Effects of zinc, copper, oregano oil and ractopamine as antibiotic alternatives on bacterial resistance and growth performance in swine

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Abstract

- Feedgrade antibiotics have been used in food animals since 1950s for growth promotion and prophylaxis.
- A worldwide increase in antibiotic resistance has prompted the exploration of alternatives to such uses in the form of heavy metals, essential oils and β-adrenergic agonists, among others.
- Our first trial on weaned piglets was arranged in a 2x2x2 complete factorial design, factors being copper (Cu), zinc (Zn), and oregano oil (OREG), and included reference diets of chlortetracycline (CTC: 22mg/kg prophylactic dose and 4mg/kg sub-therapeutic dose) for comparison purposes.
- A 2nd trial in finisher pigs was arranged in a 2x2x2 complete factorial design: Cu, Zn, & ractopamine (RAC)
- Fecal samples were collected every seven days during each study period. Quantitative and qualitative microbiological assays were performed (see methods section) on *E. coli* and *Enterococcus* spp (in progress).
- Standard growth performance and feed efficiency parameters also were recorded.
- The results of the first study did not show any significant associations between antibiotic alternatives (Cu, Zn and OREG) and bacterial resistance, with the exception of copper, which showed a strong negative association with multi-drug resistant phenotypes (including to 3rd generation cephalosporins).
- CTC and Cu added separately to the piglets' diet each showed reduction in ceftriaxone resistance, possibly tied to associations of chromosomal *pco*B and *tet*(B) genes versus plasmid-borne tet(A) and bla_{CMY-2}.
- No beneficial production effects in weaned pigs were observed for essential oils; however, other alternatives including Cu, Zn and ractopamine as well as CTC (antibiotic) improved the growth rate of nursery pigs. Carryover effects on subsequent nursery growth performance were minimal.
- The second study demonstrated that copper or zinc, alone or in combination with ractopamine, did not improve average daily gain in pigs; however, inclusion of ractopamine alone improved carcass leanness as well as feed efficiency while not measurably affecting antimicrobial resistance among *E. coli*.
- Generally speaking, alternatives to antibiotics that we assessed in two swine production stages did not demonstrably increase the risk of antibiotic resistance among coliform bacteria.
- Statistical power was limited for observing differences in any health or disease outcomes; none were noted.

Objectives

- We investigated the effects of zinc, copper, oregano oil and ractopamine in different swine production stages (as appropriate), as alternatives to growth promotion and prophylactic in-feed antibiotics.
- These two trials involved nursery and grower pigs, respectively, in full factorial experimental study designs.

Methods

Nursery Pig Trial 1

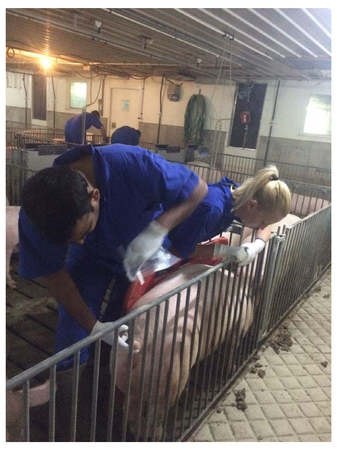
Trial design

- N=350 21 day old commercially derived nursery piglets.
- 5 piglets were randomly assigned to a pen; each pen was randomly assigned a basal swine diet with one of the 10 treatments: control group, copper, zinc, oregano oil, copper+zinc, copper+oregano oil, zinc+oregano oil, copper+zinc+oregano oil, high CTC, low CTC. There were 7 replicate pens per treatment.
- 49 day trial with 7 day acclimation period; feces collected weekly; analyzed at day 0 and day 28.
- Growth and feed efficiency data collected.

