157:H7 and 169 (3.5%) were considered high shedders; percentages by week of sampling are provided in Fig. 1. Isolates considered *E. coli* O157:H7 were positive for the *rfb*E (100%), *eae* (99.8%), *stx*1 (66.2%), *stx*2 (99.5%), *hly*A (99.7%), and *fli*C (99.8%) genes. *Escherichia coli* O157:H7 were isolated at least once from all pens (100%) and 34 pens (85%) had at least one high shedder. Within pens, unadjusted cumulative prevalence of shedding (across sampling times) ranged between 1.7% and 66.7% and high shedder prevalence ranged between 0% and 12.5%.

Analysis of within-pen prevalence of *E. coli* O157:H7 shedding data indicated no significant two- or three-way interactions among treatments and time of sampling. There also was no significant main effect of DFM (Table 1). However, a main effect of VAC was apparent, such that VAC decreased prevalence of fecal shedding (Table 2). Fig. 2 illustrates estimated efficacy (53.0%) of vaccination for reducing fecal prevalence of *E. coli* O157:H7 and means for the contrast between vaccinated and non-vaccinated pens (*P* < 0.01). A main effect of sampling time on fecal shedding was also apparent (*P* = 0.02), whereby mean prevalence on sampling week two differed from prevalence on week four; no other

week-to-week differences were detected. Means (SEM) were 24.6% (5.07), 20.7% (4.53), 27.2% (5.39) and 32.4% (5.92) for sampling weeks one through four, respectively.

Regarding high shedder prevalence, results indicated no significant two- or three-way interactions among treatments and time of sampling, and no significant main effects of DFM (Table 1) or sampling week. However, a significant effect of VAC was identified, whereby vaccination decreased the prevalence of high shedders (Table 2). Fig. 2 illustrates the difference in means for vaccinated and non-vaccinated pens (*P* < 0.01) and the estimated vaccine efficacy (77.3%) for reducing prevalence of *E. coli* O157:H7 high shedders.
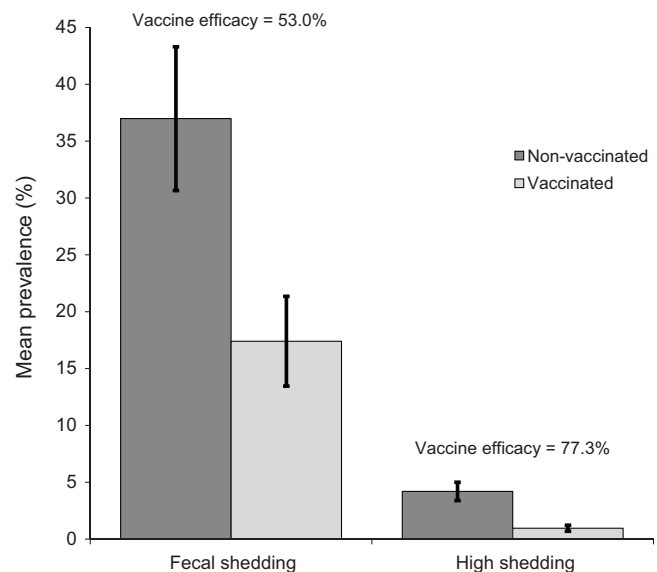
Fig. 2. Vaccine efficacy estimates and corresponding model-adjusted[1] means (with standard error bars) demonstrating the effects a siderophore receptor and porin proteins-based vaccine for controlling overall fecal prevalence of *E. coli* O157:H7 and prevalence of *E. coli* O157:H7 high shedders in pens of commercial feedlot cattle. A significant vaccine effect was demonstrated for both measures of *E. coli* O157:H7 prevalence (*P* values <0.01).[1] [1] From generalized linear mixed models accounting for allocation of pens within blocks and repeated measures on pens over time.

Table 1

Model-adjusted[a] means, standard errors (SEM), 95% confidence intervals (CI), and P values for comparisons of outcome measures between pens of feedlot cattle that were fed a direct-fed microbial[b] (DFM) containing *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* (n = 20) and pens that were not fed the DFM (n = 20).

