

Project Title:	The Use of a Yeast Fermentation Product or an Essential Oil as an Alternative to Tylosin in the Diets of Finishing Cattle
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Background

The use of medically important antimicrobial drugs in food producing animals is often linked to the development of antimicrobial resistance. As such, there is increasing pressure to reduce and remove these drugs from animal production practices. Tylosin, a macrolide antibiotic, is commonly used by the beef industry as metaphylaxis to treat and prevent liver abscesses in feedlot cattle. Over 70% of cattle in large feedlots are supplemented with Tylosin during the finishing period. The reduction of liver abscesses in feedlot cattle is significant, as they are a leading cause of liver condemnation and result in large financial losses through reduced animal performance and carcass value. As efforts to reduce antimicrobial use in beef production continue, alternatives to Tylosin must be explored. The addition of *Saccharomyces cerevisiae* fermentation products (SCFP) and essential oils to finishing diets are two potential alternatives—both of which act by altering the rumen microbial communities and plausibly reduce the incidence of liver abscesses. However, little information regarding effects of either product on the reduction of liver abscesses, the microbial community, or the presence of pathogens exists. Furthermore, to our knowledge, the effect of Tylosin supplementation on the prevalence of *Salmonella* within the lymph node has not been studied, although lymph node contamination is a significant challenge for the industry. Overall, it is imperative that the benefits to cattle performance and reduced liver abscesses afforded by Tylosin supplementation not be lost when alternatives are used. Similarly, it is of utmost importance that any alteration to current feeding strategies not result in deviations to pathogen presence or the safety of beef products.

Given the need for Tylosin alternatives and the potential benefits of including SCFP or essential oils in feedlot rations, two separate clinical trials were performed to assess the influence of Tylosin alternatives on feedlot beef cattle.

In Experiment 1, a clinical trial was performed to determine the impact of Tylosin and Tylosin alternatives on the performance, liver abscess prevalence, microbiome, and pathogen presence in feedlot cattle and derived samples.

Specifically, the objectives of this experiment were to determine the impact of Tylosin and Tylosin alternatives on:

- Prevalence of liver abscesses;
- Microbial communities of feces, liver abscesses, carcasses, beef trimmings, and related tissues, such as subiliac lymph nodes.

For Experiment 2, a clinical trial was performed to assess the efficacy of a SCFP prototype on beef cattle raised for natural-branded beef products. The objectives of this study were to evaluate the effects of SCFP supplementation on:

- Prevalence of liver abscesses;
- Microbial community of cattle feces and liver abscess microbial communities;
- Fecal resistome

Methodology

In Experiment 1, cattle were sorted into ten, 4-pen blocks. Each pen within a block was assigned to one of four treatment groups. Treatment groups were all fed corn-based rations with one of the following modifications: a) Tylosin (90 mg/head/day, Elanco Animal Health, Indianapolis, IN) fed until harvest; b) no Tylosin; c) without Tylosin, but with an essential oil (1 g/head/day of CRINA-L; source of limonene) fed until harvest; and d) without Tylosin but with a *Saccharomyces cerevisiae* fermentation product (18 g/head/day) fed until harvest.

In Experiment 2, cattle were sorted into 14, 2-pen blocks. Each pen within a block was assigned to one of two treatment groups: SCFP (18 g/head/day) mixed into the finishing ration and a control group consisting of the base ration.

Composited pen-floor fecal samples were collected at the time of cattle placement (Experiment 1) and composite fecal samples were collected prior to cattle shipment for harvest for both Experiments. At the time of harvest, liver abscess prevalence was scored and liver abscesses were collected for later characterization of the microbial community. Additionally, in Experiment 1, carcass swabs, subiliac lymph nodes (SLN), and beef trimmings were collected. Samples were subjected to cultural and genomic investigations. Specifically, traditional culture methodologies were used to detect, isolate, and characterize *Enterococcus* (fecal only), *Escherichia coli* (fecal only), and *Salmonella enterica* (fecal and SLN), as well as anaerobic liver abscess organisms. Additionally, samples were analyzed to assess changes in the microbial communities and antimicrobial, metal, and biocide resistance genes. Microbial communities were assessed using next-generation sequencing technologies.

