

# Influence of Endophyte-Infected Tall Fescue Seed on Fecal Shedding of *Escherichia coli* O157:H7 and Blood Metabolites in Experimentally Inoculated Sheep

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## Story in Brief

The objectives of this study were to determine effects of short-term feeding of endophyte-infected tall fescue seed on fecal shedding and intestinal concentrations of *E. coli* O157:H7, and concentrations of cortisol and nonesterified fatty acids (NEFA) in experimentally-inoculated sheep. Twelve ewes (mean BW = 101 ± 4 lb) were fed a diet containing either high endophyte-infected (HI-E) or low endophyte-infected (LO-E) tall fescue seed (50%, as-fed basis) for 7 days. Ewes were inoculated with *E. coli* O157:H7 on day 1 of experimental feeding, and fecal shedding of inoculated pathogens was monitored daily. On day 7, ewes were euthanized, and tissues and contents were sampled from the ileum, cecum, and rectum for quantitative enumeration of *E. coli* O157:H7. Fecal shedding of *E. coli* O157:H7 tended ( $P = 0.06$ ) to be increased in HI-E ewes [5.4 colony forming units (cfu) ( $\log_{10}$ )/gram of feces] compared with LO-E ewes [4.5 cfu ( $\log_{10}$ )/gram of feces]. Populations of *E. coli* O157:H7 in luminal contents from the ileum, cecum, and rectum did not differ ( $P > 0.36$ ) between treatments. Ileum tissues from HI-E ewes tended ( $P = 0.12$ ) to have an increased incidence of *E. coli* O157:H7. Mean concentrations of cortisol were similar ( $P = 0.49$ ) for HI-E and LO-E ewes while mean concentrations of NEFA tended ( $P = 0.11$ ) to be increased in HI-E ewes over LO-E ewes. We conclude that short-term feeding of HI-E tall fescue seed may increase concentrations of NEFA and fecal shedding of *E. coli* O157:H7 in experimentally inoculated sheep.

## Introduction

A majority of the 49 million acres of tall fescue grown in the southeastern United States is infected with an endophyte fungus causing several stressful disorders, collectively characterized as fescue toxicosis. Stress may predispose ruminants to be more susceptible to opportunistic bacteria such as *E. coli* O157:H7.

Consumption of endophyte-infected tall fescue alters metabolic hormones and enzyme activity in ruminants (Nihsen et al., 2004). Cortisol is usually associated with stress in ruminants, and nonesterified fatty acids (NEFA) are increased in nutrient-restricted animals. However, the association between these blood metabolites and *E. coli* O157:H7 shedding in ruminants is lacking.

Effects of grazing endophyte-infected tall fescue on fecal shedding of pathogenic bacteria in naturally-infected ruminants have not been consistent (Looper et al., 2003, 2006). We hypothesized that consumption of endophyte-infected tall fescue seed would induce fescue toxicosis, and consequently increase fecal shedding of *E. coli* O157:H7 from experimentally-inoculated sheep. Therefore, objectives were to determine effects of short-term (7 days) feeding of high endophyte-infected (HI-E) or low endophyte-infected (LO-E) tall fescue seed on fecal shedding and intestinal concentrations of *E. coli* O157:H7, and serum concentrations of cortisol and NEFA in experimentally-inoculated sheep.

## Experimental Procedures

The Animal Care and Use Committee of the USDA-ARS, Food and Feed Safety Research Laboratory approved the care, use, and handling of experimental animals (FFSRU IACUC 200502). Twelve

