Reduction in Antimicrobial Use and Resistance to *Salmonella, Campylobacter*, and *Escherichia coli* in Broiler Chickens, Canada, 2013–2019

Appendix

Appendix Table 1. Susceptibility testing breakpoints for *Salmonella*, *Campylobacter*, and *E. coli,* and mean (95% CI) percent resistance against each of the main antimicrobial classes tested for the three pathogens between 2013 and 2019*

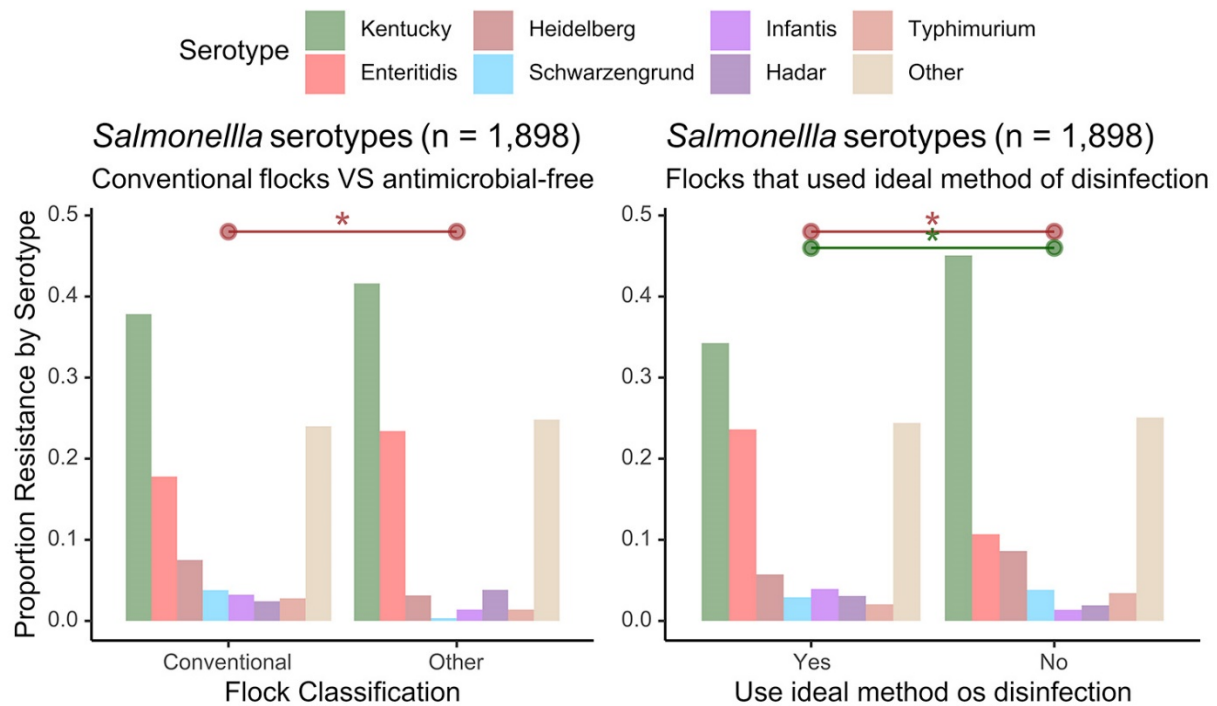| Antimicrobial	Breakpoints (µg/mL)			% Resistance (95% CI)
	Susceptible	Intermediate	Resistant	
Campylobacter (n = 769)				
Azithromycin (AZM)	≤2	4	≥8	3.77 (2.42, 5.12)
Erythromycin (ERY)	≤8	16	≥32	3.77 (2.42, 5.12)
Florfenicol (FLR)	≤4	NA	NA	0.00 (0.00, 0.00)
Tetracycline (TET)	≤4	8	≥16	38.75 (35.31, 42.19)
Ciprofloxacin (CIP)	≤1	2	≥4	16.51 (13.89, 19.14)
Gentamicin (GEN)	≤2	4	≥8	0.00 (0.00, 0.00)
Clindamycin (CLI)	≤2	4	≥8	2.08 (1.07, 3.10)
Nalidixic Acid (NAL)	≤16	32	≥64	16.38 (13.77, 19.00)
E. coli (n = 3,671)				
Amoxicillin-clavulanic acid (AMC)	≤8/4	16/8	≥32/16	14.11 (12.98, 15.23)
Chloramphenicol (CHL)	≤8	16	≥32	5.86 (5.09, 6.62)
Gentamicin	≤4	8	≥16	18.41 (17.16, 19.67)
Sulfisoxazole (SSS)	≤256	NA	≥512	39.42 (37.83, 41.00)
Ampicillin (AMP)	≤8	16	≥32	40.53 (38.95, 42.12)
Ciprofloxacin	≤0.06	0.12–0.5	≥1	0.29 (0.12, 0.47)
Streptomycin (STR)	≤16	NA	≥32	46.33 (44.72, 47.94)
Trimethoprim-sulfonamides (SXT)	≤2/38	NA	≥4/76	16.10 (14.91, 17.29)
Azithromycin	≤16	NA	≥32	0.41 (0.20, 0.61)
Ceftriaxone (CRO)	≤1	2	≥4	13.73 (12.62, 14.84)
Cefoxitin (FOX)	≤8	16	≥32	14.06 (12.93, 15.18)
Nalidixic Acid	≤16	NA	≥32	6.18 (5.40, 6.96)
Tetracycline	≤4	8	≥16	46.82 (45.21, 48.44)
Salmonella (n = 1,898)				
Amoxicillin-clavulanic acid	≤8/4	16/8	≥32/16	11.43 (10.00, 12.86)
Chloramphenicol	≤8	16	≥32	0.42 (0.13, 0.71)
Gentamicin	≤4	8	≥16	1.32 (0.80, 1.83)
Sulfisoxazole	≤256	NA	≥512	6.48 (5.37, 7.59)
Ampicillin	≤8	16	≥32	11.43 (10.00, 12.86)
Ciprofloxacin	≤0.06	0.12–0.5	≥1	0.16 (0.00, 0.33)
Streptomycin	≤16	NA	≥32	43.57 (41.34, 45.80)
Azithromycin	≤16	NA	≥32	0.00 (0.00, 0.00)
Ceftriaxone	≤1	2	≥4	11.49 (10.05, 12.92)
Cefoxitin	≤8	16	≥32	10.22 (8.86, 11.58)
Nalidixic Acid	≤16	NA	≥32	2.32 (1.64, 3.00)
Tetracycline	≤4	8	≥16	44.73 (42.50, 46.97)

*Breakpoints stablished by Clinical and Laboratory Standards Institute were used when available. If unavailable, Not Applicable (NA) was assigned.

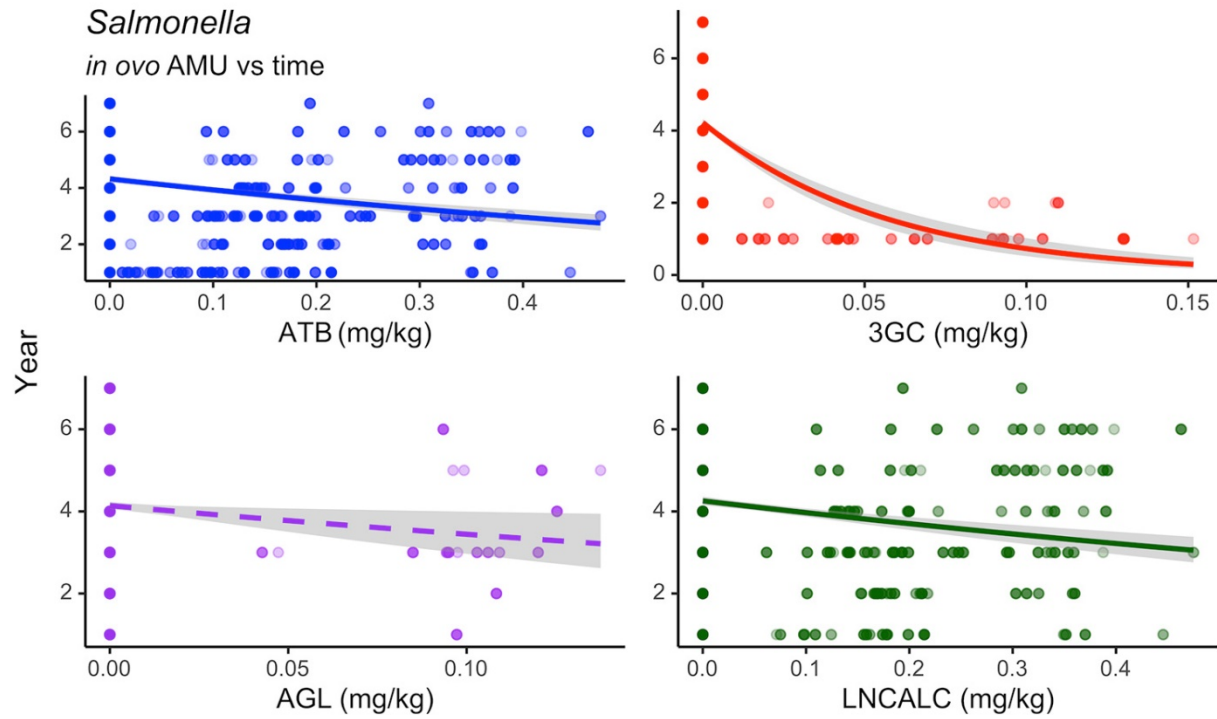**Appendix Table 2.** Variables evaluated by the LASSO regression.

