Influence of toxic endophyte-infected tall fescue on the prevalence of *E. coli* O157:H7 from ruminants, a review

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Abstract

Ruminants consuming toxic endophyte-infected (E+) tall fescue are exposed to ergot alkaloids that usually result in a malady of problems identified as fescue toxicosis. Grazing studies investigating the effects of E+ tall fescue on the prevalence of *E. coli* O157:H7 from naturally-infected cattle have not been consistent. Further, *in vitro* studies with selected ergot alkaloids did not affect growth of *E. coli* O157:H7. Dietary stressors usually associated with E+ tall fescue consumption did result in increased faecal shedding of *E. coli* O157:H7 from sheep experimentally-inoculated. Animal species, animal model (naturally vs experimentally-infected), environmental conditions, type of diet, and length of exposure to E+ tall fescue may influence the association between grazing E+ tall fescue and *E. coli* O157:H7 shedding. A better understanding of how E+ tall fescue influences shedding of *E. coli* O157:H7 from ruminants will aid in the development of management strategies for on-farm pathogen control.

Keywords: cattle, *E. coli* O157:H7, sheep, tall fescue

Introduction

Approximately 70% of the more than 20 million hectares of tall fescue (*Festuca arundinacea* (Schreb), syn, *Lolium arundinaceum* (Schreb.) Darbysh) grown in the southeastern United States is infected with the toxic endophyte fungus, *Neotyphodium coenophialum*, which produces numerous ergot alkaloids (Shelby & Dalrymple 1987). Ruminants grazing toxic endophyte-infected (E+) tall fescue generally exhibit various stressful signs of fescue toxicosis (Hoveland *et al.* 1983; Paterson *et al.* 1995). It is possible that stress may cause ruminants to become more susceptible to pathogenic bacteria such as *E. coli* O157:H7 (Fitzgerald *et al.* 2003; Looper *et al.* 2006a). Reduced dry matter intake is common in ruminants consuming E+ tall fescue (Paterson *et al.* 1995; Parish *et al.* 2003), and alterations in the diet may influence faecal shedding of pathogenic bacteria from ruminants (Callaway *et al.* 2003).

Studies have generally focused on faecal shedding of *E. coli* O157:H7 from grain-fed cattle due to the possibility of contaminated beef entering the human food supply (Elder *et al.* 2000; Smith *et al.* 2001); however, grazing cattle are also infected with *E. coli* O157:H7 (Laegreid *et al.* 1999; Riley *et al.* 2003; Dunn *et al.* 2004). Investigation of how consumption of E+ tall fescue influences the prevalence of *E. coli* O157:H7 from ruminants is necessary to reduce the number of animals shedding pathogenic bacteria on entering the feed-yard or processing facility. This review examines a series of recent experiments that our laboratories have conducted to investigate the influence of E+ tall fescue on shedding of *E. coli* O157:H7 from ruminants.

Methods

The Animal Care and Use Committees at the USDA-ARS, Food and Feed Safety Laboratory and (or) the USDA-ARS, Dale Bumpers Small Farms Research Center approved care, use, and handling of animals in the following experiments.

Influence of E+ tall fescue on *E. coli* O157:H7 from naturally-infected cattle

In our initial experiment, faecal samples were collected from Angus x Hereford cows (n=49) and their spring-born calves (n=45) grazing E+ tall fescue or endophyte-free (E-) tall fescue during summer. Our objectives were to examine 1) effects of forage type on shedding of *E. coli* O157:H7 from naturally-infected cattle, and 2) influence of *E. coli* shedding from the cow on the incidence of *E. coli* shedding from the calf. Faecal samples were collected at 0700 on each collection date (5 August and 26 August); mean ambient temperature and relative humidity at time of faecal collections were 27°C and 77%, respectively.

A second grazing experiment at two locations was conducted to compare the prevalence of faecal shedding of *E. coli* O157:H7 in growing beef cattle consuming different forage diets. At location 1, faecal samples were collected thrice from steers grazing either E+ tall fescue or common bermudagrass (*Cynodon dactylon;* CB). Steers grazing E+ tall fescue were confined to a dry-lot pen and fed CB hay *ad libitum* for 10 days following the E+ grazing period. At location 2, faecal samples were collected twice from steers grazing E+ or novel endophyte-infected [NE; HiMag tall fescue with strain 4 and marketed as the variety ArkPlus™ from 2002 to 2004 (West *et al.* 1998)].