non-lactating hair-type sheep ( $n = 6$  each of Katadhin and St. Croix; mean BW = 101 ± 4 lb; mean age 2.6 ± 1.5 yr) were blocked by body weight and breed, and housed indoors in individual pens. Ewes had not been exposed to endophyte-infected tall fescue the 6 months prior to initiation of experiment. Ewes were acclimated to the basal diet (cracked corn substituted for fescue seed) for 7 days prior to the initiation of the experiment. On day 0 of the experiment, ewes were fed a diet containing either high endophyte-infected (HI-E) or low endophyte-infected (LO-E) tall fescue seed for 7 days. Ewes were offered the diet at 3.5% BW, and orts were weighed daily to calculate DMI. Concentrations of ergovaline in fescue seed and in total diets were determined by HPLC (Moubarak et al., 1996). Ewes were experimentally-inoculated with an antibiotic-resistant *E. coli* O157:H7 strain BDMS T4169 (ATCC 700728) on day 1 of feeding treatment, and fecal shedding of the inoculated pathogen was monitored by assay of fecal grab samples daily for 5 days (day 2 through day 6). Sheep were weighed and euthanized (Euthasol®, euthanasia solution, Delmarva Laboratories, Inc., Midlothian, Va.) on day 7, and intestinal contents (10 to 15 grams) and tissues from the ileum, cecum, and rectum were aseptically collected for qualitative enrichment and quantification of the inoculated strain of *E. coli* O157:H7 (described below). Care was taken to ensure that each tissue and lumen content sample was removed from approximately the same location in each animal. Urine was collected from each animal at euthanization (day 7) to determine total ergot alkaloid concentrations via immunoassay.

Blood serum samples were collected on days 1, 2, 4, and 7 from each ewe by venipuncture of the jugular vein, allowed to clot for 24 hours at 40°F, and centrifuged (1,500 x g for 25 min). Serum was frozen and stored until concentrations of cortisol were quantified by radioimmunoassay (Coat-A-Count®, Diagnostic Products, Los Angeles, Calif.). Serum concentrations of NEFA were determined

<sup>1</sup> Names are necessary to report factually on available data; however, the USDA does not guarantee or warrant the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that also may be suitable.

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by an enzymatic colorimetric procedure (NEFA-C, Wako Chemicals Inc., Dallas, Texas) adapted for use in a 96-well microtiter plate system and expressed as microequivalents of palmitate per liter. Intra-assay coefficients of variation were 8.9 and 2.3% for cortisol and NEFA, respectively.

**Bacterial cultures.** *Escherichia coli* O157:H7 strain BDMS T4169 (ATCC 700728) was obtained from the American Type Culture Collection (Manassas, Va.) and was cultivated in anoxic tryptic soy broth (TSB) medium at 99°F. This strain was made resistant to novobiocin and nalidixic acid (20 and 25 µg/mL, respectively) via successive cultivation in TSB containing up to 20 µg/mL of novobiocin and 25 µg/mL nalidixic acid. Overnight cultures (1 L) were harvested by centrifugation (7,500 x g, 10 min) and the cell pellets were re-suspended in TSB medium (150 mL total volume). Sheep were individually inoculated with 10 mL of TSB containing *E. coli* O157:H7 ( $4 \times 10^{11}$  cfu) via oral gavage. Fecal samples were collected 3 days prior to dosing and screened for the presence of wild-type *E. coli* O157:H7 and generic *E. coli* resistant to novobiocin and nalidixic acid. On each of the subsequent 5 days after initiation of feeding experimental diets, fecal samples were collected and shedding of inoculated *E. coli* O157:H7 was qualitatively analyzed and populations were enumerated daily as described below.

**Bacterial Enumeration.** Ten to 15 grams of fecal material were collected from each ewe daily. One gram of each fecal sample was serially diluted (10-fold increments) in sterile phosphate buffered saline (PBS, pH 6.5) and plated on MacConkey's agar that was supplemented with novobiocin (20 µg/mL) and nalidixic acid (25 µg/mL). Plates were incubated for 24 hours at 99°F and colonies that grew on agar plates directly counted (quantitative enumeration). To qualitatively confirm the presence of inoculated *E. coli* O157:H7, daily fecal samples, intestinal contents, and epithelial tissue samples (2 grams) were incubated (24 hours, 99°F) in 20 mL GN Hajna with novobiocin/naladixic acid and streaked on MacConkey's agar plates as above. Plates showing colony growth were classified as positive for the inoculated bacteria (qualitative enumeration).