Variable	Distribution	Levels	Unit
Production system	Binary	Conventional, antimicrobial-free*	NA
Disinfection method	Binary	Ideal,† Other	NA
All-in-all-out	Binary	Yes, No	NA
Province	Categorical (5 levels)	Alberta, Saskatchewan, Ontario, British Columbia, and Québec	NA
Year of sample collection	Categorical (7 levels)	2013, 2014, 2015, 2016, 2017, 2018, 2019	NA
AMU *in ovo*	Continuous	NA	mg/kg
AMU *in ovo*	Binary	Yes, No	NA
AMU via feed	Continuous	NA	mg/kg
AMU via feed	Binary	Yes, No	NA
AMU via water	Continuous	NA	mg/kg
AMU via water	Binary	Yes, No	NA

*Farms that are not exposed to medically important antimicrobials, but that may allow the use of ionophore and chemical coccidiostats.

†Ideal method for cleaning and disinfection recommended by the World Organization for Animal Health.
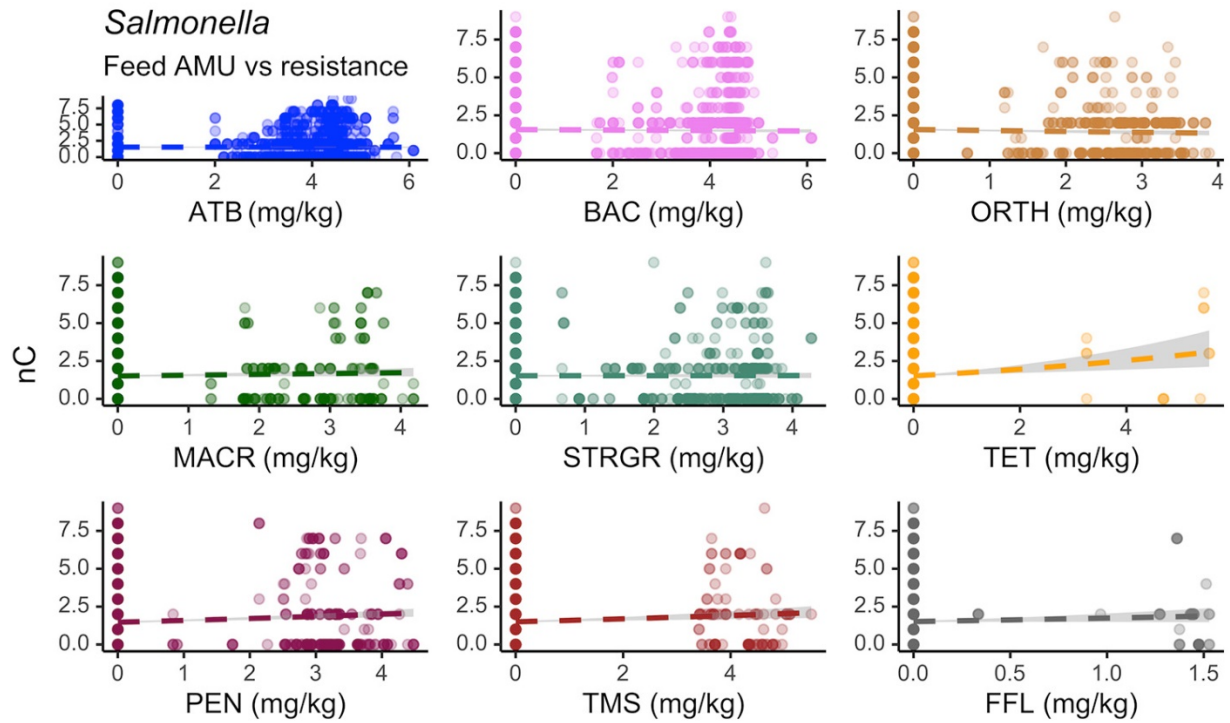

Appendix Figure 1. Differences in *Salmonella* (n = 1,898) serotypes proportions in chicken flocks that are classified as conventional versus antimicrobial-free; and between farms reporting to have used the ideal method of disinfection before sample collection versus farms that did not use this method of disinfection. Color-coded * by serotype represents p<0.05 using Z test to compare two population proportions.
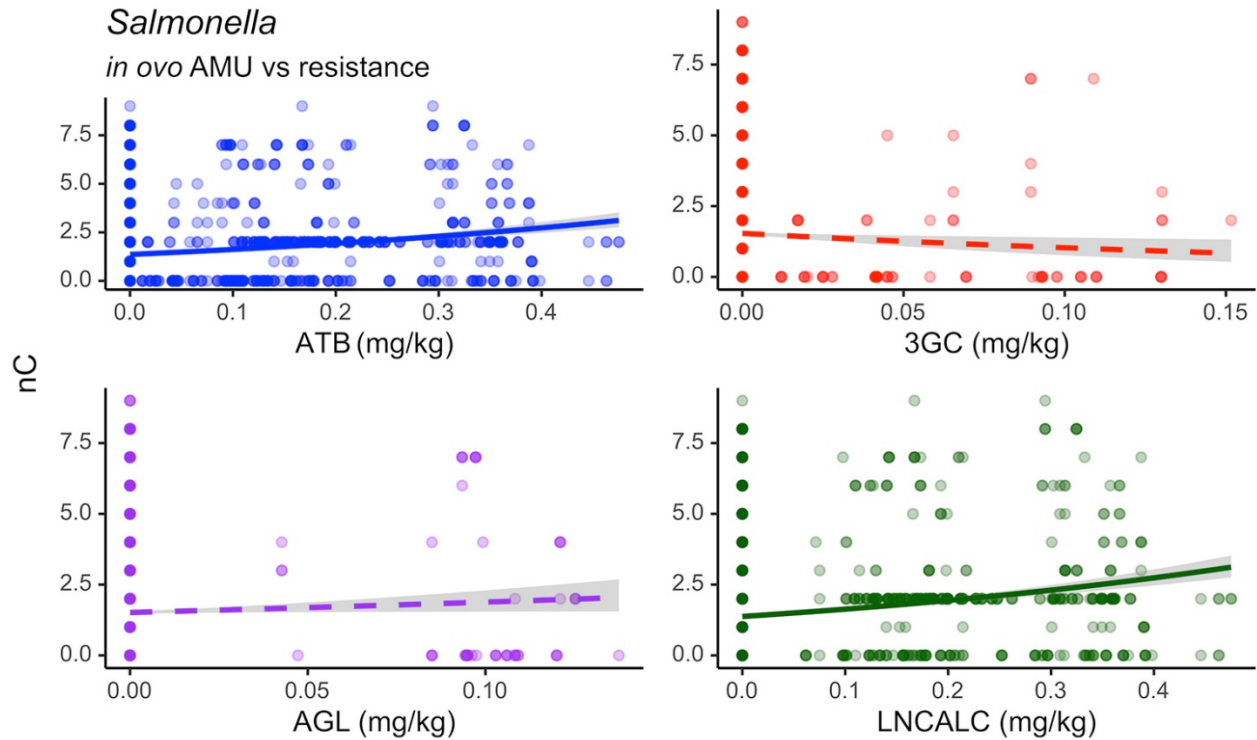
Appendix Figure 2. Generalized linear models demonstrating the change in overall (log-transformed mg/kg) use of antimicrobials in flocks where *Salmonella* was isolated, and stratified by the use of third-generation cephalosporins, aminoglycosides and lincosamides *in ovo* over time. Continuous lines represent significance (p<0.05).
