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# Vaccination and Direct Fed Microbials as Intervention Strategies for Reduction of *E. coli* O157:H7 in Feedlot Steers

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## Summary

*A clinical trial was conducted to evaluate effects of two intervention strategies on the prevalence of E. coli O157:H7 shedding by feedlot steers using 384 steers and 48 pens. Intervention strategies were a direct fed microbial or vaccination against E. coli O157:H7. No differences in performance or carcass yield were observed for direct fed microbial or vaccination treatments, compared to the control. Vaccination significantly reduced the prevalence of E. coli O157:H7. In addition, we also observed a non-significant decrease in the prevalence of E. coli O157:H7 with inclusion of the direct fed microbial.*

## Introduction

Preliminary Nebraska research has indicated inclusion of direct fed microbials such as (*Lactobacillus* spp.) in the diet of beef animals, may reduce numbers of *E. coli* O157:H7 shed in the feces (Moxley, et al., 2000, unpublished observations; Brashears et al., 2003, Journal of Food Protection 66:748-754). Also, previous research indicated vaccination against *E. coli* O157:H7 type III secreted proteins reduced fecal shedding of this organism in experimentally inoculated cattle (6-month old calves and adult yearlings; Potter, et al., 2001 unpublished observations). Therefore, an experiment was conducted to evaluate effects of vaccination, and

or inclusion of a direct fed microbial as intervention strategies on the prevalence and of *E. coli* O157:H7 being shed in the feces.

## Procedure

Three hundred eighty-four medium framed steer calves (768 lb) were used in a feedlot finishing experiment. Steers were blocked into three weight groups and stratified by weight within block and assigned randomly into forty-eight pens (8 steers / pen). Pens within each block were assigned randomly to a 2 x 2 factorial treatment design; factors being with or without direct fed microbial (NPC 747) (Nutrition Physiology Corp.), or with or without vaccination treatment. Direct fed microbial product was mixed with water and applied to the feed truck mixing box and fed at a rate of 1x10<sup>9</sup> colony forming units / steer / day. Steers were fed once daily with the control steers fed with a control feed truck and treatment steers fed with another treatment feed truck to minimize cross contamination. The finishing diet dry matter composition was 55% high moisture corn, 35% wet corn gluten feed, 5% corn silage, 2% alfalfa hay, 2 % supplement, and 1 % water (used to mix the direct fed microbial) (Table 1). Steer weights were taken for two consecutive days at the start of the experiment after a 3-day period of limit-feeding to equalize gut fill. Steers were fed for an average of 121 days. Steers were sampled one block per week in three-week experimental periods, resulting in one pre-treatment period and 5 experimental periods. Rectal fecal grab samples were obtained from each steer in each period. Vaccinations were given three times, beginning with the pre-treatment sampling and twice more during

Table 1. *E. coli* O157:H7 intervention experiment finishing diets.

Ingredient (% DM basis)	
Wet Corn Gluten Feed	35.0
High Moisture Corn	55.0
Corn Silage	5.0
Alfalfa Hay	2.0
Supplement <sup>a</sup>	2.0
Water <sup>b</sup>	1.0
Nutrient Composition, % of DM <sup>c</sup>	
Crude Protein	12.5
Calcium	0.7
Potassium	0.7
Phosphorus	0.3

<sup>a</sup>Supplement formulated to deliver 30 g/ton Rumensin® and 10g/ton Tylan® and meet NRC requirements for trace minerals and vitamins.

<sup>b</sup>Used to mix the direct fed microbial.

<sup>c</sup>Calculated from 1996 NRC Model.

the first two experimental period samplings.

All fecal samples were taken immediately to the UNL *E. coli* lab and analyzed for presence of *E. coli* O157:H7 using procedures previously described (Smith, et al., 1999) with modifications. Ten-gram fecal samples were incubated 6 hr in Gram Negative (GN) broth containing vancomycin, cefixime and cefsulodin. An aliquot of culture material was then subjected to immunomagnetic bead separation and plated onto sorbitol-MacConkey agar containing cefixime and tellurite (CT-SMAC). After an 18-24 hr incubation, three non-sorbitol-fermenting colonies were picked and subcultured onto CT-SMAC to ensure purity then were subcultured onto MacConkey and Fluorocult agars. After an 18-24 hr incubation, lactose-fermenting colonies that yielded a negative MUG (4-methylumbelliferyl-β-D-glucuronide) reaction were streaked for isolation on blood agar. After an overnight incubation, one colony per isolate on blood agar was tested for *E. coli* O157 and H7 antigens by latex agglutination. Isolates that

(Continued on next page)

were positive for O157 antigen, regardless of H7 results, were tested in a 5-primer-pair multiplex polymerase chain reaction (PCR) assay that detected genes for *E. coli* O157, H7 Shigatoxins 1 and 2, and intimin. Detection of genes for O157, H7 and at least one other target in the assay was considered to be confirmation of an isolate as *E. coli* O157:H7.

Pen was considered the experimental unit, and the performance and proportion of culture-positive animals per pen during the period were the outcomes of interest. *E. coli* O157:H7 data were analyzed on a pen basis using repeated measures in the Mixed procedure of SAS assuming random block and compound symmetry. Performance data were statistically analyzed with the mixed procedures of SAS.