Outcome measures	Fed DFM			No DFM			P value
	Mean	SEM	CI	Mean	SEM	CI	
Fecal prevalence (%)	25.8	5.24	16.6–37.9	26.2	5.26	16.9–38.3	0.94
High shedder prevalence (%)	2.16	0.49	1.36–3.42	1.87	0.47	1.12–3.11	0.59
Average daily weight gain (kg/head/day)	1.48	0.02	1.43–1.53	1.46	0.02	1.40–1.51	0.09
Feed to gain ratio	6.01	0.08	5.84–6.17	6.14	0.08	5.99–6.31	0.03
Mortality risk (%)	1.14	0.11	0.93–1.40	1.08	0.11	0.87–1.34	0.70
Culling risk (%)	0.42	0.15	0.21–0.86	0.41	0.14	0.21–0.86	0.78

[a] From linear mixed models accounting for allocation of pens within blocks (and repeated measures on pens over time for fecal and high shedder prevalence).

[b] Bovamine® (labeled for 10^6 CFU/animal/day of *Lactobacillus acidophilus* and 10^9 CFU/animal/day of *Propionibacterium freudenreichii*), Nutrition Physiology Corp., Guymon, OK, USA.

Effects of treatment were apparent on both ADG and F:G, but there were no significant interactions between VAC and DFM. For ADG, there was no significant DFM effect (Table 1), but the VAC effect was significant (Table 2). For F:G, effects of DFM (Table 1) and VAC (Table 2) were both statistically significant. There was no evidence of VAC and DFM interactions, main effect of DFM (Table 1), or main effect of VAC (Table 2) on either mortality or culling risks.

4. Discussion

An important finding of this study is that two doses of the SRP® vaccine applied in a commercial feedlot reduced *E. coli* O157:H7 shedding by more than 50% and reduced high shedders by more than 75%. These results from a cattle population with relatively high levels of *E. coli* O157:H7 have important practical implications since efficacy of pre-harvest interventions is most important when prevalence is high [13]. Another important finding is that the commercial DFM (Bovamine®) had no effect on *E. coli* O157:H7 fecal shedding. These results also have practical significance since end-users of pre-harvest interventions may wonder whether these commercially available products – the SRP® vaccine and the Bovamine® DFM – are equally efficacious. Results also indicate that DFM-fed cattle may have improved performance whereas cattle in vaccinated pens had relatively poorer performance. Performance effects need to be further quantified since cattle performance affects beef production costs, and the adoption of pre-harvest control programs will be affected by all costs associated with implementation.

Study cattle were fed a diet with 25% DG during the summer; thus, the interventions were tested in a situation when fecal shedding of *E. coli* O157:H7 was expected to be high. Feeding DG to cattle can increase fecal shedding of *E. coli* O157:H7 approximately two to threefold [9,11,12]. Seasonal effects associated with *E. coli* O157:H7 shedding (higher in the summer) also has been well documented; study data (Fig. 1) demonstrate a well-described seasonal pattern [4,16,19]. The sample-level prevalence for high shedders (3.5%) and overall fecal shedding (31.7%) were relatively high, but numerically similar to estimates from comparable populations. Reports on summer-harvested cattle included prevalence estimates for high shedders of 3.7% [7] and 3.3% [8]. Recent estimates of overall fecal prevalence in summer-fed feedlot cattle have ranged between 37% and 10%, but within-pen prevalence is highly variable [16,20,21]. Thus, the range in cumulative within-pen prevalence (1.7–66.7%) reported in this current study is consistent with previous reports. While diagnostic sensitivity and specificity of culture methods used in this study are not perfect for identifying fecal shedding and high shedding [22], any misclassification would be expected to be non-differential with respect to treatments. Further, these methods have previously provided useful data on fecal shedding relative to important food safety parameters such as *E. coli* O157:H7 carcass and hide prevalence [7,8]. Gene profiles of isolates recovered in this study are similar to those previously reported; indicating that the *E. coli* O157:H7 isolates have potential for human virulence [23,24].

This is the first published study demonstrating efficacy of a 2-dose regimen of the commercially available SRP® vaccine for reducing both the prevalence of *E. coli* O157:H7 shedding and high shedding in a large-pen commercial feedlot setting. Although vaccine efficacy has been demonstrated previously [15,25,26], key features differ between previous studies and the study reported here. The SRP® vaccine was first shown to reduce fecal shedding in young calves orally inoculated with *E. coli* O157:H7 [28]. Cattle that were naturally shedding *E. coli* O157:H7 in a research feedlot were used to show that 3 mL doses of vaccine reduced fecal shedding; a dose–response trend was also observed [25]. In one feedlot study, a 2-dose regimen of the vaccine reduced fecal prevalence, and in another study, a 3-dose regimen reduced fecal concentration [26]. A cow-calf study found no significant vaccine effects, but cattle were vaccinated at much different production phases [27]. In addition to differing study designs, vaccine dosages, or study populations, this commercial feedlot study reported here utilized very large pens while others used smaller pens (≤70 animals/pen) [15,25,26]. A recent systematic review indicating efficacy of the SRP® vaccine