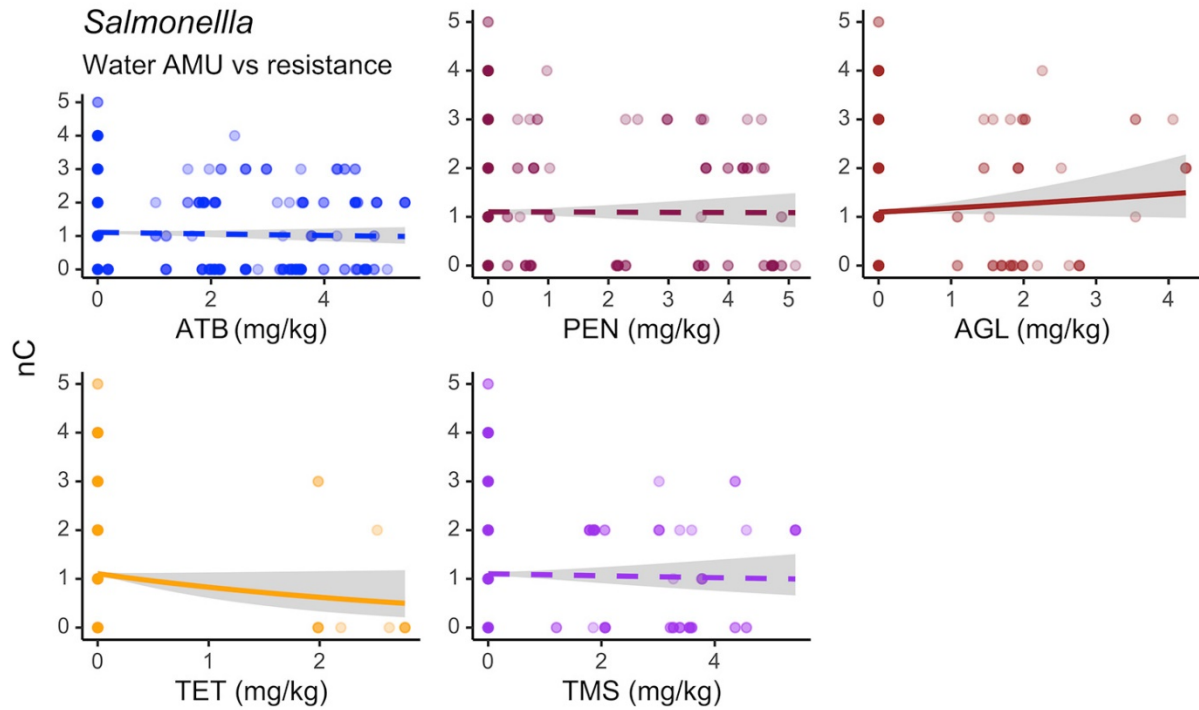
Appendix Figure 3. Generalized linear models demonstrating the change in overall (log-transformed mg/kg) use of antimicrobials in flocks where *Salmonella* was isolated, and stratified by the use of penicillins, aminoglycosides, tetracyclines, and trimethoprim-sulfonamides via water over time. Continuous lines represent significance (p<0.05).
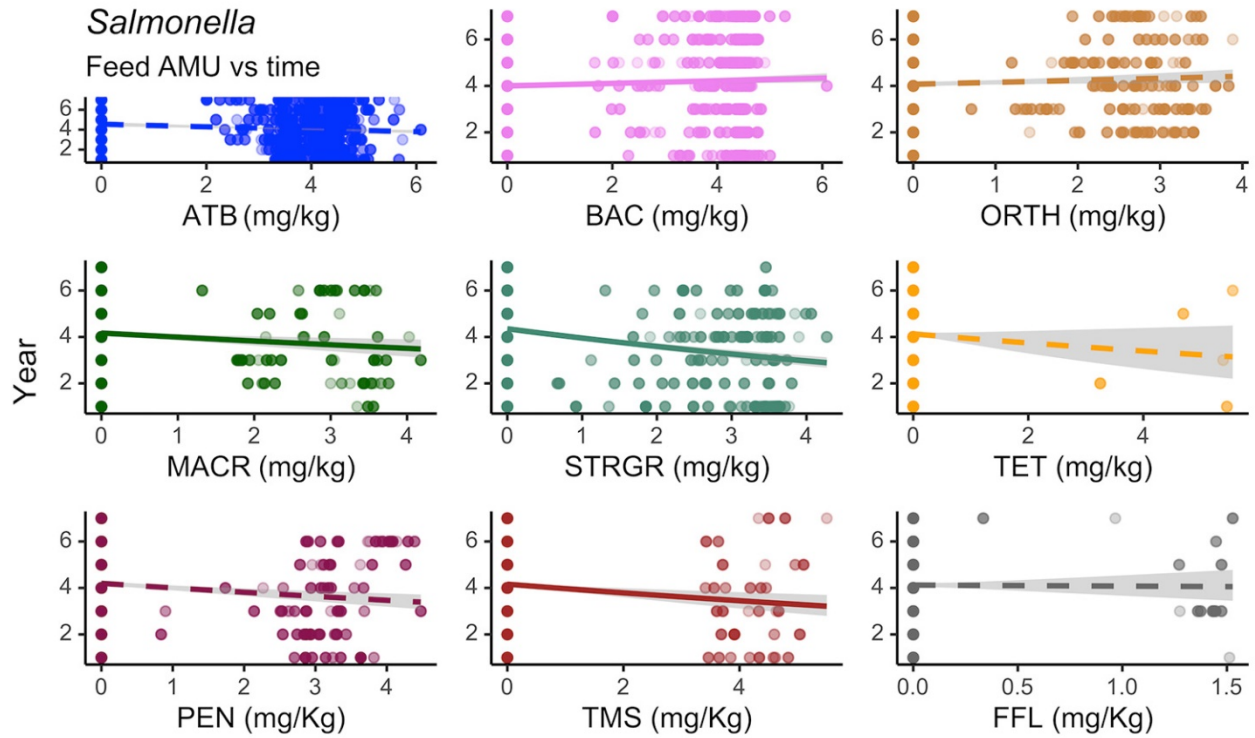
Appendix Figure 4. Generalized linear models demonstrating the change in overall (log-transformed mg/kg) use of antimicrobials in flocks where *Salmonella* was isolated, and stratified by the use of bacitracins, orthosomycins, macrolides, streptogramins, tetracyclines, penicillins, trimethoprim-sulfonamides and flavophospholipids via feed over time. Continuous lines represent significance (p<0.05).