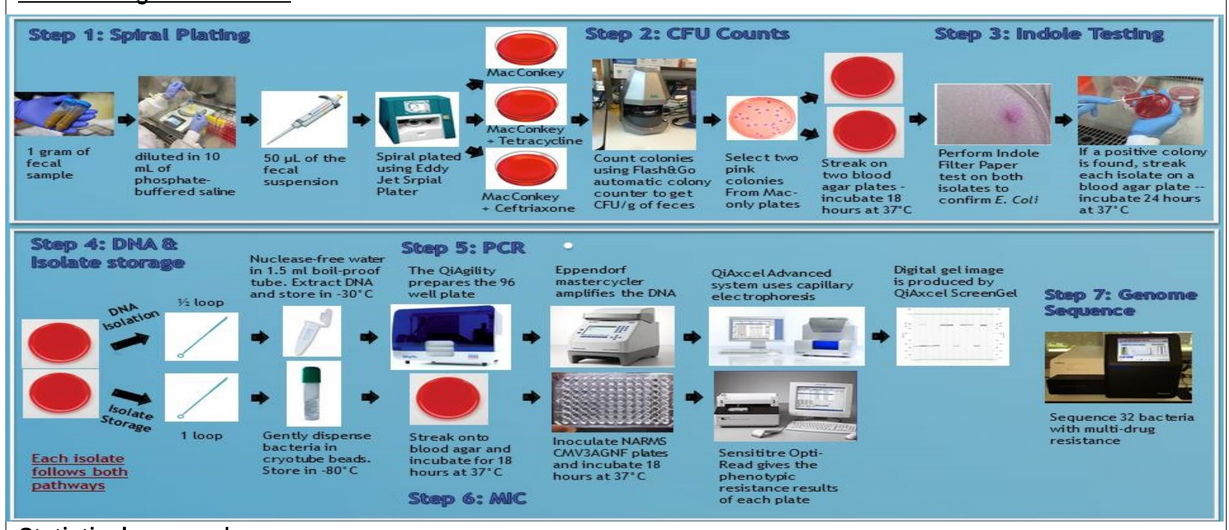
Finisher Pig Trial 2

Trial design

- N=480 (PIC 327 × 1050; initially 50 kg) pigs were used to determine the interactive effects of supplemental Cu, Zn, and Ractopamine HCl on finishing pig growth performance, carcass characteristics, and antimicrobial susceptibility of enteric bacteria.
- Dietary treatments were arranged in a 2 × 2 × 2 factorial with main effects of added copper sulfate (CuSO4; 0 vs. 125 ppm Cu), added zinc oxide (ZnO; 0 vs. 150 ppm Zn), and Ractopamine HCl (0 vs. 10 ppm during the last 28 d prior to marketing; Paylean®; Elanco Animal Health, Greenfield, IN).
- 83-90 day trial with acclimation period; feces collected as baseline and right before harvest.
- Growth and feed efficiency data collected.



Microbiological methods



Statistical approaches

- Descriptive statistics
- Multivariable analyses
  - Multi-level mixed linear regression models (log10 CFU)
  - Multi-level mixed logistic regression models (single resistance endpoints and MDR count >=3)
  - Ordinal logistic regression (MDR count = 0, 1, 2, 3, 4+)

Results

Nursery Pig Trial 1

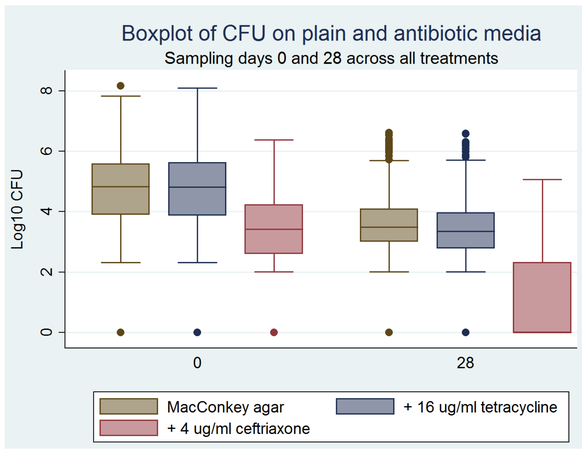


Figure 1. Boxplots showing the distribution of colony forming units (CFU) for all three media on Day 0 and Day 28.