Influence of selected ergot alkaloids on growth of *E. coli* O157:H7 *in vitro*

Three commercially available ergot alkaloids (dihydroergotamine, ergonovine, and ergotamine) that are found in E+ tall fescue were evaluated for their effects on the growth of two strains of *E. coli* O157:H7. A range of concentrations of each ergot alkaloid (10 pM, 1 nM, 100 nM, 10 μM, 1 mM and a methanol control) were added to pure and mixed ruminal fluid cultures of *E. coli* O157:H7.

Influence of E+ tall fescue seed on *E. coli* O157:H7 from experimentally-inoculated ewes

Ewes were acclimated to the experimental diet (cracked corn substituted for fescue seed) for 7 days before initiation of the experiment and then fed diets containing either high endophyte-infected (HI-E; 0.75 mg ergovaline/kg) or low endophyte-infected (LO-E; 0.11 mg of ergovaline/kg) tall fescue seed (50%, as-fed basis) for 7 days. Ewes were inoculated with antibiotic-resistant selected *E. coli* O157:H7 on day 1 of feeding treatment,

and faecal shedding of inoculated pathogens was monitored daily (day 2 to 6). On day 7, ewes were weighed, euthanased, and tissues and contents sampled from the ileum, caecum, and rectum for quantitative enumeration of *E. coli* O157:H7. Urine also was collected at euthanasation to determine total ergot alkaloid concentrations via immunoassay (Hill *et al.* 2000).

Bacterial culture and isolation

In all experiments, an immunomagnetic separation technique was used to isolate *E. coli* O157:H7. Faeces (10 g) were enriched in 90 ml of gram-negative broth containing vancomycin (8 µg/ml), cefixime (0.5 µg/ml), and cefsoludin (10 µg/ml). Following incubation for 6 hours at 37°C, 20 µl of anti-*E. coli* O157:H7 antibody-labeled paramagnetic beads (Neogen Corp., Lansing, MI, USA) were added to 1 ml volumes of the above enrichments, mixed and washed thrice in 1 ml of phosphate-buffered saline (PBS) with 0.05% Tween 20. Fifty microliters of the resulting suspension was plated on CHROMagar™ O157 (DRG International, Mountain Side, NJ, USA) plates (containing 2.5 µg/ml potassium tellurite) and incubated overnight at 37°C. Pink colonies exhibiting *E. coli* O157:H7 colony morphology were resuspended in PBS and confirmed as *E. coli* O157:H7 using the Reveal® microbial screening test (Neogen Corp., Lansing, MI, USA).

Results and Discussion

Influence of E+ tall fescue on *E. coli* O157:H7 from naturally-infected cattle

Overall, incidence of *E. coli* O157:H7 shedding averaged 8.4% and 7.6% for calves and cows, respectively. The percentage of cows positive for *E. coli* O157:H7 was lower (P<0.05) in cows grazing E+ than the percentage of cows grazing E- (1.8% vs 17% for E+ and E- cows, respectively). Likewise, the percentage of calves shedding *E. coli* O157:H7 tended (P=0.11) to be reduced in E+ calves (4.3%) compared with E- calves (13.9%). The presence of *E. coli* O157:H7 in the faeces of the cow did not influence (P>0.10) shedding of the bacterium from the calf. These preliminary results suggested that consumption of E+ tall fescue might reduce the incidence of faecal shedding of *E. coli* O157:H7 in naturally-infected cattle (Looper *et al.* 2003).

Sixteen percent of steers were positive for *E. coli* O157:H7 at location 1 in the second grazing experiment. Faecal shedding of *E. coli* O157:H7 was reduced in E+ tall fescue steers fed CB hay compared with steers continuously grazing CB at location 1. At location 2, the percentage of steers positive for *E. coli* O157:H7 was 3.3%. Consumption of E+ tall fescue did not influence faecal shedding of *E. coli* O157:H7 at either location; however, hay feeding of steers previously grazing E+ tall fescue did reduce the prevalence of *E. coli* O157:H7 (Looper *et al.* 2006b).

Prevalence of faecal shedding of *E. coli* O157:H7 from naturally-infected cattle in both experiments was highly variable ranging from 1.8% to 17%. Further, influence of E+ tall fescue on the prevalence of *E. coli* O157:H7 was inconsistent; however, altering the diet by feeding hay after grazing might be a possible management strategy to reduce fecal shedding of *E. coli* O157:H7 (Looper *et al.* 2006b).

