Dietary Impact on Antibiotic Resistance in Feedlot Manure

Mara Zelt
Amy Millmier Schmidt
Noelle Mware
Xu Li
Galen Erickson

Summary with Implications

There is a growing public concern regarding antibiotic resistance and the use of antibiotics, including in livestock management. Understanding the ecology of antibiotic resistance among microbes, identifying resistance gene reservoirs, and implementing antibiotic resistance mitigation practices in livestock production are critical to protecting animal and human health while meeting increasing food demands. This research is one of several studies seeking to assess risk for livestock-to-human transfer of antibiotic resistance and to identify mechanisms for reducing that risk where possible. This study evaluated the impact of forage concentration and supplemental essential oil in beef cattle finishing diets on antibiotic resistance in freshly excreted and consolidated beef feedlot manure. Results indicate that antibiotic resistance in manure was not impacted by either of the two dietary treatments considered.

Introduction

Antibiotics are widely used in agricultural livestock production and human medicine for the treatment of infectious diseases. However, the use of antibiotics applies selective pressure to the gut microbiome of animals and humans, resulting in excretion of antibiotic resistant (AR) bacteria in animal and human feces. The wide spread use of animal manures as fertilizers in agricultural production has resulted in growing concerns about the potential risks of antibiotics, AR bacteria and AR genes present in animal manures and their impact on public, animal, and environmental health.

Forage is included in feedlot diets to improve microbial protein synthesis in the gut but inclusion is minimized because the economic gains from improved ruminal health do not generally outweigh the losses due to a lower average daily weight gain. However, the documented benefits of forage on the ruminal microbiome suggest that increasing forage in finishing diets could reduce AR development in the animals, thereby influencing potential AR-related food safety and environmental exposure risks to people. Essential oils are believed to possess strong antimicrobial effects, suggesting that the addition of essential oils to animal feed may be a viable alternative to antibiotics in animal feed and a means to prevent the development AR in the animal gut.

The objectives of this study were to quantify the effect of essential oil and forage concentration in beef finishing diets on the concentrations of four AR bacterial populations important to human and animal health—azithromycin (AZ)- and tetracy-

Table 1. Effect of essential oil and silage concentration on proportion of *E.coli* resistant to azithromycin or tetracycline in freshly excreted manure and pen surface material

	Fresh Manure		Pen Surface Material	
Variable	AZ^R *E. coli*/ Total *E. coli*	TET^R *E. coli*/ Total *E. coli*	AZ^R *E. coli*/ Total *E. coli*	TET^R *E. coli*/ Total *E. coli*
Essential Oil	P = 0.087	P = 0.148	P = 0.579	P = 0.723
Yes	0.68	0.25	0.74	0.21
No	0.72	0.20	0.75	0.19
Forage Conc.	P = 0.459	P = 0.003	P = 0.743	P = 0.041
80%	0.72	0.21 [b]	0.76	0.15 [a]
47%	0.69	0.18 [a]	0.74	0.25 [b]
14%	0.69	0.17 [a]	0.73	0.19 [ab]

Table 2. Effect of essential oil and silage concentration on proportion of *Enterococci* resistant to tetracycline or tylosin in fresh manure and pen surface material

	Fresh Manure		Pen Surface Material	
Variable	TET^R *Enterococci*/ Total *Enterococci*	TY^R *Enterococci*/ Total *Enterococci*	TET^R *Enterococci*/ Total *Enterococci*	TY^R *Enterococci*/ Total *Enterococci*
Essential Oil	P = 0.622	P = 0.133	P = 0.450	P = 0.185
Yes	0.52	0.94	0.73	0.89
No	0.52	0.94	0.72	0.87
Forage Concentration	P = 0.073	P = 0.519	P = 0.686	P = 0.357
80%	0.08	0.75	0.23	0.55
47%	0.11	0.74	0.23	0.58
14%	0.22	0.68	0.27	0.53

cline (TET)-resistant *Escherichia coli* and tylosin (TY)- and TET-resistant *Enterococci spp*.—in freshly excreted manure and consolidated pen surface material from a beef feedlot operation.