**Statistical analyses.** Dry matter intake, daily fecal shedding, and concentrations of cortisol and NEFA were analyzed by repeated measures using the MIXED procedure of SAS (SAS Inst. Inc., Cary, N.C.) with a compound symmetry covariance structure. Treatment, day, and the interactions were included in the model. Effects of treatment on ADG, urinary concentrations of total ergot alkaloid, and bacterial counts from luminal contents (quantitative) were determined by the MIXED procedure of SAS. Chi-square analysis, using the FREQ procedure of SAS, was used to determine influence of treatment on qualitative bacterial enumeration of epithelial tissue samples. Least squares means were compared using the PDIF option of MIXED when protected by a significant ( $P < 0.05$ ) treatment effect.

## Results and Discussion

Concentration of ergovaline was 1,626 and 260 parts per billion (ppb) for HI-E and LO-E tall fescue seed, respectively. Concentrations of ergovaline were 1,051 ppb in HI-E tall fescue seed diet and 184 ppb in LO-E tall fescue seed diet. Urinary concentrations of total ergot alkaloids were increased ( $P < 0.001$ ) in ewes fed HI-E tall fescue seed (67.3 ng/mg creatinine) compared with

LO-E ewes (5.3 ng/mg creatinine). Concentrations of urinary ergot alkaloids were increased within 2 days of steers grazing endophyte-infected tall fescue (Stuedemann et al., 1998). Concentrations of ergovaline in the HI-E tall fescue seed diet in the current experiment were similar to or exceeded published concentrations of ergovaline capable of causing physiological symptoms of fescue toxicosis. Further, a 12-fold increase in urinary alkaloids in HI-E ewes compared with LO-E ewes demonstrates HI-E ewes were exposed to high levels of toxic fescue.

Ewes fed HI-E seed diets had lower ( $P < 0.05$ ) DMI than LO-E ewes (1.8 and 3.5 lb/day DMI for HI-E and LO-E ewes, respectively). Consequently, there was a tendency ( $P = 0.06$ ) for HI-E ewes to lose 0.7 lb/day and LO-E ewes to gain 0.4 lb/day during the 7-day study. Mean concentrations of NEFA tended ( $P = 0.11$ ) to be greater in HI-E ( $181 \pm 18$  mEq/L) than in LO-E ( $140 \pm 18$  mEq/L) ewes. Nonesterified fatty acids are the by-product of body fat breakdown and are released into circulation during periods of nutrient restriction.

Fecal grab samples collected from all sheep prior to inoculation with *E. coli* O157:H7 were negative for wild-type *E. coli* strains. Fecal shedding data of *E. coli* O157:H7 during the 5-day collection (day 2 through day 6) are shown in Figure 1. There was no treatment x day interaction ( $P = 0.18$ ); however, overall mean shedding of *E. coli* O157:H7 tended ( $P = 0.06$ ) to be increased in HI-E ewes [5.4 cfu ( $\log_{10}$ )/gram of feces] compared with LO-E ewes [4.5 cfu ( $\log_{10}$ )/gram of feces]. Luminal contents from the ileum, cecum, and rectum contained similar ( $P > 0.36$ ) populations of *E. coli* O157:H7 between treatments (Table 1). Tissue samples (after a 24-hour enrichment) from the cecum and rectum had a similar ( $P = 0.30$ ) occurrence of *E. coli* O157:H7; however, ileum tissues from HI-E ewes tended ( $P = 0.12$ ) to have an increased incidence of *E. coli* O157:H7 (Table 1). Stress may predispose animals to be more susceptible to opportunistic bacteria such as *E. coli* O157:H7. Cattle grazing endophyte-infected tall fescue have increased body temperature during summer months, reduced milk production and reproductive performance, and decreased growth rate. Ewes consuming HI-E tall fescue seed diets exhibited signs/symptoms of fescue toxicosis including reduced DMI and subsequent BW loss, and increased urinary ergot alkaloids. Further, HI-E ewes tended to have increased fecal shedding of *E. coli* O157:H7 and a tendency for a greater incidence of *E. coli* O157:H7 in ileum tissue than LO-E ewes.

