Are Antibiotics a Concern in Distiller's Co-products?

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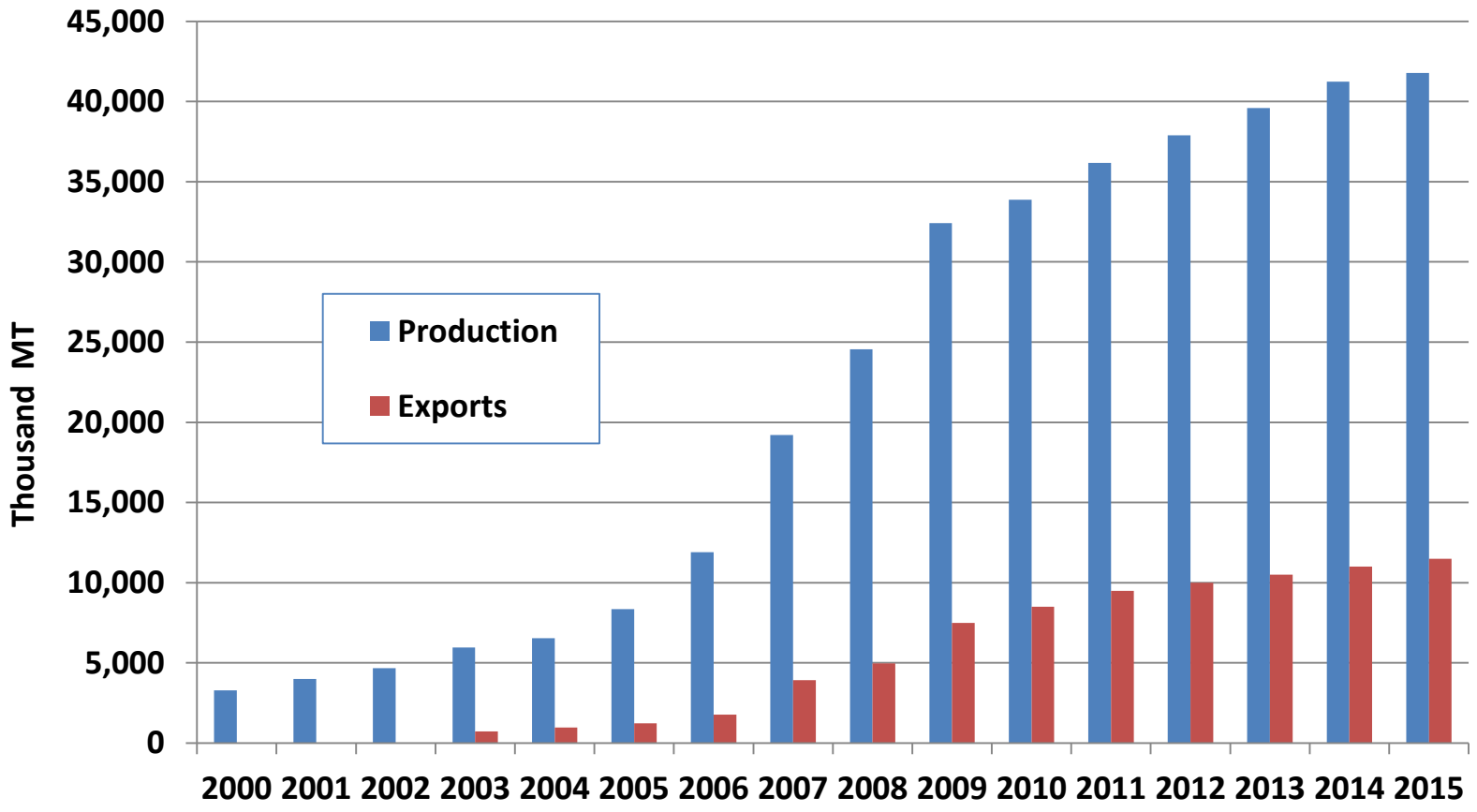
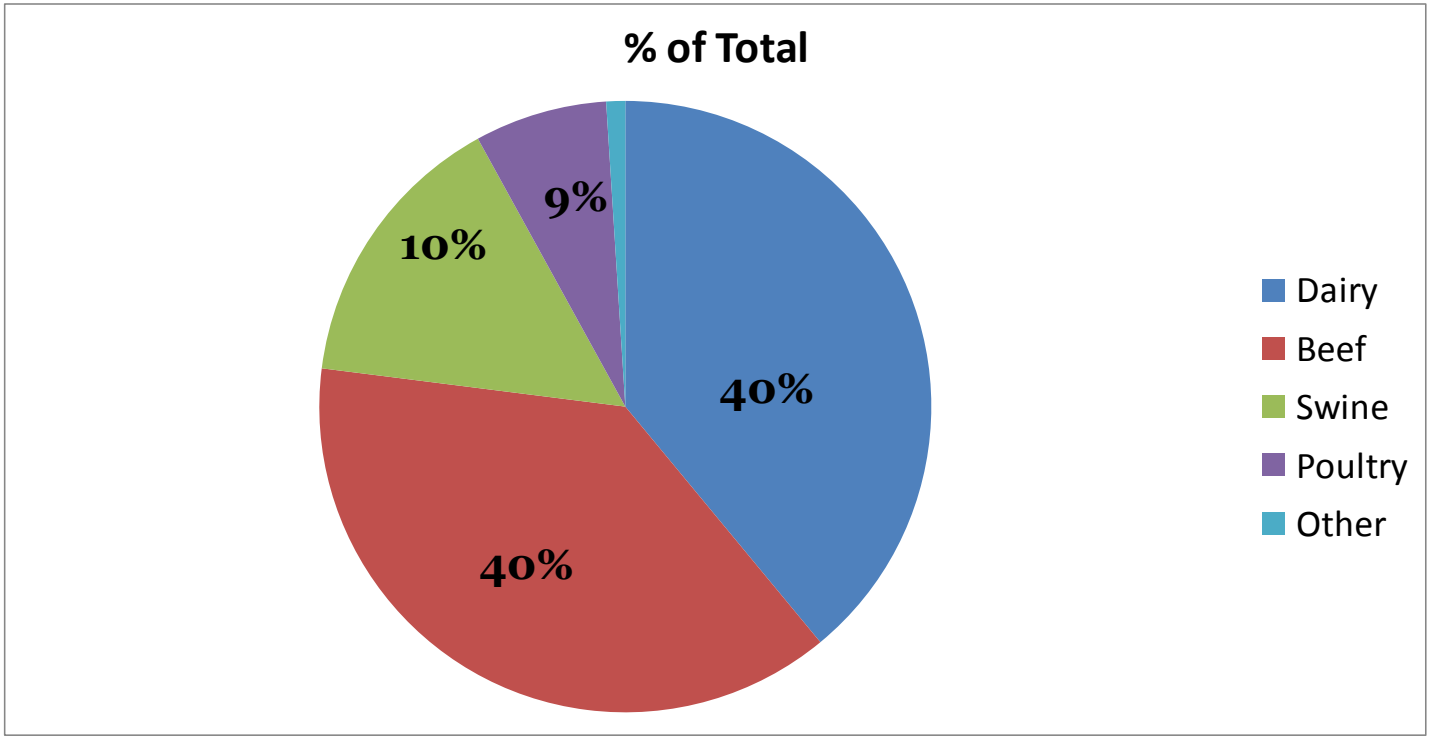
U.S. DDGS production