Findings

In Experiment 1, treatment group had largely no effect ($P > 0.05$) on the microbial populations of feces, liver abscesses, whole carcass, or beef trimmings. Overall, 84.6% of the lymph nodes were positive for *Salmonella*. However, the prevalence of *Salmonella enterica* within the SLN did not differ ($P = 0.8402$) among treatment groups, nor did the microbiome of the SLN differ among treatment groups ($P = 0.31$). Although there were no differences in microbial populations, cattle in Treatment group 1 were less likely ($P = 0.0036$) to develop liver abscesses. The fecal resistome comprised 0.17% of the entire microbiome, with 99.72% of the resistome displaying resistance to antimicrobial drugs, particularly tetracycline ribosomal protection proteins.

In Experiment 2, liver abscess prevalence was not different between treatment groups ($P = 0.82$). Similarly, no differences in microbial composition or diversity existed between

treatment groups; however, the microbiome and resistome were influenced by block. The most common phyla within cattle feces were Firmicutes, Bacteroidetes, Tenericutes, Proteobacteria, and Spirochaetes. The most common phyla in liver abscesses were Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, and Fusobacteria. There was a correlation between high ratios of Firmicutes to Bacteroidetes with increased liver abscess prevalence, but this did not differ between treatment groups. We detected 151 unique resistance genes in cattle feces. The most abundant drug classes of antibacterial resistance in these natural cattle conferring resistance to tetracyclines ribosomal protection proteins and macrolide resistance efflux pumps.

Implications

Liver abscesses result in significant economic losses in the beef industry. Although the inclusion of Tylosin in finishing rations is effective, efforts to reduce antimicrobial use demand the identification of alternatives. In that regard, the Tylosin alternatives utilized in either of these clinical trials had little impact on liver abscess rates, fecal pathogen prevalence, lymph node *Salmonella* prevalence, microbial communities or resistance gene profiles. As alternatives to Tylosin continue to be explored, it is imperative that the alternatives not only effectively reduce liver abscesses, but also do not negatively alter the microbial populations in the beef continuum—particularly with regard to the presence of pathogens. The information gained in this study will assist in the further identification of alternatives which have the desired benefits to cattle performance and liver abscess rates, but do not sacrifice beef quality or safety.

Table 1. The influence¹ of supplementation with Tylosin (90 mg/head/day), an essential oil (1 g/head/day), or a *Saccharomyces cerevisiae* fermentation byproduct (18 g/head/day) on the prevalence of liver abscesses in feedlot cattle (Exp 1).

Treatment	Liver Characteristics		
	Abscessed Livers	Total Livers Scored	Mean % Abscessed
Trt 1	212	1353	14.97 ^a
Trt 2	311	1359	23.72 ^b
Trt 3	314	1349	24.48 ^b
Trt 4	313	1354	22.32 ^b

^{a,b} Within column, mean values with the same letter do not differ.

¹ P-value: 0.0036; SEM = 1.879

Table 2. The impact of pre-harvest feeding strategies on the prevalence of *Salmonella enterica* in the subiliac lymph nodes of feedlot beef cattle (Exp1).

Treatment	Percentage (%) of Subiliac Lymph Nodes positive for <i>Salmonella enterica</i>
Tylosin	86.00
No Tylosin	83.33
Treatment 3	86.67
Treatment 4	82.00

¹ Percentage did not differ among treatment groups ($P = 0.8402$).



Table 3. Liver abscess prevalence by grade and treatment (Exp 2).

Abscess Grade	Treatment 1 (n = 2,171)			Treatment 2 (n = 2,153)		
	Number	Prevalence	95% CI	Number	Prevalence	95% CI
No abscess	1326	61%	59 to 63%	1,335	62%	36 to 40%
A minus	382	18%	16 to 19%	375	17%	16 to 19%
A	122	6%	5 to 7%	106	5%	4 to 6%
A plus	341	16%	14 to 17%	337	16%	14 to 17%
Total Abscess	845	39%	37 to 41%	818	38%	36 to 40%