Table 2

Model-adjusted[a] means, standard errors (SEM), 95% confidence intervals (CI), and P values for comparisons of outcome measures between pens of feedlot cattle that were vaccinated twice with a siderophore receptor and porin proteins-based vaccine[b] (n = 20) and pens that were not vaccinated (n = 20).

Outcome measures	Vaccinated			Non-vaccinated			P value
	Mean	SEM	CI	Mean	SEM	CI	
Fecal prevalence (%)	17.4	3.95	10.7–27.0	37.0	6.32	25.2–50.6	<0.01
High shedder prevalence (%)	0.95	0.26	0.54–1.67	4.19	0.81	2.82–6.20	<0.01
Average daily weight gain (kg/head/day)	1.45	0.02	1.39–1.50	1.49	0.02	1.44–1.54	0.01
Feed to gain ratio	6.14	0.08	5.99–6.31	6.01	0.08	5.85–6.18	0.04
Mortality risk (%)	1.14	0.11	0.93–1.40	1.08	0.11	0.87–1.34	0.70
Culling risk (%)	0.41	0.14	0.20–0.83	0.43	0.15	0.21–0.87	0.78

[a] From linear mixed models accounting for allocation of pens within blocks (and repeated measures on pens over time for fecal and high shedder prevalence).

[b] *E. coli* SRP® vaccine, Pfizer Animal Health, New York, NY, USA.

suggested that further studies in commercial settings were needed [14].

No evidence for any DFM (Bovamine®) effect on *E. coli* O157:H7 fecal shedding was observed, contradicting some results of empirical studies and a systematic review indicating efficacy of this *L. acidophilus* strain (NP51) [5,10,28–32]. Possibly larger pen sizes and a lower dose of product in the current study compared to previous studies could explain seemingly contradictory results. This commercial feedlot study utilized large pens while many other studies used much smaller ($\leq$10 animals/pen) pens [28–32]. Further, efficacy of this DFM for reducing *E. coli* O157:H7 may be improved at a higher dose [10,29,32]. The commercial low-dose Bovamine® product (106 CFU/head/day of *Lactobacillus*) was utilized in the current study because of the perception that this product can reduce fecal shedding and also improve cattle performance. Indeed, there are important practical implications if a pre-harvest control program could reduce *E. coli* O157:H7 fecal shedding while improving animal performance.

A meta-analysis demonstrated that this DFM can improve feedlot cattle performance [33]; reported summary effects were similar to effects reported here. However, results indicating lower weight gain per day and less efficient feed conversion for vaccinated versus unvaccinated pens are novel. Previous feedlot studies with this vaccine did not detect significant differences in cattle performance [15]. However, in previous studies both vaccine and control groups were handled on re-vaccination days and controls were given a placebo. Further, previous studies had much smaller sample sizes to detect differences with half as many pens (20) and much fewer animals overall (<1300) than the current study (40 and >17,000, respectively). For the current study, controls were not re-handled or given a placebo during vaccinations times because the feedlot would not normally do these procedures; the intent of assessing performance was to evaluate the total program (not just the vaccine) as compared to normal production practices. Since production costs must be considered for implementation of a vaccination program, further research specifically designed for evaluating performance effects may be warranted.

5. Conclusions

We found the overall fecal prevalence of *E. coli* O157:H7 and prevalence of high shedders in this large commercial feedlot population were relatively high as expected for summer-fed cattle supplemented with distiller's grains. We conclude that this DFM, Bovamine® (labeled for 10^6 CFU/head/day of *Lactobacillus*), administered alone or in combination with the SRP® vaccine, does not significantly affect fecal shedding. However, the SRP® vaccine significantly reduces fecal prevalence of *E. coli* O157:H7 and prevalence of high shedders, and therefore may be an effective intervention for *E. coli* O157:H7 control in commercial feedlots.

References

[1] Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. Emerg Infect Dis 2005;11(April (4)):603–9.