- Currently 207 ethanol plants in 29 states
  - 16 plants idle, 5 under construction
  - Majority are dry-grind vs. wet mill
  - Most have capacity to produce 378 million L ethanol produced/yr
  - Plants operate 354 days/yr
  - 378 million L plants produce 5,625 MT tons of DDGS/week
  - Plant DDGS storage capacity is <1 wk
- 2012 – 36.5 million MT wet and dried distillers grains will be produced

U.S. DDGS production and exports (Sep-Aug Marketing Year)



Distribution of DDGS use in food animal production in the U.S.



Antibiotics are used in ethanol and DDGS production

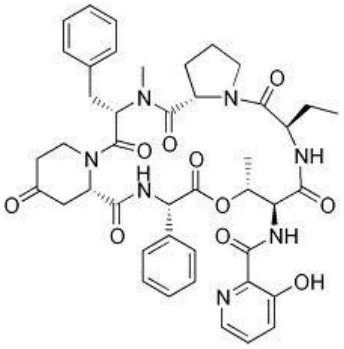
- Bacterial contamination during fermentation is a challenge in ethanol production.
  - Lactic acid producing bacteria (*Lactobacillus, Pediococcus, Leuconostoc, and Weissella*) are most common
    - Bischoff et al. (2009).
  - Bacteria compete with yeast for sugars and micronutrients
    - Reduce ethanol yield by 1 to 5% (Narendranath et al., 1997)
    - Reduce DDGS quality and nutritional value
- Antibiotics have been used to manage this problem for many years.