Influence of E+ tall fescue seed on *E. coli* O157:H7 from experimentally-inoculated ewes

Ewes fed HI-E had lower (P<0.001) dry matter intake (DMI) than ewes fed LO-E. There was a tendency (P=0.06) for HI-E ewes to lose weight and LO-E ewes to gain weight during the experiment. Urinary ergot alkaloids were increased (P<0.001) in ewes fed HI-E compared with the LO-E ewes. Faecal shedding of *E. coli* O157:H7 tended (P=0.06) to be increased in HI-E ewes compared with LO-E ewes (Fig. 1). Population of *E. coli* O157:H7 in luminal contents from the ileum, caecum, and rectum did not differ (P>0.36) between treatments. Treatment did not influence (P=0.30) the occurrence of *E. coli* O157:H7 in caecal or rectal tissues; however, ileal tissues from HI-E ewes tended (P=0.12) to have an increased incidence of *E. coli* O157:H7.

Feeding HI-E tall fescue seed for 7 days induced signs of fescue toxicosis including decreased DMI, increased urinary concentrations of total ergot alkaloids, and reduced body weights. Ewes fed HI-E tall fescue seed diets tended to shed more antibiotic-resistant *E. coli* O157:H7 in their faeces and tended to have an increased incidence of antibiotic-resistant *E. coli* O157:H7 in their ileum than LO-E ewes (Looper *et al.* 2006a).

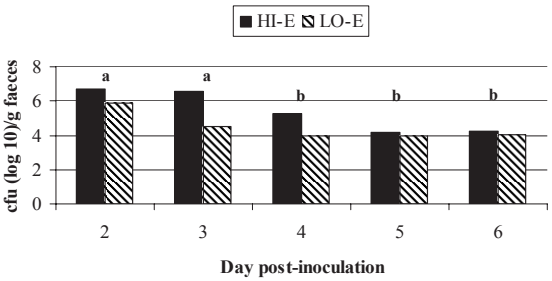
Influence of selected ergot alkaloids on growth of *E. coli* O157:H7 *in vitro*

No differences (P>0.10) were found in growth rates in pure culture of *E. coli* O157:H7 when comparing increasing concentrations of the same ergot alkaloid or when comparing different ergot alkaloids. Similarly, increasing ergot alkaloid concentrations had no effect (P>0.10) on colony-forming units for *E. coli* O157:H7 after incubation in mixed ruminal fluid. There was minimal, if any, effect on the growth of *E. coli* O157:H7 from these three ergot alkaloids. Ergot alkaloids, at the various concentrations used in this experiment would have included the range of concentrations that occur in the rumen of cattle grazing E+ tall fescue. Other factors such as different ergot alkaloids (i.e. ergovaline), exposure to a combination of alkaloids, characteristics of the grazed forage, or management strategies may influence faecal shedding of *E. coli* O157:H7 in cattle grazing E+ tall fescue (Looper *et al.* 2004). Currently, experiments are being conducted that include the alkaloid, ergovaline, as well as combinations of these four alkaloids to more closely mimic the whole plant exposure that grazing ruminants would experience.

Conclusions

Differences in faecal shedding patterns between experimentally-inoculated animals and naturally infected animals may explain variations among our studies. Shedding of *E. coli* O157:H7 in naturally-infected animals is sporadic, with an animal testing positive at one sample collection and negative at the subsequent collection (Callaway *et al.* 2004). Length of exposure to the toxic effects of E+ tall fescue also may attribute to differences between studies. Ewes were exposed to a diet containing E+ seed for 7 days (Looper *et al.* 2006a) while cattle utilised in our grazing experiments (Looper *et al.* 2003, 2006b) were exposed to E+ tall fescue from 87 days to approximately 2 years. It is possible that animals may become acclimated to the toxic effects of fescue after prolonged exposure (Spiers *et al.* 2005). Differences in diets (total mixed ration vs grazing) also may have contributed to differences in faecal shedding among experiments. Sudden alterations in diet, specifically a shift from concentrate to forage feeding may decrease pathogen shedding (Callaway *et al.* 2003) which is similar to our results (Looper *et al.* 2006b). Possible differences in the pattern of faecal shedding between cattle and sheep also may have influenced results within studies. Management strategies that prevent livestock from grazing E+ tall fescue and (or) alleviate dietary stressors (i.e. reduced DMI) associated with consumption of E+ tall fescue before entry into the feed-yard or harvest facility may reduce faecal shedding of pathogenic bacteria from livestock.

Figure 1 Faecal shedding (colony-forming units, cfu (log_{10})/g faeces) of *E. coli* O157:H7 from ewes 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 diet effect (P=0.06; standard error=0.30); [a,b]day effect (P<0.001); treatment x day interaction (P=0.18) (Looper *et al.* 2006a).



ACKNOWLEDGEMENTS

The authors gratefully acknowledge L. Huddleston, W. Jackson, G. Robson, and D. Jones, USDA-ARS, for technical assistance and daily animal management; and D. Kreider, M. Lamb, S. Reiter, and Feed Mill personnel, University of Arkansas, for technical assistance.

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