Procedure

This study was conducted at the Eastern Nebraska Research and Extension Center (ENREC), near Mead, NE. Four-hundred, twenty beef cattle were assigned to 42 pens with each pen assigned randomly to one of six treatments: feed containing 14%, 47% or 80% corn silage with or without essential oil supplement. The remainder of the diet consisted of dry-rolled corn, 16% wet distillers' grains, monensin (30 g/ton), and tylosin (Tylan®) (90 mg/steer/day). Samples of freshly-excreted cattle manure and consolidated feedlot surface material from two areas of each pen—near waterers and at the backs of pens—were retrieved from each pen four times (February through June) during the finishing period. Samples were spiral-plated in duplicate on agar to select for four types of antibiotic resistant bacteria: azithromycin (AZ^R)- and tetracycline (TET^R)-resistant *Escherichia coli* and tylosin (TY^R)- and TET^R-resistant *Enterococci*. Colony-forming units per gram of sample were enumerated by manual plate counting.

Results and Discussion

Examination of the ratio of AR bacteria to total bacterial concentration (Table 1 and Table 2) reveals that the concentration of TY^R *Enterococci* and AZ^R *E.coli* were quite high relative to the measured total concentration of each bacteria in samples throughout this study. These high concentrations are not surprising given that the animals were fed tylosin, which suggests that bacteria with resistance to tylosin would have had an advantage over other bacteria. Perhaps more surprising is that AR bacteria were present in all the manure samples collected in this study, including bacteria that were resistant to antibiotics not administered to the animals (tetracycline) indicating either a certain degree of baseline resistance must be expected or an environmental selection for tetracycline resistance not directly related to antibiotic use.

When the impact of dietary forage concentration was averaged for both presence and absence of essential oils TET^R *E.coli* showed significant (α=0.05) differences due to forage concentration in both the freshly excreted manure and pen surface material (Table 1). In freshly excreted manure the mean ratio of TET^R *E.coli* was lower in manure samples from pens where cattle received a 14% forage diet and the highest bacterial concentrations in manure from cattle receiving a 80% forage diet. However in consolidated pen surface material the mean ratio of TET^R *E.coli* was lowest in samples from pens where cattle received an 80% forage diet and highest in samples from cattle receiving a 47% forage diet, the 14% diet was not significantly different from either of the two higher concentration diets. The results of this study indicate that a beef cattle finishing diet low in dietary forage concentration produces the same effect on AR bacteria concentrations in manure as high forage, and in one population (TET^R *E.coli* in pen fresh manure) a low dietary forage concentration was the most effective for reducing AR in manure.

Inclusion of a proprietary blend of essential oils to the finishing diets of cattle in this study did not impact any of the AR bacterial concentrations in freshly excreted manure or consolidated feedlot pen surface material (Table 1 and 2).

Implications/Conclusions

The results of this research indicate that beef finishing diets with low silage concentrations (14%) are equally or more effective than diets with higher silage concentrations for reducing AR bacteria concentrations in manure. The presence of bacteria resistant to antibiotics not given to the animals during the study also indicates that co-selection for multiple resistances inside the animal's digestive tract or environmental factors at the feedlot may have more impact on AR in manure than dietary treatments. Furthermore, because there was little impact by dietary changes on AR bacteria in manure, it will be important to continue to examine manure treatment, storage and application strategies that may mitigate potential human health risks from manure-borne AR bacteria.

Acknowledgments

Funding for this research was provided by USDA-NIFA Award No. 2017-68003-26497.

We wish to extend our gratitude to Dr. Bing Wang, Linda Schott, Erin Stevens, Ece Bulut, Eric Henning, Autumn Dunn, Bridget Gile and Zhe Zhang for help with sample collection, preparation and analyses.

Mara Zelt, research associate, Biological Systems Engineering, University of Nebraska–Lincoln

Amy Schmidt, associate professor Biological Systems Engineering and Animal Science, University of Nebraska–Lincoln

Noelle Mware, graduate student, Civil Engineering, University of Nebraska–Lincoln

Xu Li, professor, Civil Engineering, University of Nebraska–Lincoln

Galen Erickson, professor, Animal Science, University of Nebraska–Lincoln