What antibiotics are used in ethanol production?

- PhibroChem
  - Lactrol™ – virginiamycin
- Lallemand
  - Lactoside 247™ – penicillin and virginiamycin
  - Lactoside V™ – virginiamycin
  - Allpen Special™ - penicillin
- FermSolutions
  - Fermguard™
  - Fermguard Sentry™ – virginiamycin
  - Fermguard Extreme™ – erythromycin, penicillin, virginiamycin
- North American Bioproducts Corp.
  - Bactenix® V60 – penicillin
  - Bactenix® V300 - erythromycin

What antibiotics are used?

- No published data are available
- Virginiamycin and penicillin are GRAS listed.



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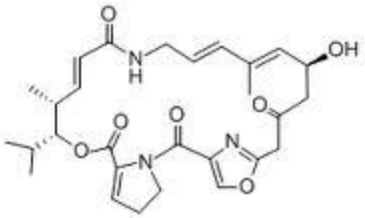

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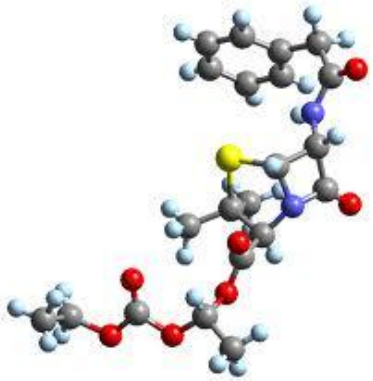
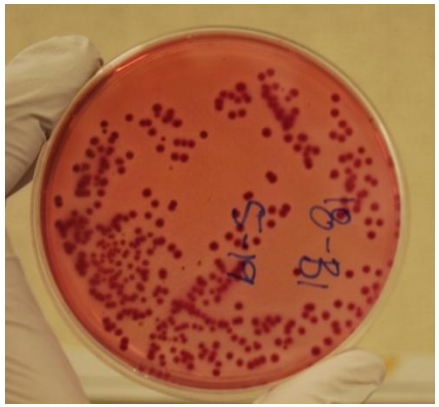


Results from 2008 FDA multi-state distiller's grains sampling survey - unpublished

- A multi-analyte HPLC residue detection method was used
  - de Alwis and Heller (2010)
  - Detection level $\geq$ 0.1 ppm (DM basis)
- Antibiotic residues were detected in 24 of 45 samples (53%)
  - Virginiamycin residues (33%)
  - Erythromycin residues (27 %)
  - Tylosin residues (11%)
- No determinations were made for biological activity (bacterial inhibition) of residues
- Currently, there is no regulatory monitoring or enforcement of antimicrobial residues in distillers co-products produced by fuel ethanol plants.

Antibiotic residues in distillers grains study – University of Minnesota

- Objectives
  - Collect wet and dried distillers co-products samples from multiple geographical locations and dry-grind ethanol plants in the U.S.
  - Analyze for antibiotic residues
    - Virginiamycin
    - Penicillin
    - Erythromycin
    - Tetracycline
    - Tylosin
  - Determine the extent of any antimicrobial activity of samples using the sentinel bacteria
    - *Escherichia coli* (ATCC 8739)
    - *Listeria monocytogenes* (ATCC 19115)



Antibiotic residues in distillers grains study – University of Minnesota

- Sample collection (4 quarters/year)
  - 20 wet and 20 dried distillers grain samples/quarter
    - 34 dry-grind ethanol plants
    - 8 Midwestern U.S. states
  - Collected by independent nutritional consultants
    - frozen (-21 °C) upon arrival

- Sample analysis
  - SGS North America (Brookings, SD) determined:
    - Presence and level of residues (de Alwis and Heller, 2010)
      - Erythromycin
      - Penicillin
      - Tylosin
      - Tetracycline
    - Antimicrobial inhibition using sentinel bacteria
      - *E. coli* ATTC 8739
      - *L. monocytogenes* ATTC 19115
  - PhibroChem EPG determined:
    - Presence and level of virginiamycin residues using the FDA approved bioassay