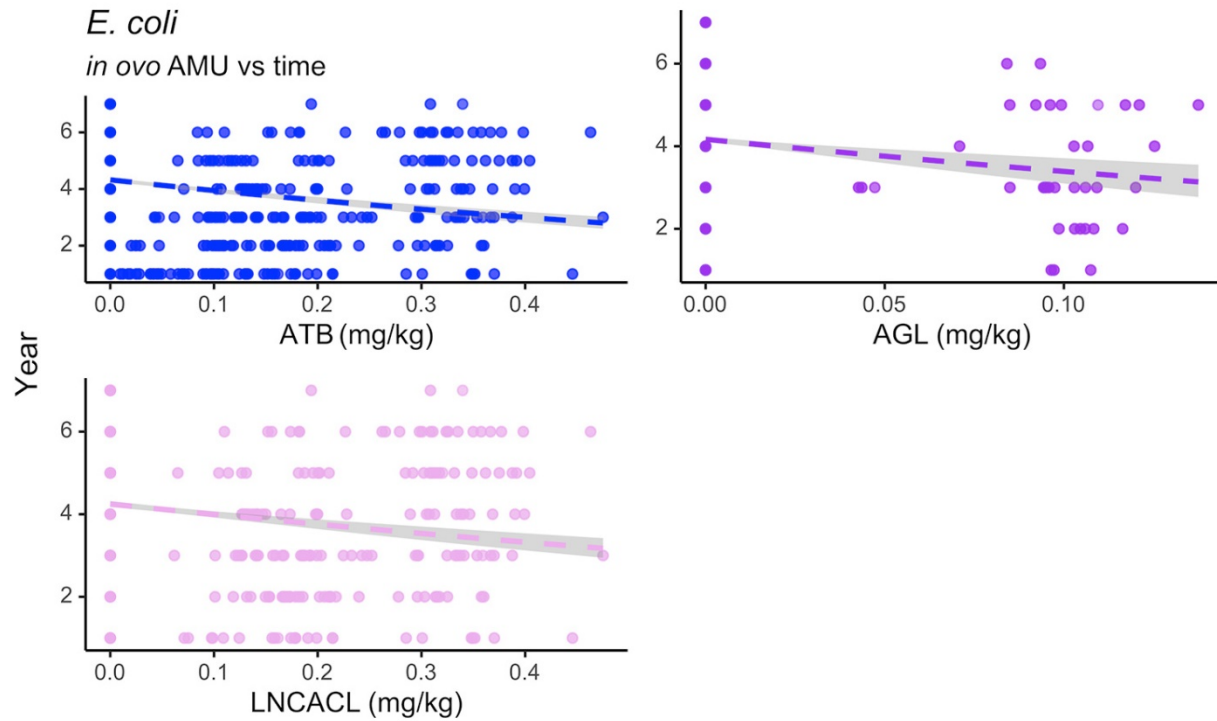
Appendix Figure 5. Generalized linear models illustrating the change in the number of antimicrobial classes a *Salmonella* isolate is resistant to (nC) in response to overall (log transformed mg/kg) use of antimicrobials, and stratified by the use of third generation cephalosporins, aminoglycosides, and lincosamides *in ovo*. Continuous lines represent significance (p<0.05).
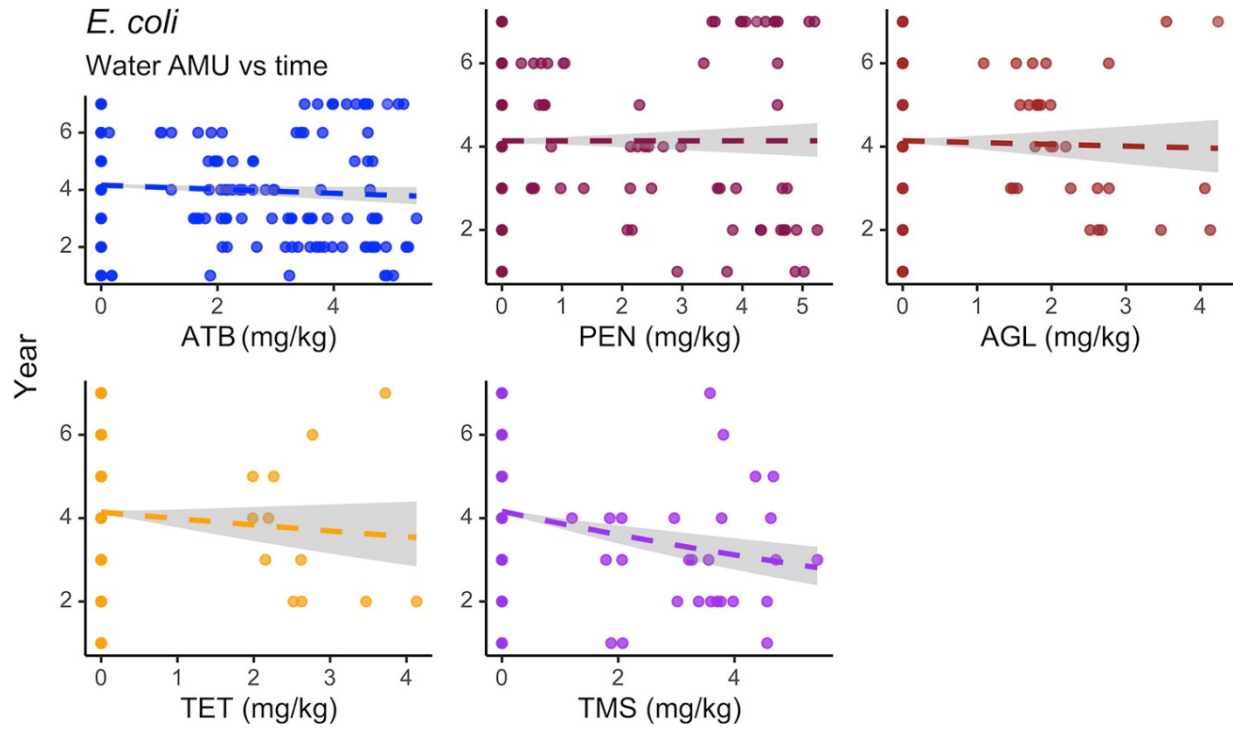
Appendix Figure 6. Generalized linear models illustrating the change in the number of antimicrobial classes a *Salmonella* isolate is resistant to (nC) in response to overall use of antimicrobials (log transformed mg/kg), and stratified by the use of penicillins, aminoglycosides, tetracyclines, and trimethoprim-sulfonamide combinations via water. Continuous lines represent significance (p<0.05).
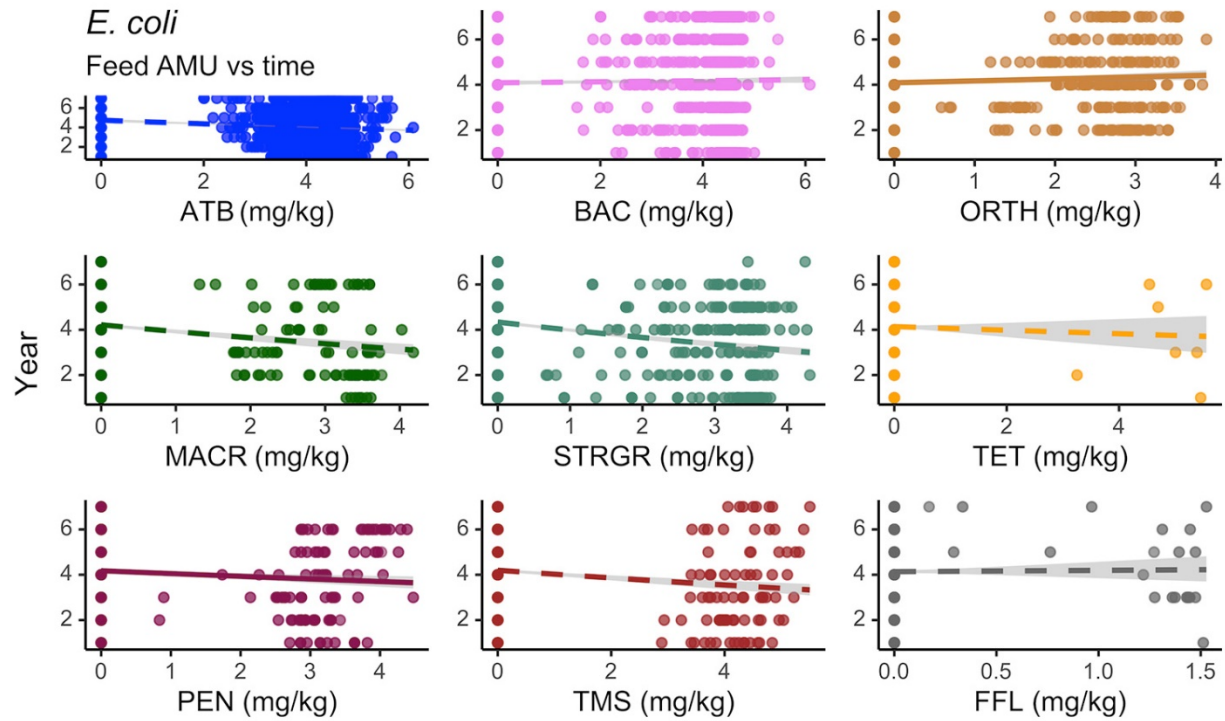
Appendix Figure 7. Generalized linear models illustrating the change in the number of antimicrobial classes a *Salmonella* isolate is resistant to (nC) in response to overall use of antimicrobials (log transformed mg/kg), and stratified by the use of bacitracins, orthosomycins, macrolides, streptogramins, tetracyclines, penicillins, trimethoprim-sulfonamides and flavophospholipids via feed. Continuous lines represent significance (p<0.05).