[2] U.S. Department of Agriculture, Economic Research Service. Food-borne illness cost calculator: STEC O157:H7. p. 1. Available from: http://www.ers.usda.gov/Data/FoodBorneIllness/ecoli_intro.asp [updated 24.06.11; cited 15.11.11].

[3] U.S. Department of Agriculture, Food Safety and Inspection Service. Pre-harvest management controls and intervention options for reducing *Escherichia coli* O157:H7 shedding in cattle. p. 28. Available from: http://www.fsis.usda.gov/pdf/reducing_ecoli_shedding_in_cattle_0510.pdf; May 2010 [cited 15.11.11].

[4] Renter DG, Sargeant JM. Enterohemorrhagic *Escherichia coli* O157: epidemiology and ecology in bovine production environments. Anim Health Res Rev 2002;3(2):83–94.

[5] Sargeant JM, Amezcua MR, Rajic A, Waddell L. Pre-harvest interventions to reduce the shedding of *E. coli* O157 in the feces of weaned domestic ruminants: a systematic review. Zoonoses Public Health 2007;54(6–7):260–77.

[6] Elder RO, Keen JE, Siragusa GR, Barkocy-Gallagher GA, Koohmaraie M, Laegreid WW. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc Natl Acad Sci USA 2000;97(March (7)):2999–3003.

[7] Fox JT, Renter DG, Sanderson MW, Nutsch AL, Shi X, Nagaraja TG. Associations between the presence and magnitude of *Escherichia coli* O157 in feces at harvest and contamination of preintervention beef carcasses. J Food Protect 2008;71(9):1761–7.

[8] Jacob ME, Renter DG, Nagaraja TG. Animal- and truckload-level associations between *Escherichia coli* O157:H7 in feces and on hides at harvest and contamination of preevisceration beef carcasses. J Food Protect 2010;73(6):1030–7.

[9] Jacob ME, Callaway TR, Nagaraja TG. Dietary interactions and interventions affecting *Escherichia coli* O157 colonization and shedding in cattle. Foodborne Pathog Dis 2009;6(September (7)):785–92.

[10] Loneragan GH, Brashears MM. Pre-harvest interventions to reduce carriage of *E. coli* O157 by harvest-ready feedlot cattle. Meat Sci 2005;71(1):72–8.

[11] Jacob ME, Paddock ZD, Renter DG, Lechtenberg KF, Nagaraja TG. Inclusion of dried or wet distillers' grains at different levels in diets of feedlot cattle affects fecal shedding of *Escherichia coli* O157:H7. Appl Environ Microbiol 2010;76(November (21)):7238–42.

[12] Wells JE, Shackelford SD, Berry ED, Kalchayanand N, Guerini MN, Varel VH, et al. Prevalence and level of *Escherichia coli* O157:H7 in feces and on hides of feedlot steers fed diets with or without wet distillers grains with soluble. J Food Protect 2009;72(August (8)):1624–33.

[13] Dodd CC, Sanderson MW, Jacob ME, Renter DG. Modeling preharvest and harvest interventions for *Escherichia coli* O157 contamination of beef cattle carcasses. J Food Protect 2011;74(9):1422–33.

[14] Snedeker KG, Campbell M, Sargeant JM. A systematic review of vaccinations to reduce the shedding of *Escherichia coli* O157 in the feces of domestic ruminants. Zoonoses Public Health 2011;59(June (2)):126–38.

[15] Thomson DU, Loneragan GH, Thornton AB, Lechtenberg KF, Emery DA, Burkhardt DT, et al. Use of a siderophore receptor and porin proteins-based vaccine to control the burden of *Escherichia coli* O157:H7 in feedlot cattle. Foodborne Pathog Dis 2009;6(7):871–7.

[16] Renter DG, Smith DR, King R, Stilborn R, Berg J, Berezowski J, et al. Detection and determinants of *Escherichia coli* O157:H7 in Alberta feedlot pens immediately prior to slaughter. Can J Vet Res 2008;72(April (3)):217–27.

[17] Bai J, Shi X, Nagaraja TG. A multiplex PCR procedure for the detection of six major virulence genes in *Escherichia coli* O157:H7. J Microbiol Methods 2010;82(July (1)):85–9.

[18] Measures of association.Dohoo IR, Martin SW, Stryhn H, editors. Veterinary epidemiologic research. Charlottetown: AVC, Inc., PEI; 2003. p. 121–38.

