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 plain 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*

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

Methods

- Feeder Cattle Trial 1
- Trial Design
- 2x2 full factorial design, factors being zinc (300 ppm) and menthol (0.3%)
- 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 9 ml PBS
- Suspension plated on MacConkey, MacConkey with 16mg/L tetracycline, MacConkey with 4 mg/L ceftriaxone
  - 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

Figure 1. Individual cattle pens at Kansas State Beef Cattle Research Center

Figure 2. Lactose-fermenting presumptive *E. coli* on MacConkey agar

Figure 3. Positive indole test confirmation of *E. coli*

- 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

Figure 4. Group cattle pens at Kansas State Beef Cattle Research Center

Figure 5. Sensititre® plate post inoculation and incubation. Accumulation in well indicates resistance

Figure 6. TREK Autoinoculator ®system

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

Results

- Feeder Cattle Trial 1

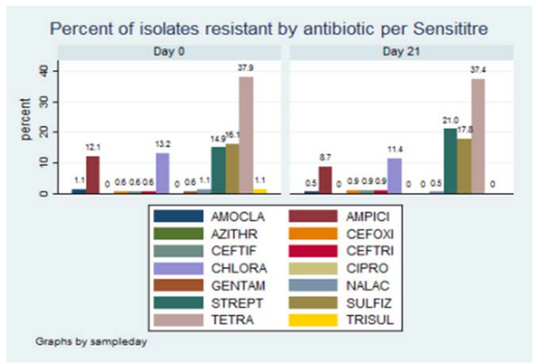

Figure 7. Figure shows the distribution of antibiotic resistance by sample day as determined by Sensititre. The percentage of isolates resistant to ampicillin, cefoxitin, ceftiofur, ceftriaxone, streptomycin, and sulfisoxasole increases.

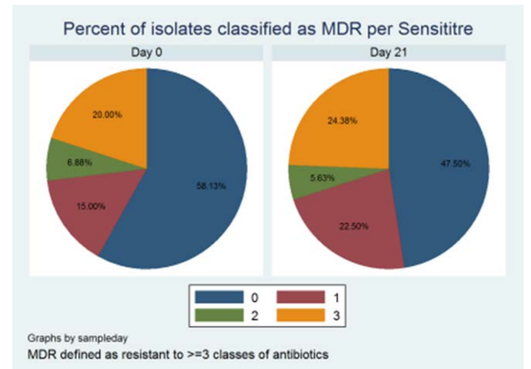

Figure 8. Figure shows the distribution of isolates classified as multi-drug resistant (MDR) as determined by Sensititre susceptibilities. The percentage of MDR and single-resistance isolates increases from day 0 to day 21. Percentage of pan-susceptible isolates decreases.

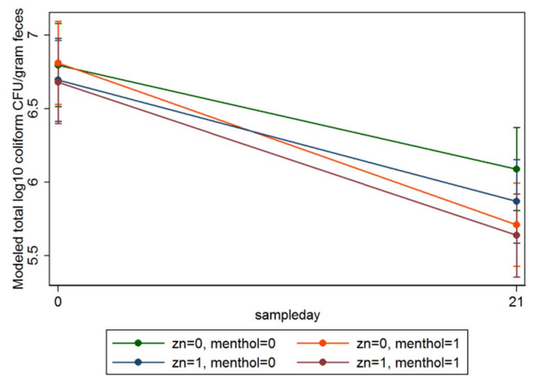

Figure 9. Figure shows the predicted means for log10 CFU of *E. coli* on plain MacConkey agar. The growth of *E. coli* significantly decreased independent of treatment group.

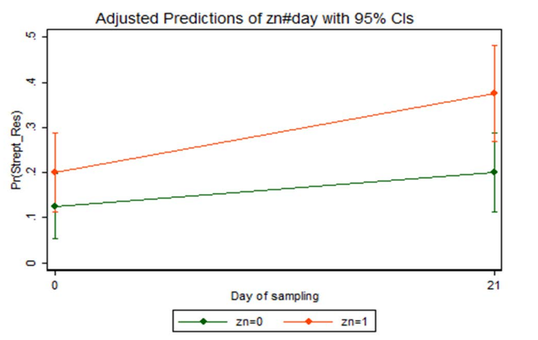

Figure 10. Figure shows the predicted means for isolates from zinc treatment group resistant to streptomycin. The resistance to streptomycin moderately increases, indicating possibility of co-selection with zinc that should be further explored.

- Finisher Cattle Trial 2

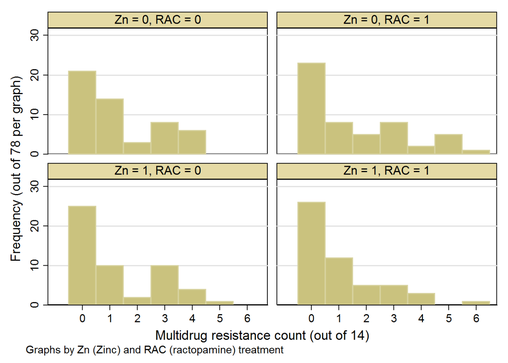

Figure 11. Figure shows the distribution of multi-drug resistant (MDR) isolates by treatment group. Frequency of MDR isolates increases in the zinc treated group

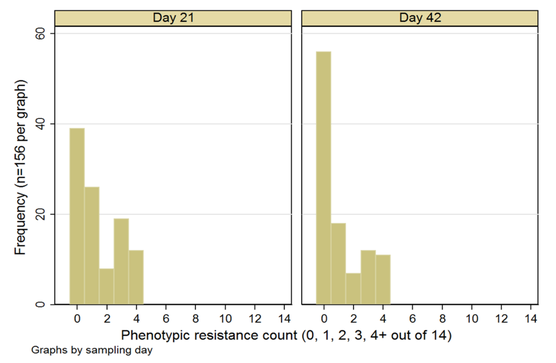

Figure 12. Figure shows the distribution of multi-drug resistant (MDR) isolates by day. Frequency of MDR isolates decreases from day 21 to day 42, while frequency of pan-susceptibile isolates increases.

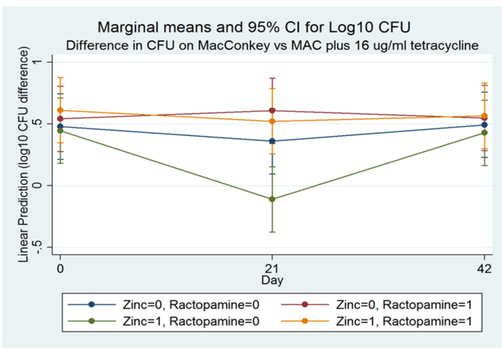

Figure 13. Figure shows the predicted means of log10 cfu for *E. coli* for the difference between plain MacConkey and MacConkey with tetracycline. Graph shows zinc treatment group is associated with increased levels of tetracycline resistance

Discussion

Feeder Cattle Trial 1

- Overall *E. coli* log10 CFU on plain MacConkey significantly decreased with period effects alone (day, independent of treatment)
- No major effects on coliform counts
- Some evidence of effects of zinc on tetracycline, streptomycin, sulfisoxazole and chloramphenicol
- No effects of menthol on resistance of *E. coli*

Finisher Cattle Trial 2

- Generally, ractopamine showed no associations with either endpoint (quantitative or prevalence) as main effect or modifier
- Elevated levels of Zn by itself are associated with increased relative and absolute levels of tetracycline resistance on day 21

Future Work

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

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