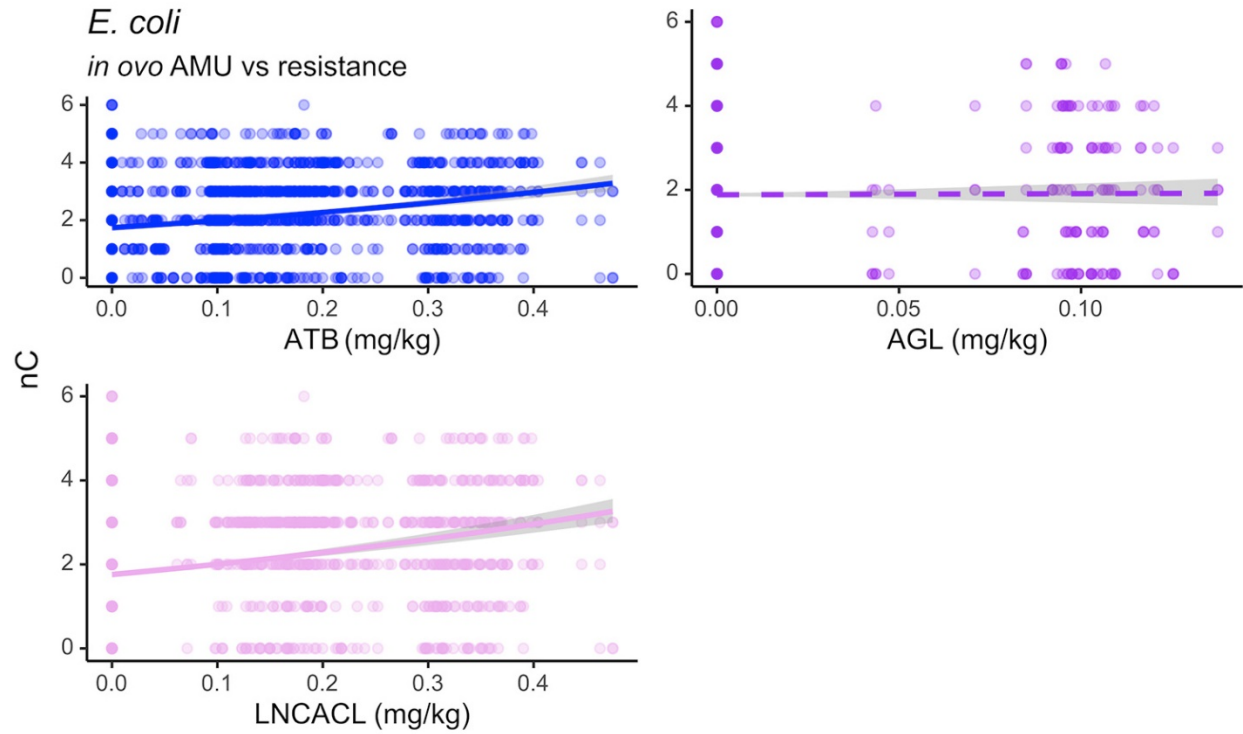
Appendix Figure 8. Generalized linear models demonstrating the change in overall (log-transformed mg/kg) use of antimicrobials in flocks where *E. coli* was isolated, and stratified by the use of aminoglycosides and lincosamides *in ovo* over time. Continuous lines represent significance (p<0.05).
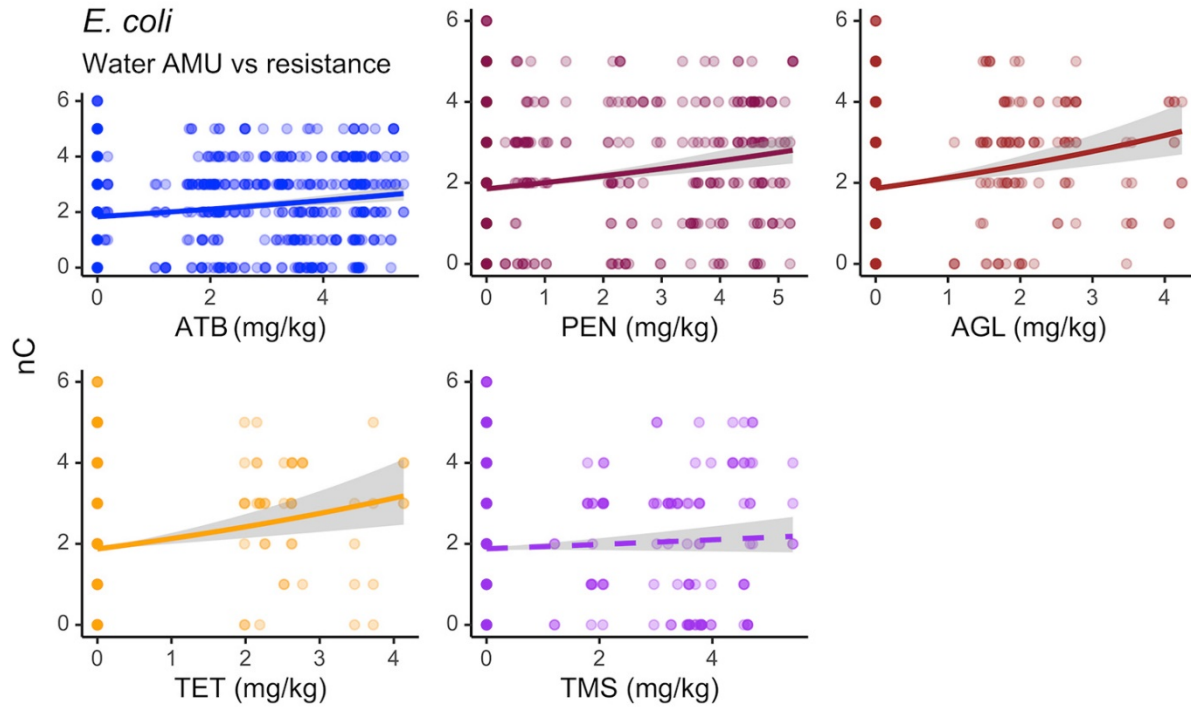
Appendix Figure 9. Generalized linear models demonstrating the change in overall (log-transformed mg/kg) use of antimicrobials in flocks where *E. coli* was isolated, and stratified by the use of penicillins, aminoglycosides, tetracyclines, and trimethoprim-sulfonamide combinations via water over time. Continuous lines represent significance (p<0.05).
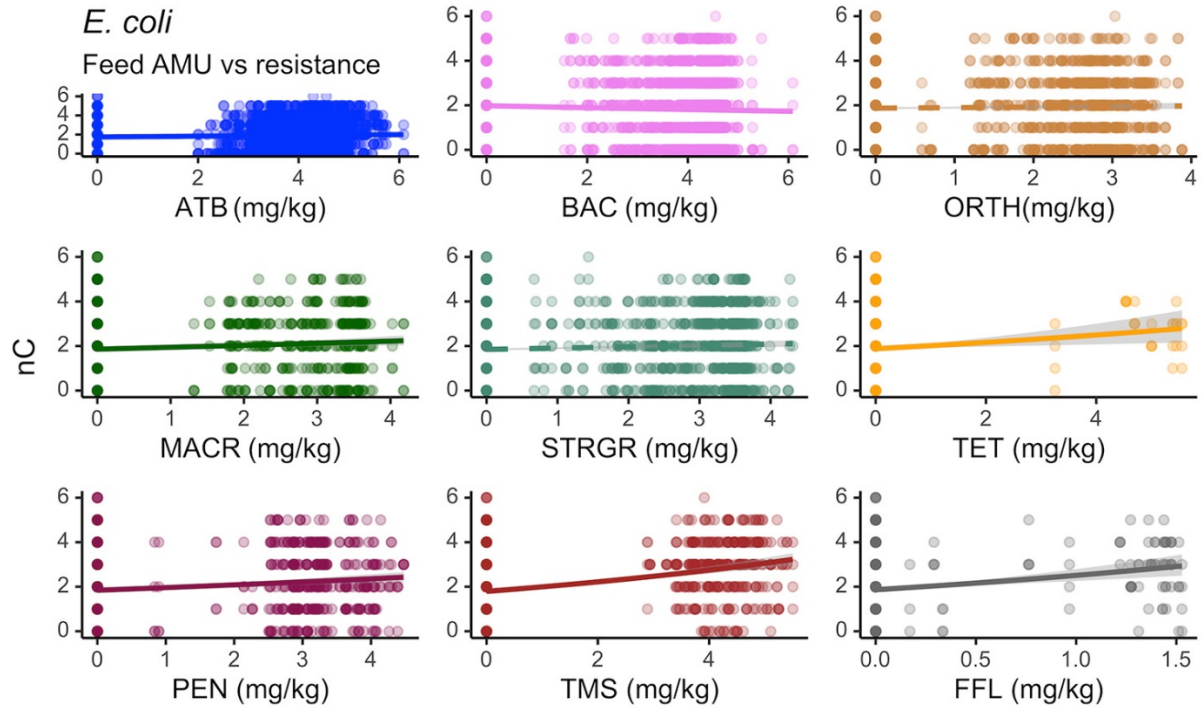
Appendix Figure 10. Generalized linear models demonstrating the change in overall (log-transformed mg/kg) use of antimicrobials in flocks where *E. coli* was isolated, and stratified by the use of bacitracins, orthosomycins, macrolides, streptogramins, tetracyclines, penicillins, trimethoprim-sulfonamide combinations, and flavophospholipids via feed over time. Continuous lines represent significance (p<0.05).