[19] Williams MS, Withee JL, Ebel ED, Bauer Jr NE, Schlosser WD, Disney WT, et al. Determining relationships between the seasonal occurrence of *Escherichia coli* O157:H7 in live cattle, ground beef, and humans. Foodborne Pathog Dis 2010;7(October (10)):1247–54.

[20] Smith D, Blackford M, Younts S, Moxley R, Gray J, Hungerford L, et al. Ecological relationships between the prevalence of cattle shedding *Escherichia coli* O157:H7 and characteristics of the cattle or conditions of the feedlot pen. J Food Protect 2001;64(December (12)):1899–903.

[21] Dewell GA, Ransom JR, Dewell RD, McCurdy K, Gardner IA, Hill AE, et al. Prevalence of and risk factors for *Escherichia coli* O157 in market-ready beef cattle from 12 U.S. feedlots. Foodborne Pathog Dis 2005;2(Spring 1):70–6.

[22] Fox JT, Renter DG, Sanderson MW, Thomson DU, Lechtenberg KF, Nagaraja TG. Evaluation of culture methods to identify bovine feces with high concentrations of *Escherichia coli* O157. Appl Environ Microbiol 2007;73(August (16)):5253–60.

[23] Paddock ZD, Walker CE, Drouillard JS, Nagaraja TG. Dietary monensin level, supplemental urea, and ractopamine on fecal shedding of *Escherichia coli* O157:H7 in feedlot cattle. J Anim Sci 2011;89(September (9)):2829–35.

[24] Renter DG, Sargeant JM, Oberst RD, Samadpour M. Diversity, frequency, and persistence of *Escherichia coli* O157 strains from range cattle environments. Appl Environ Microbiol 2003;69(January (1)):542–7.

[25] Fox JT, Thomson DU, Drouillard JS, Thornton AB, Burkhardt DT, Emery DA, et al. Efficacy of *Escherichia coli* O157:H7 siderophore receptor/porin proteins-based vaccine in feedlot cattle naturally shedding *E. coli* O157. Foodborne Pathog Dis 2009;6(7):893–9.

[26] Thornton AB, Thomson DU, Loneragan GH, Fox JT, Burkhardt DT, Emery DA, et al. Effects of a siderophore receptor and porin proteins based vaccination on fecal shedding of *Escherichia coli* O157:H7 in experimentally inoculated cattle. J Food Protect 2009;72(4):866–9.

[27] Wileman BW, Thomson DU, Olson KC, Jaeger JR, Pacheco LA, Bolte J, et al. *Escherichia coli* O157:H7 shedding in vaccinated beef calves born to cows vaccinated prepartum with *Escherichia coli* O157:H7 SRP vaccine. J Food Protect 2011;74(October (10)):1599–604.

[28] Younts-Dahl SM, Galyean ML, Loneragan GH, Elam NA, Brashears MM. Dietary supplementation with Lactobacillus- and Propionibacterium-based direct-fed microbials and prevalence of *Escherichia coli* O157 in beef feedlot cattle and on hides at harvest. J Food Protect 2004;67(May (5)):889–93.

[29] Younts-Dahl SM, Osborn GD, Galyean ML, Rivera JD, Loneragan GH, Brashears MM. Reduction of *Escherichia coli* O157 in finishing beef cattle by various doses of *Lactobacillus acidophilus* in direct-fed microbials. J Food Protect 2005;68(January (1)):6–10.

[30] Brashears MM, Galyean ML, Loneragan GH, Mann JE, Killinger-Mann K. Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given Lactobacillus direct-fed microbials. J Food Protect 2003;66(May (5)): 748–54.

[31] Stephens TP, Loneragan GH, Chichester LM, Brashears MM. Prevalence and enumeration of *Escherichia coli* O157 in steers receiving various strains of Lactobacillus-based direct-fed microbials. J Food Protect 2007;70(May (5)):1252–5.

[32] Stephens TP, Loneragan GH, Karunasena E, Brashears MM. Reduction of *Escherichia coli* O157 and *Salmonella* in feces and on hides of feedlot cattle using various doses of a direct-fed microbial. J Food Protect 2007;70(10): 2386–91.

[33] Hanford KJ, Kreikemeier WM, Ware DR. The effect of Bovamine on feedlot performance of finishing cattle: a meta-analysis. Proceedings of the American Dairy Science Association and American Society of Animal Science Conference, 2011 July 10–14, New Orleans, Louisiana. J Anim Sci 2011; 89(1):258.