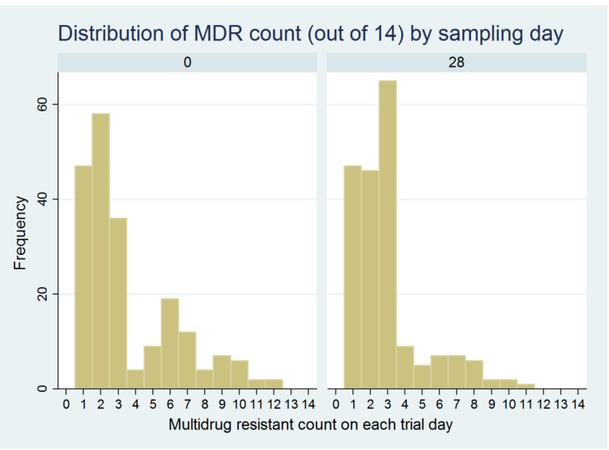


Figure 2. Bar graph that shows the phenomenon of a drop in median resistance count as the pigs age.

NARMS Code	Antimicrobial	Isolates (n)	% Resistant	L95%	U95%	Distribution of MICs in ug/ml (%)													
						0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512
AUG	Amoxicillin/Clavulanic Acid*	403	15.1	11.6	18.7							0.0	3.7	45.2	34.7	1.2	1.7	13.4			
AMP	Ampicillin	403	44.7	39.8	49.5							0.4	23.6	27.5	3.5	0.2	0.2	44.4			
AZI	Azithromycin	403	2.2	0.8	3.7							1.5	38.4	50.9	5.7	1.2	2.2				
FOX	Cefoxitin	403	14.9	11.4	18.4						0.0	0.0	20.6	51.1	12.9	0.5	2.0	12.9			
XNL	Ceftiofur	403	18.4	14.6	22.2				0.2	30.0	51.1	0.5	0.0	0.0	5.7	12.7					
AXO	Ceftriaxone	403	18.4	14.6	22.2					80.1	0.5	1.0	0.0	0.2	3.5	11.2	0.5	0.0	3.0		
CHL	Chloramphenicol	403	13.6	10.3	17.0								0.2	25.3	58.6	2.2	5.7	7.9			
CIP	Ciprofloxacin	403	5.7	3.4	8.0	89.1	2.7	0.2	0.0	2.2	0.0	1.5	0.0	0.0	4.2						
GEN	Gentamicin	403	10.2	7.2	13.1					0.2	26.1	58.6	4.0	1.0	0.0	5.0	5.2				
NAL	Nalidixic Acid	403	6.5	4.0	8.9						0.0	3.2	69.0	20.3	1.0	0.0	0.0	6.5			
STR	Streptomycin	403	42.9	38.1	47.8								0.0	12.7	25.6	14.7	4.2	16.7	26.3		
FIS	Sulfisoxazole	403	35.7	31.0	40.4											56.3	7.4	0.0	0.0	0.5	35.7
TET	Tetracycline	403	99.5	98.8	1.0									0.1	0.0	0.2	2.5	96.8			
SXT	Trimethoprim/Sulfa*	403	2.0	0.6	3.4				69.0	16.3	12.2	0.5	0.0	0.2	1.7						

Table 1. This table shows the distribution of MIC's for 14 antimicrobials tested. White areas indicate the dilution range of the Sensititre® plate used to test isolates. Red vertical bars indicate the CLSI resistance breakpoint when available or else NARMS consensus breakpoint. Yellow highlighting indicates the growth beyond the CLSI breakpoint (or the NARMS consensus breakpoint). Numbers indicate the number of bacteria that grew in each concentration of antibiotic.