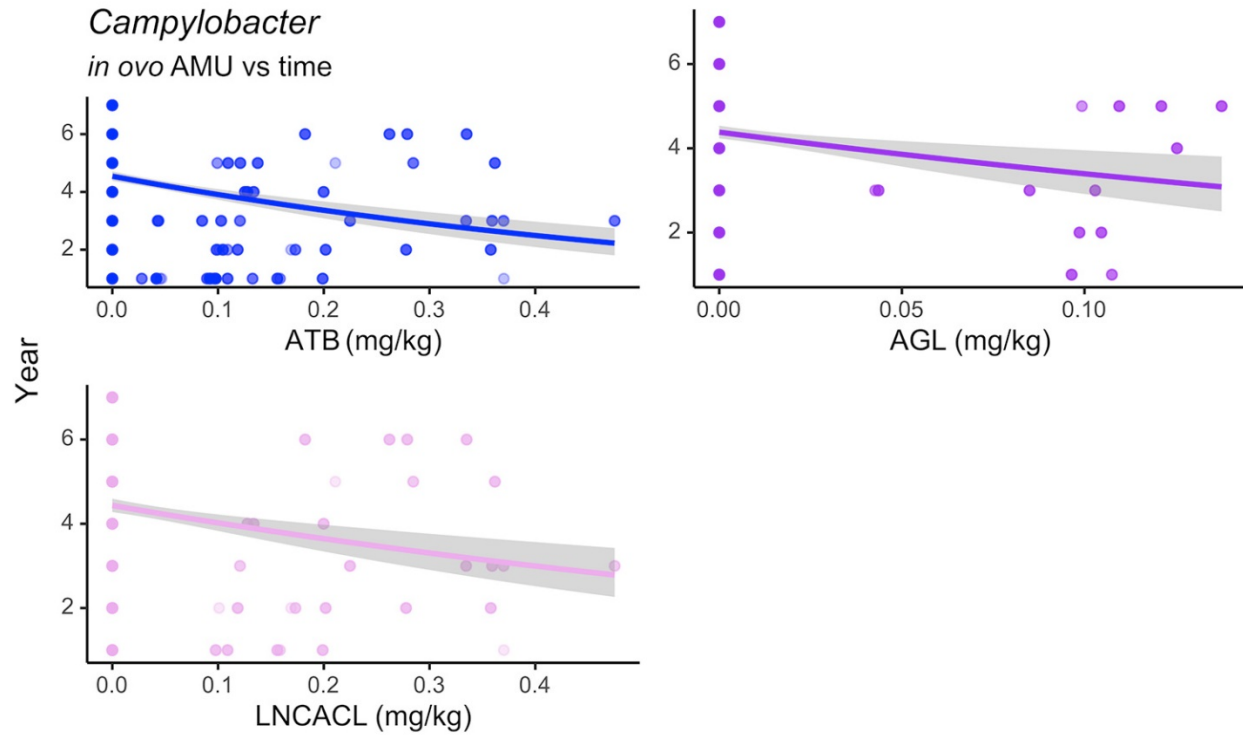
Appendix Figure 11. Generalized linear models illustrating the change in the number of antimicrobial classes a *E. coli* isolate is resistant to (nC) in response to overall use of antimicrobials (log transformed mg/kg), and stratified by the use of aminoglycosides and lincosamides *in ovo*. Continuous lines represent significance (p<0.05).
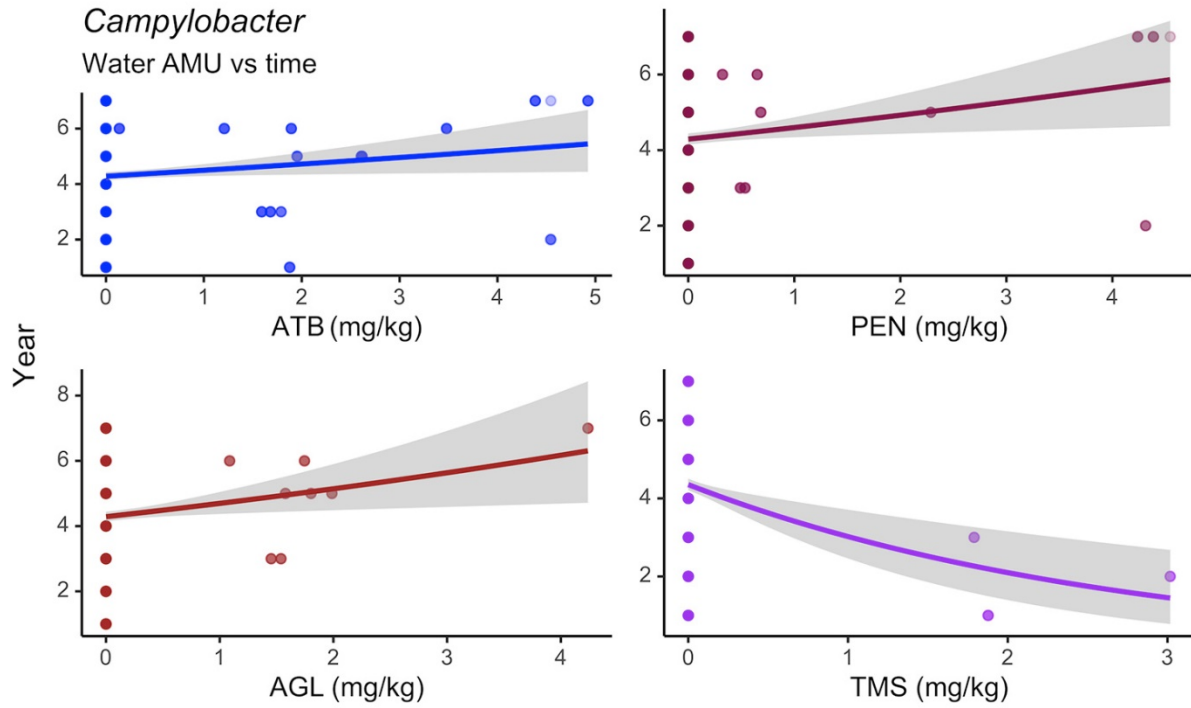
Appendix Figure 12. Generalized linear models illustrating the change in the number of antimicrobial classes a *E. coli* isolate is resistant to (nC) in response to overall use of antimicrobials (log transformed mg/kg), and stratified by the use of penicillins, aminoglycosides, tetracyclines, and trimethoprim-sulfonamide combinations via water. Continuous lines represent significance ($p<0.05$).