Mean concentrations of cortisol were not different ( $P = 0.49$ ) for HI-E ewes ( $3.2 \pm 0.7$  µg/dL) and LO-E ewes ( $4.1 \pm 0.7$  µg/dL) in the current experiment. Concentrations of cortisol were increased in heifers and cows 3 to 6 hours after ergot alkaloid infusion (Browning et al., 2000). However, concentrations of cortisol in heifers (Aldrich et al., 1993) and lambs (Fiorito et al., 1991) adapted to endophyte-infected tall fescue diet for 10 to 14 days were similar to cortisol in animals consuming endophyte-free tall fescue diets. Concentrations of cortisol were similar between sheep fed HI-E or LO-E tall fescue seed diets and may not be a good indicator of stress induced by endophyte-infected tall fescue seed consumed for 7 days.

## Implications

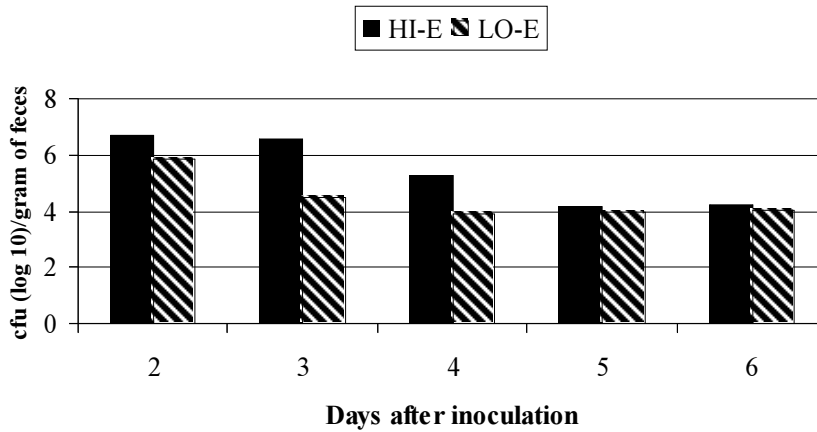
Sheep consuming high endophyte-infected tall fescue seed diets exhibited signs/symptoms of fescue toxicosis and tended to

shed more *E. coli* O157:H7. Management strategies that prevent livestock from grazing endophyte-infected tall fescue and/or alleviate stressors associated with consumption of endophyte-infected tall fescue prior to harvest may reduce fecal shedding of pathogenic bacteria from livestock.

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**Fig. 1. Fecal shedding [cfu (log<sub>10</sub>)/gram of feces] of *E. coli* O157:H7 in sheep experimentally inoculated with *E. coli* O157:H7 and fed diets of high endophyte-infected (HI-E) or low endophyte-infected (LO-E) tall fescue seed; treatment effect (P = 0.06; SE = 0.49; treatment x day interaction, P = 0.18)**

**Table 1. Luminal contents [cfu (log<sub>10</sub>)/gram of feces] and tissue samples (number of ewes) of gastrointestinal tract positive for *E. coli* O157:H7 in sheep 7 days after inoculation with antibiotic-resistant *E. coli* O157:H7 and fed diets of high endophyte-infected (HI-E) or low endophyte-infected (LO-E) tall fescue seed.**

Item	HI-E	LO-E	SEM
Luminal contents			
Ileum	2.10	2.75	0.61
Cecum	2.27	3.06	0.57
Rectum	2.64	2.09	0.73
Tissue samples			
Ileum	6/6 <sup>a</sup>	4/6 <sup>b</sup>	--
Cecum	5/6 <sup>a</sup>	6/6 <sup>a</sup>	--
Rectum	6/6 <sup>a</sup>	5/6 <sup>a</sup>	--

<sup>a,b</sup>Numbers in a row with no superscript in common differ (P = 0.12).