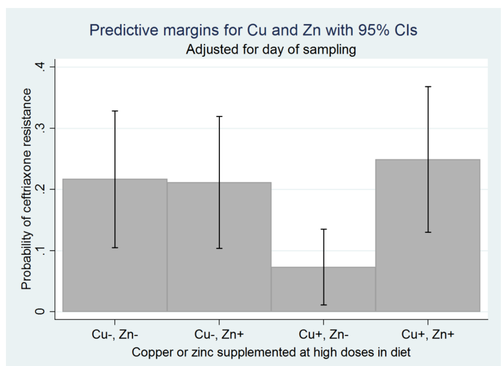


Figure 3. Prevalence of ceftriaxone resistance for Cu &/or Zn

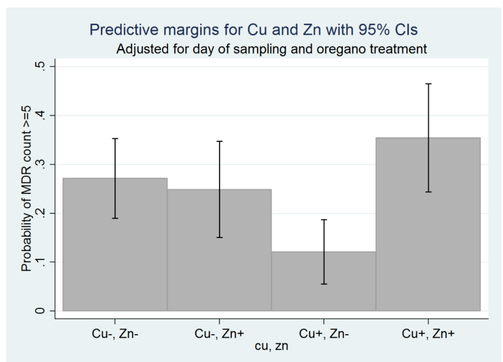


Figure 4. Probability of multi-drug resistance for Cu &/or Zn

Finisher Pig Trial 2

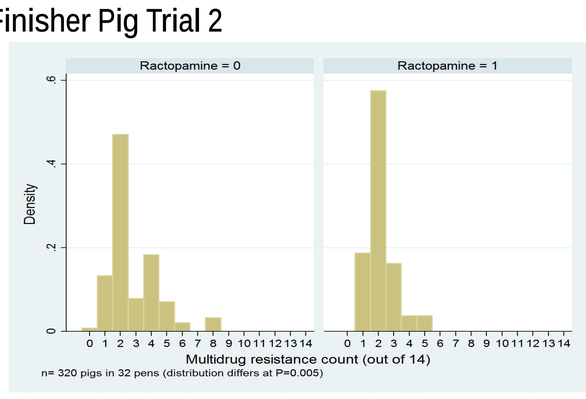


Figure 5. Distribution of MDR count by ractopamine (differ at P = 0.005)

Discussion

Nursery Pig Trial 1

- The alternatives (copper, zinc, oregano oil) did not show a significant association with drug resistance (P>0.05), with a few notable exceptions involving copper (see Graphs 3-4).
- 5.7% of *E. coli* were resistant or exhibited reduced susceptibility to ciprofloxacin, which is an important broad spectrum antibiotic for humans
- 99% of *E. coli* were resistant to tetracycline, which shows the saturation of this resistance in the swine industry. The widely reported phenomenon of multi-drug resistance decreasing with age was confirmed.
- Addition of CTC and copper separately to the piglet's diet each showed a paradoxical reduction of ceftriaxone resistance likely tied to associations of *pco*B and *tet(*B) genes versus *tet(*A) and bla_{CMY}.
- The role of the pco operon in conferring *E. coli* resistance to copper is questionable based on our results.

Finisher Pig Trial 2

- Supplementation of 125 mg/kg Cu or 150 mg/kg Zn in diets containing RAC did not improve finishing pig growth performance of pigs with high feed intake levels as observed in this study.
- Inclusion of 10 mg/kg RAC in the diet for 28 d prior to marketing dramatically improved carcass leanness as well as the feed and caloric efficiencies of pigs.
- RAC did not adversely affect resistance among fecal bacterial isolates, possible reduction in MDR.
- There were no adverse effects of feeding added Zn or Cu on antimicrobial resistance.

Future Work

- Fully explore the MDR genotypes in depth and examine Cu and Zn with regards to antibiotic r-gene presence; thus, more fully understanding the interplay of molecular biology and microbial ecology of metal and antibiotic resistance genes in pig production environments. An unexplained effect of ractopamine on MDR counts exists.

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