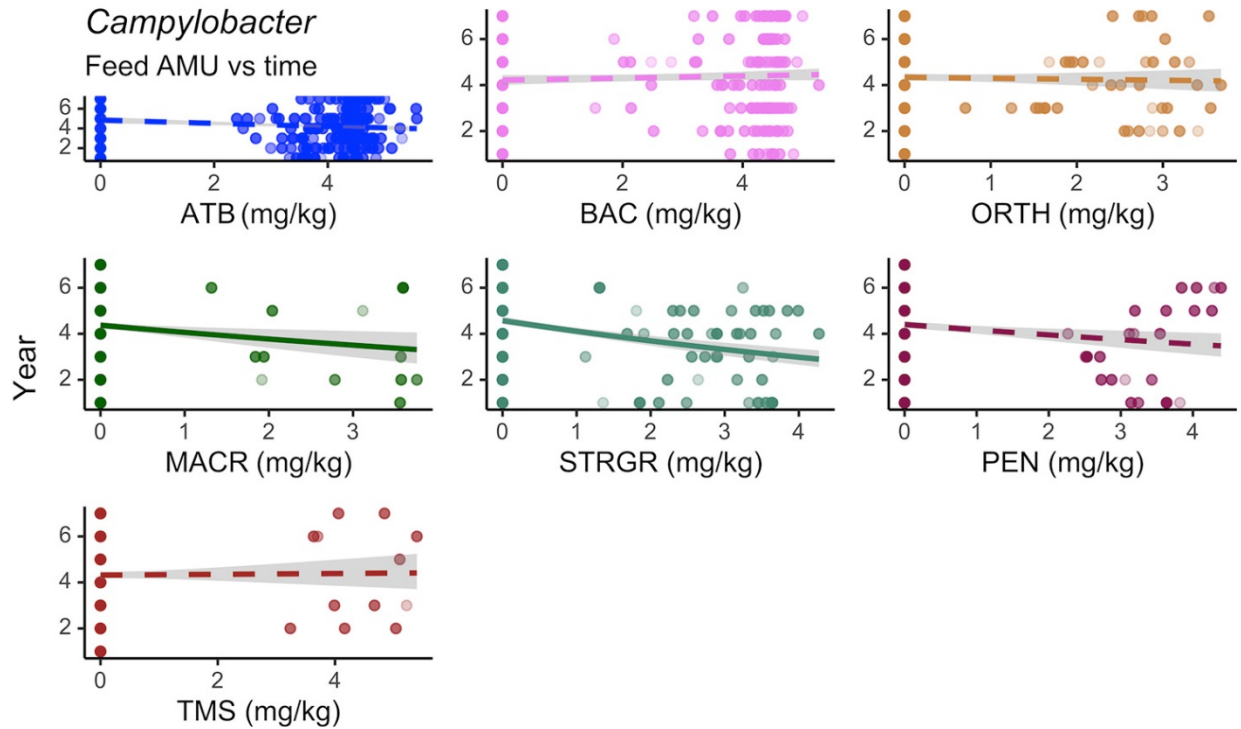
Appendix Figure 13. Generalized linear models illustrating the change in the number of antimicrobial classes a *E. coli* isolate is resistant to (nC) in response to overall use of antimicrobials (log transformed mg/kg), and stratified by the use of bacitracins, orthosomycins, macrolides, streptogramins, tetracyclines, penicillins, trimethoprim-sulfonamides, and flavophospholipids via feed. Continuous lines represent significance (p<0.05).
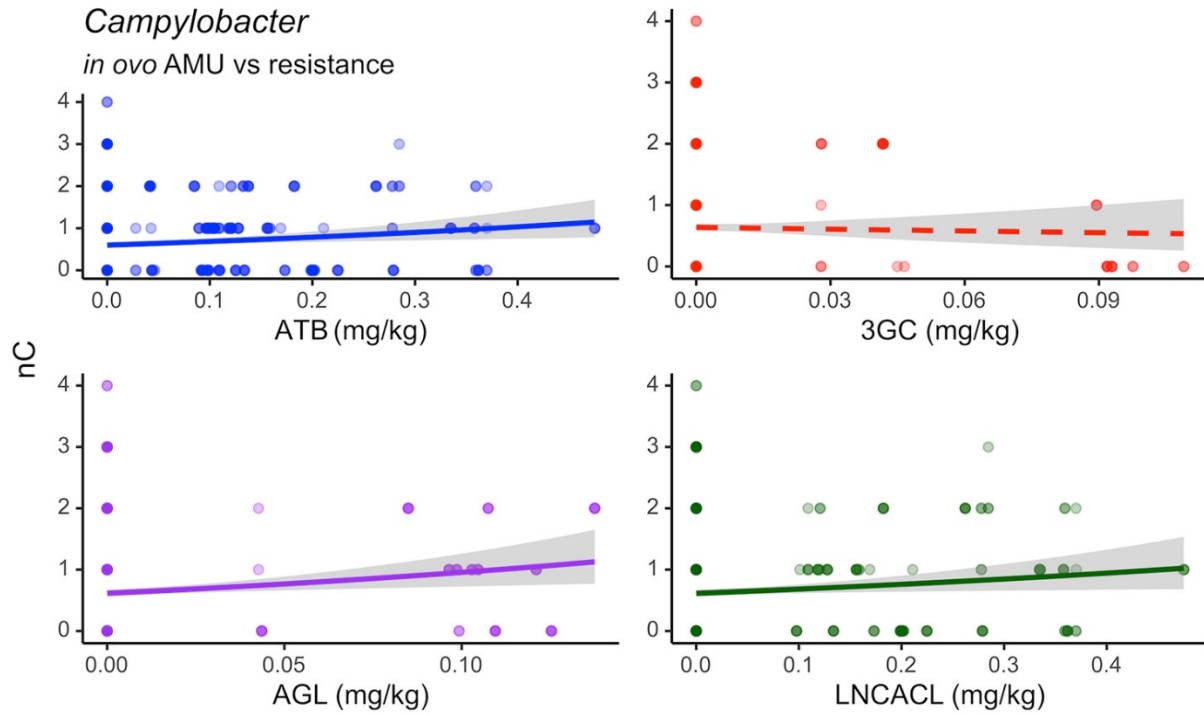
Appendix Figure 14. Generalized linear models demonstrating the change in overall (log-transformed mg/kg) use of antimicrobials in flocks where *Campylobacter* was isolated, and stratified by the use of aminoglycosides and lincosamides *in ovo* over time. Continuous lines represent significance (p<0.05).
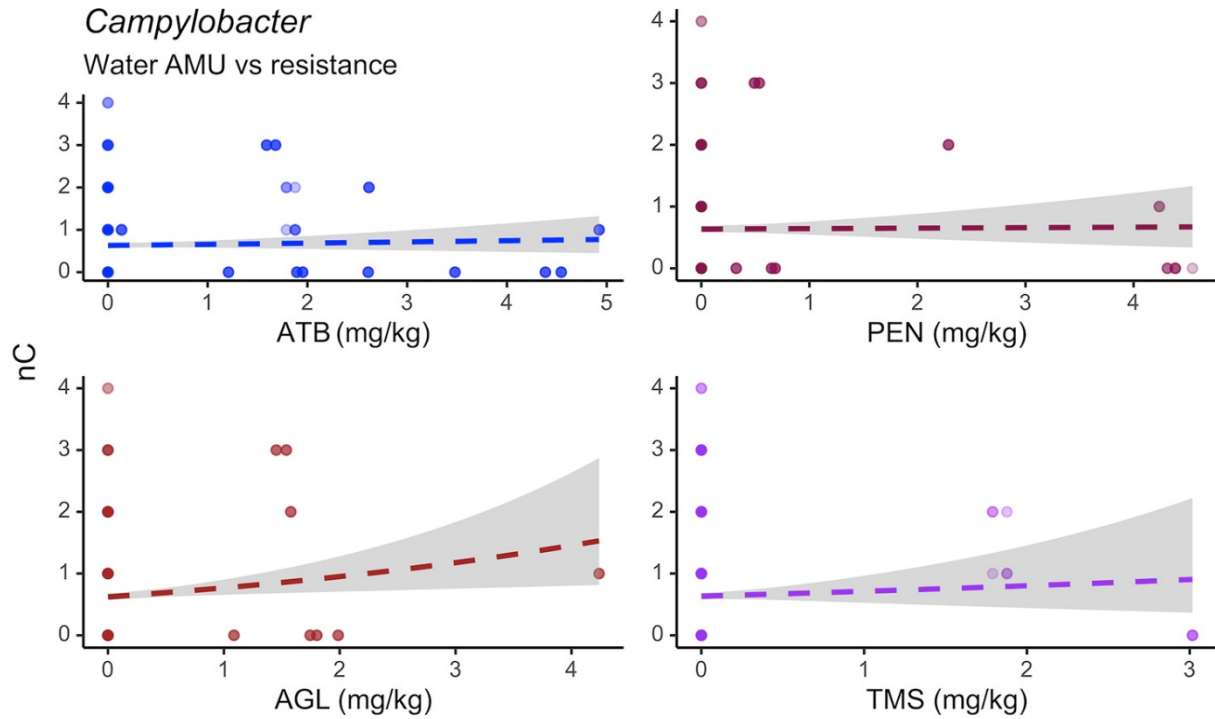
Appendix Figure 15. Generalized linear models demonstrating the change in overall (log-transformed mg/kg) use of antimicrobials in flocks where *Campylobacter* was isolated, and stratified by the use of penicillins, aminoglycosides, tetracyclines, and trimethoprim-sulfonamides via water over time. Continuous lines represent significance (p<0.05).
