Heavy metals, essential oils, and β-adrenergic agonists as alternatives to antibiotics and their impacts on bacterial resistance in beef cattle


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Abstract

- Sub-therapeutic antibiotics have been used in animal agriculture to increase feed efficiency, promote growth, and prevent disease.
- Public health risks relating to the global rise of antimicrobial resistance have led to research on alternative feed supplements, including heavy metals, essential oils, and β-adrenergic agonists.
- We aimed to measure the impact of antibiotic alternatives by investigating the effects of zinc, menthol, and ractopamine on antimicrobial resistance among commensal enteric bacteria of cattle in two trials.
- Escherichia coli and Enterococcus spp. are used as indicator organisms by the U.S. National Antimicrobial Resistance Monitoring System (NARMS) to track Gram negative and Gram positive bacterial resistance patterns respectively.
- In the first trial, E. coli log10 CFU on plan MacConkey significantly decreased with period effects alone, independent of treatment.
- In the second trial, ractopamine exhibited no associations with either endpoint, whether as a main effect or modifier.
- In both trials, elevated levels of zinc were associated with increased relative and absolute levels of E. coli tetracycline resistance on day 21 (mid-trial) as measured both via log10 CFU and resistance prevalence among isolates.
- Similar studies are ongoing for Enterococcus faecium

Methods

Objectives

- The objectives of this study:
  - Examine the impacts of supra-nutritional zinc, menthol, and ractopamine as alternatives to antibiotics
  - Further understand the mechanisms for distribution and co-selection of resistance factors

Feeder Cattle Trial 1

- **Trial Design**
  - 2x2 full factorial design, factors being zinc (300 ppm) and menthol (0.39%)
  - Sample collection from 80 feeder cattle every 7 days over a 35 day period, with day 21 being the height of treatment
  - Feces collected on day 0 and day 21 analyzed

- **Microbiological methods**
  - Feces stored in 5 ml tubes, with and without glycerol at -80C
  - 1 g feces suspended in 5 ml PBS
  - Suspension plated on MacConkey, MacConkey with 16mg/L tetracycline, MacConkey with 4 mg/L cefoxitane
  - Colony counted using Flash & Go®
  - Two magenta colonies picked from plain MacConkey plate
  - Confirmed as E. coli with indole test
  - Preserved on cryobeads for further analysis
  - Phenotypic MIC obtained on each isolate using TREK Sensititre® system

- **Statistical approaches**
  - Descriptive statistics
  - Multivariate analyses
  - Multi-level mixed linear regression models
  - Ordinal logistic regression (MDR count 0, 1, 2, 3, 4+)

Feeder Cattle Trial 2

- **Trial Design**
  - 2x2 full factorial design, factors being zinc (100 ppm) and ractopamine (200 mg/head/day)
  - Sample collection from 104 feeder cattle every 7 days over a 42 day period, with day 21 being the height of treatment for zinc while ractopamine was continued through the trial
  - Feces collected on day 0, 21 and 42 analyzed

- **Microbiological Methods**
  - E. coli isolated using same methods as above
  - One isolate from each plain MacConkey plate
  - Preserved on cryobeads for further analysis

Finisher Cattle Trial 2

- **Trial Design**
  - 2x2 full factorial design, factors being zinc (100 ppm) and ractopamine (200 mg/head/day)
  - Sample collection from 104 finisher cattle every 7 days over a 42 day period, with day 21 being the height of treatment for zinc while ractopamine was continued through the trial
  - Feces collected on day 0, 21 and 42 analyzed

- **Microbiological Methods**
  - E. coli isolated using same methods as above
  - One isolate from each plain MacConkey plate
  - Phenotypic MIC obtained on each isolate using TREK Sensititre® System
  - Sensititre performed on isolates from day 21 and 42

Statistical approaches

- Descriptive statistics
- Multivariate analyses
- Multi-level mixed linear regression models
- Multi-level mixed logistic regression models (single resistance endpoints and MDR count 1-3)
- Ordinal logistic regression (MDR count 0, 1, 2, 3, 4+)

Results

- Feeder Cattle Trial 1
  - E. coli log10 CFU on plan MacConkey significantly decreased with period effects alone, independent of treatment
  - No major effects on coliform counts
  - Some evidence of effects of zinc on tetracycline, streptomycin, sulfisoxazole and chloramphenicol
  - No effects on menthol on resistance of E. coli

- Finisher Cattle Trial 2
  - Elevated levels of Zn by itself is associated with increased levels of tetracycline resistance on day 21.
  - Percentage of pan-susceptible isolates decreases.
  - Percentage of MDR and single-resistance isolates increases from day 0 to day 21.
  - Percentage of pan-susceptible isolates decreases.

Discussion

- Exploring the MDR genotypes and examining Zn-gene presence is needed
- Similar work ongoing for Enterococcus faecium

Future Work

- Exploring the MDR genotypes and examining Zn-gene presence is needed
- Similar work ongoing for Enterococcus faecium

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