Antibiotic residues study – University of Minnesota

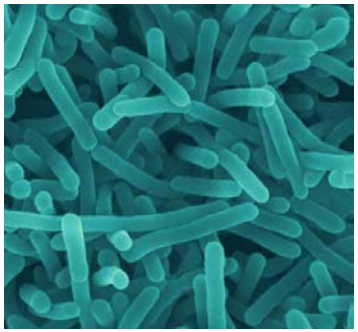


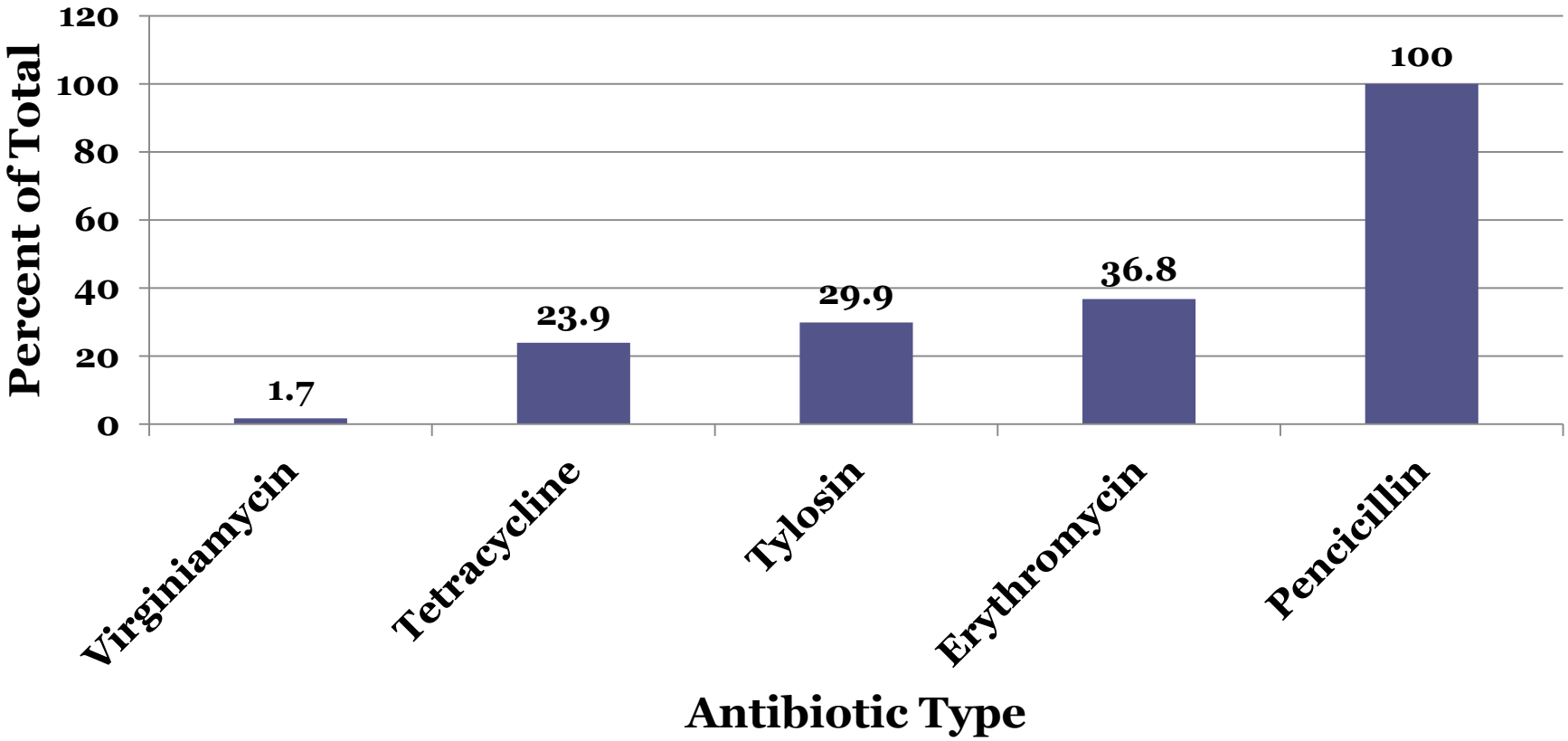
Image by Limoge

- Bacterial Thresholds
  - Determined for residues with sentinel bacterial concentrations of 10^4, 10^5, 10^6, and 10^7
  - Sentinel bacteria cultured with the antibiotic extract in broth for 18 to 24 h at 37°C
  - Examined for bacterial growth
- Bacterial Inhibition
  - Antibiotic extracts plated with sentinel bacterial concentrations of 10^4, 10^5, 10^6, and 10^7
  - Plated on tryptic soy agar and incubated at 37°C for 18 to 24 h
  - Bacterial colonies counted and recorded as colony forming units (CFU) per mL