## Results

### *E. coli* Results

During the pre-treatment sampling period, the prevalence of *E. coli* O157:H7 was 31.0% (Table 2) and there were no significant ( $P=.19$ ) differences among treatment groups. Average pen prevalence by treatment and period is summarized in Figure 1. During the experimental periods prevalence varied significantly over time ( $P=0.01$ ), with period 1 having an overall prevalence of 18.5%. Period 2 declined in overall prevalence with 10.2%. Period 3 was static having an overall prevalence of 11.7%. Prevalence again declined in period four having an overall prevalence of

**Table 2. *E. coli* O157:H7 results over time and treatment.**

Experiment Periods	Prevalence, %
Pre-Treatment	31.0
Experimental Periods	
Period 1	18.5
Period 2	10.2
Period 3	11.7
Period 4	4.4
Period 5	18.8
Treatment	Prevalence, %
Control	21.3
DFM <sup>a</sup>	13.3
Vacc. <sup>b</sup>	8.8
DFM + Vacc.	7.7

<sup>a</sup>DFM = Direct fed microbial treatment.

<sup>b</sup>Vacc. = Vaccination treatment.

**Table 3. Steer finishing and carcass performance.**

Item	Vacc. <sup>a</sup>	DFM <sup>b</sup>	Vacc.+ DFM	Control	SE	DFM P <sup>c</sup>	Vacc. x DFM <sup>d</sup>
Daily Gain, lb	3.94	4.03	3.94	3.98	.05	0.72	0.70
Feed / Gain	6.20	6.04	6.16	6.15	.07	0.37	0.55
DMI lb/d	24.3	24.3	24.2	24.5	.2	0.39	0.80
Fat, in	0.50	0.50	0.50	0.47	.13	0.81	0.84
Marbling <sup>e</sup>	476	471	477	478	6	0.60	0.47

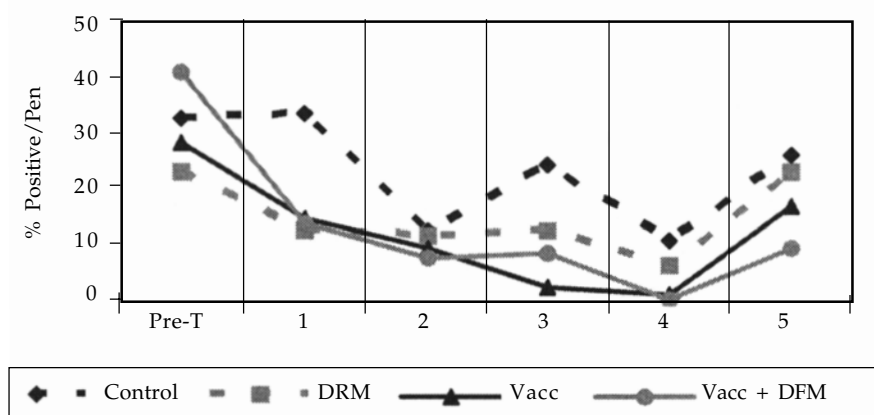
<sup>a</sup>Vacc. = Vaccination treatment.

<sup>b</sup>DFM = Direct fed microbial treatment.

<sup>c</sup>DFM P = P-value for main effect of DFM.

<sup>d</sup>Vacc by DFM interaction.

<sup>e</sup>Marbling = Marbling score = 400 = Slight<sup>0</sup>, 450 = Slight<sup>50</sup>, 500 = Small<sup>0</sup>, etc.



**Figure 1. Prevalence of *E. coli* O157:H7 with treatment over time.**

4.4%. However in period 5 we observed an increase in overall prevalence with 18.8% of the steers being positive at market time. There were no significant interactions between time and treatment.

Over the five experimental periods, control steers averaged 21.3% (Table 2) positive for *E. coli* O157:H7. Steers treated with direct-fed microbials showed decreased prevalence with 13.3% of the steers positive, but the response was non-significant ( $P=.21$ ). However, steers vaccinated with three injections of vaccine displayed a significant decrease ( $P=.03$ ) in prevalence of *E. coli* O157:H7 with an average of 8.8% positive. The addition of the direct fed microbial treatment to the vaccinated cattle showed slight, but not significant improvement with an average prevalence of 7.7%. There was no interaction between vaccination and direct fed microbial.

### Finishing Performance

There were no effects of direct-fed microbial or vaccination on any aspect of steer finishing performance or carcass merit (Table 3).

In conclusion, vaccination of steers with three injections of *E. coli* O157:H7 vaccine significantly reduced fecal shedding of *E. coli* O157:H7. We observed a non-significant trend to decrease the prevalence of *E. coli* O157:H7 in cattle feces, with the addition of a direct-fed microbial to the feed. A similar non-significant decrease in prevalence was observed in at least two recent experiments in other research facilities. This suggests the direct-fed microbial is effective in reducing prevalence. Therefore, because we saw no interaction we believe the two intervention strategies may be useful separately or in concert to reduce fecal shedding of *E. coli* O157:H7.

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