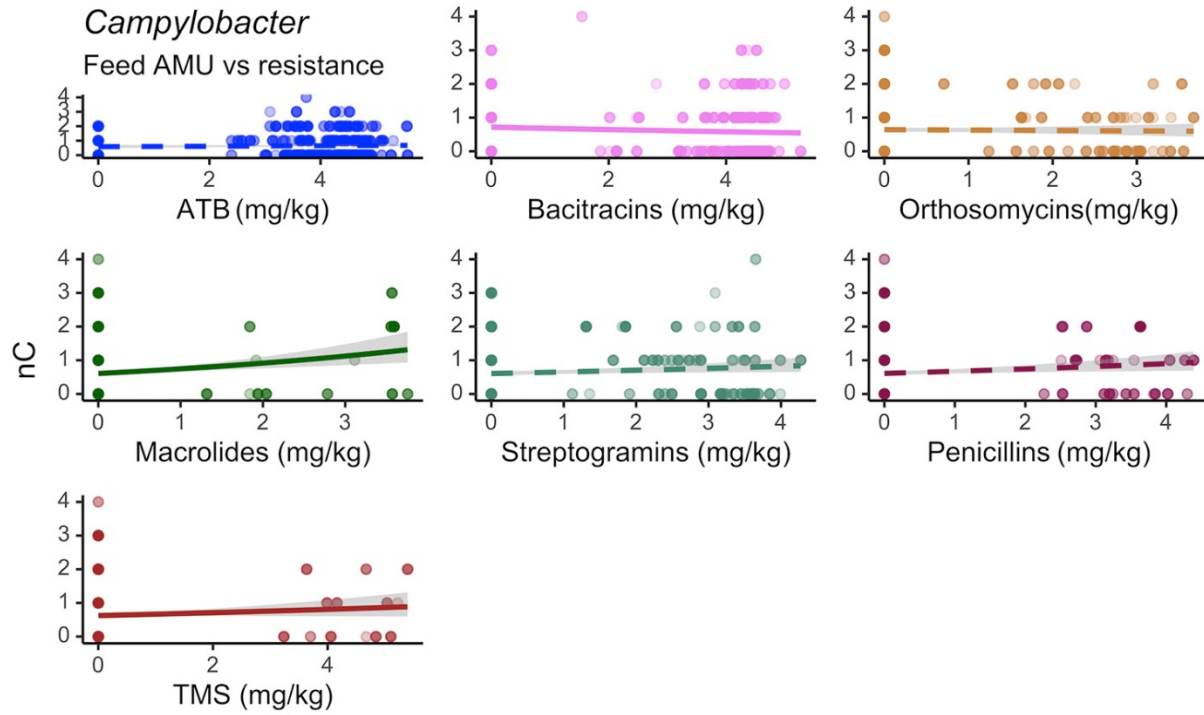
Appendix Figure 16. Generalized linear models demonstrating the change in overall (log-transformed mg/Kg) use of antimicrobials in flocks where *Campylobacter* was isolated, and stratified by the use of bacitracins, orthosomycins, macrolides, streptogramins, tetracyclines, penicillins, and trimethoprim-sulfonamide combinations via feed over time. Continuous lines represent significance (p<0.05).
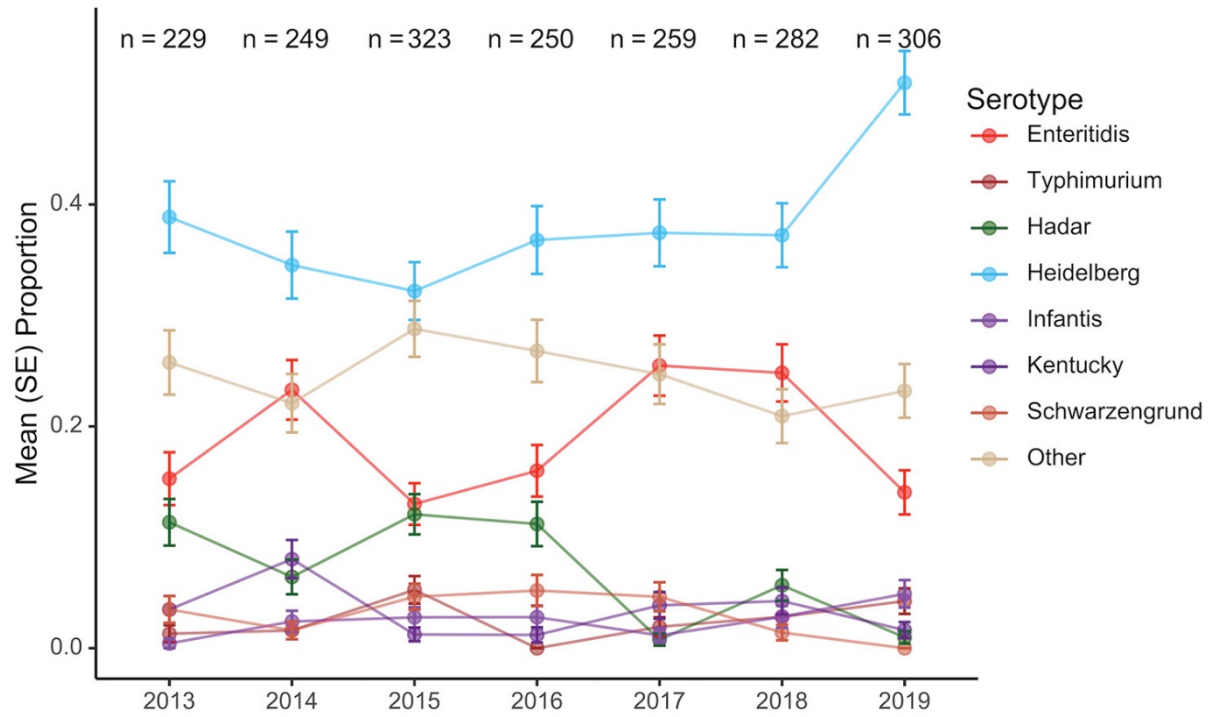
Appendix Figure 17. Generalized linear models illustrating the change in the number of antimicrobial classes a *Campylobacter* isolate is resistant to (nC) in response to overall use of antimicrobials (log transformed mg/kg), and stratified by the use of third generation cephalosporins, aminoglycosides, and lincosamides *in ovo*. Continuous lines represent significance (p<0.05).

Appendix Figure 18. Generalized linear models illustrating the change in the number of antimicrobial classes a *Campylobacter* isolate is resistant to (nC) in response to overall use of antimicrobials (log transformed mg/kg), and stratified by the use of penicillins, and trimethoprim-sulfonamide combinations via water. Continuous lines represent significance ($p<0.05$).

Appendix Figure 19. Generalized linear models illustrating the change in the number of antimicrobial classes a *Campylobacter* isolate is resistant to (nC) in response to overall use of antimicrobials (log transformed mg/kg), and stratified by the use of bacitracins, orthosomycins, macrolides, streptogramins, penicillins, and trimethoprim-sulfonamides via feed. Continuous lines represent significance (p<0.05).

Appendix Figure 20. Mean proportion and standard errors of the *Salmonella* (n = 1,898) serotypes isolated from chicken flocks in Canada over the study period (2013–2019).