Preliminary results

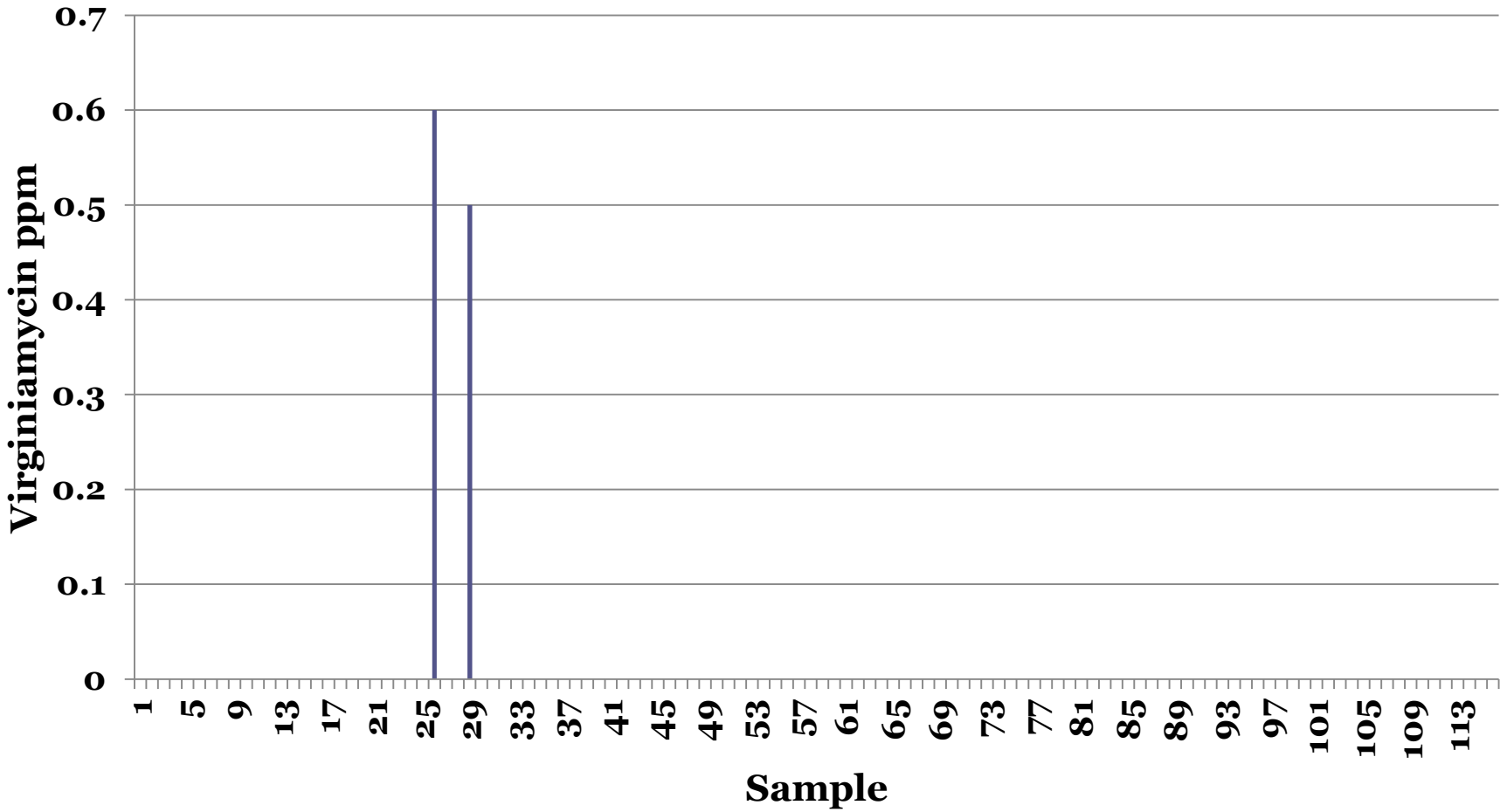
- Residue data from first 3 quarters of sampling
  - **116** samples have been analyzed for Virginiamycin
  - **116** samples (58 wet and 58 dried) have been analyzed for:
    - Tetracycline
    - Tylosin
    - Erythromycin
    - Penicillin
  - **116** sample extracts tested for inhibitory properties with sentinel bacteria

Results - percentage of samples containing antibiotic residues



* Using HPLC method (de Alwis and Heller, 2010) resulted in 85.7% of samples containing virginiamycin residues.
* No samples had virginiamycin residue concentrations > 1 ppm (GRAS limit)

Results - virginiamycin residue concentrations

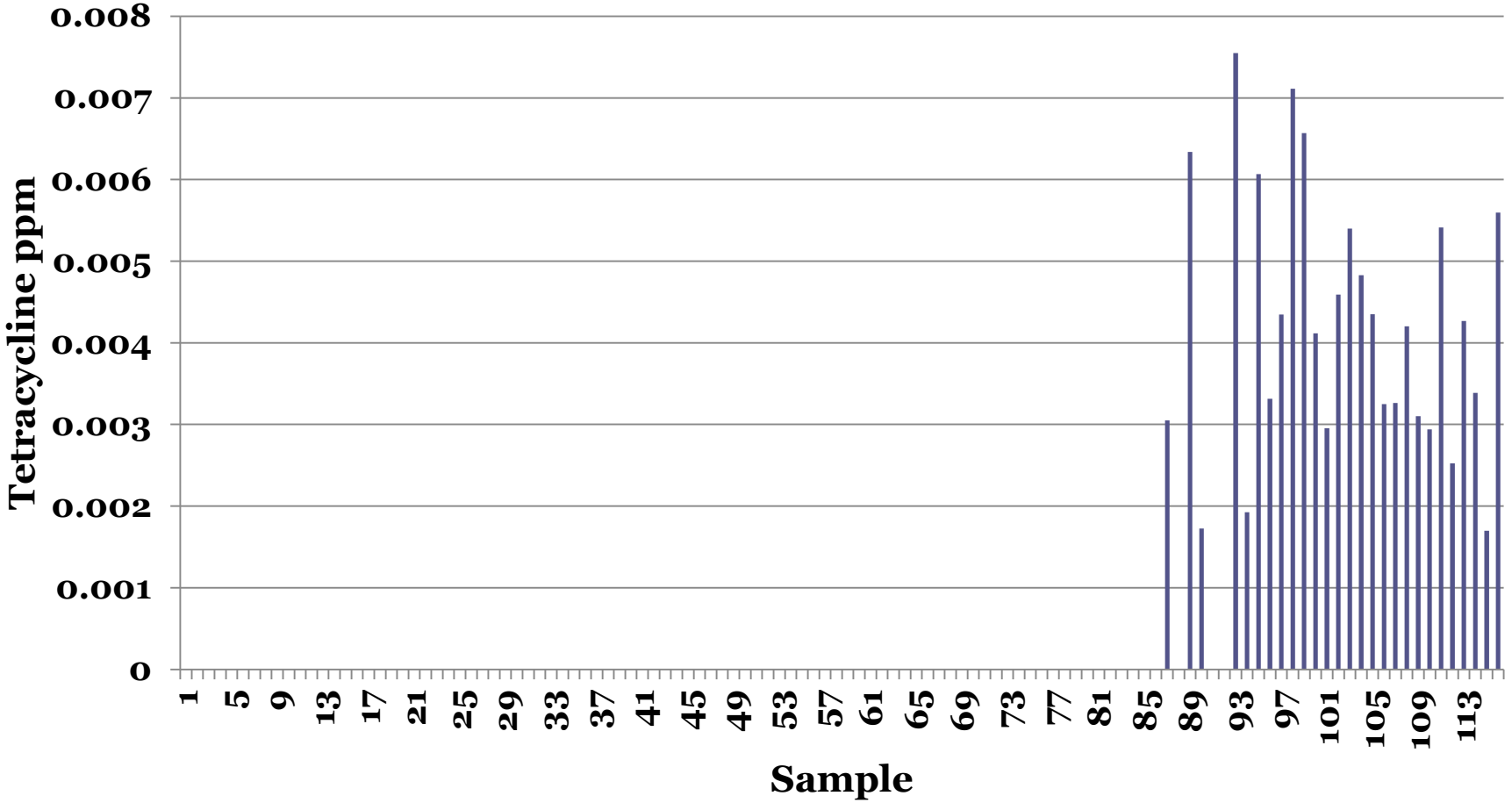


FDA approved feeding levels of virginiamycin for various species vs. levels detected in distillers grains samples

Antibiotic	Species	Min (ppm)	Max (ppm)
Virginiamycin	Chicken	5.5	22.0
Virginiamycin*	Swine	5.5	11.0
Virginiamycin	Turkey	11.0	22.0
Virginiamycin	Distillers grains samples	0	0.60

*Values presented as ppm for minimum and maximum allowed for swine derived from FDA clearance stated as g/ton for finishing swine consuming an average of 5.4 lbs feed/day.

Results – tetracycline residue concentrations

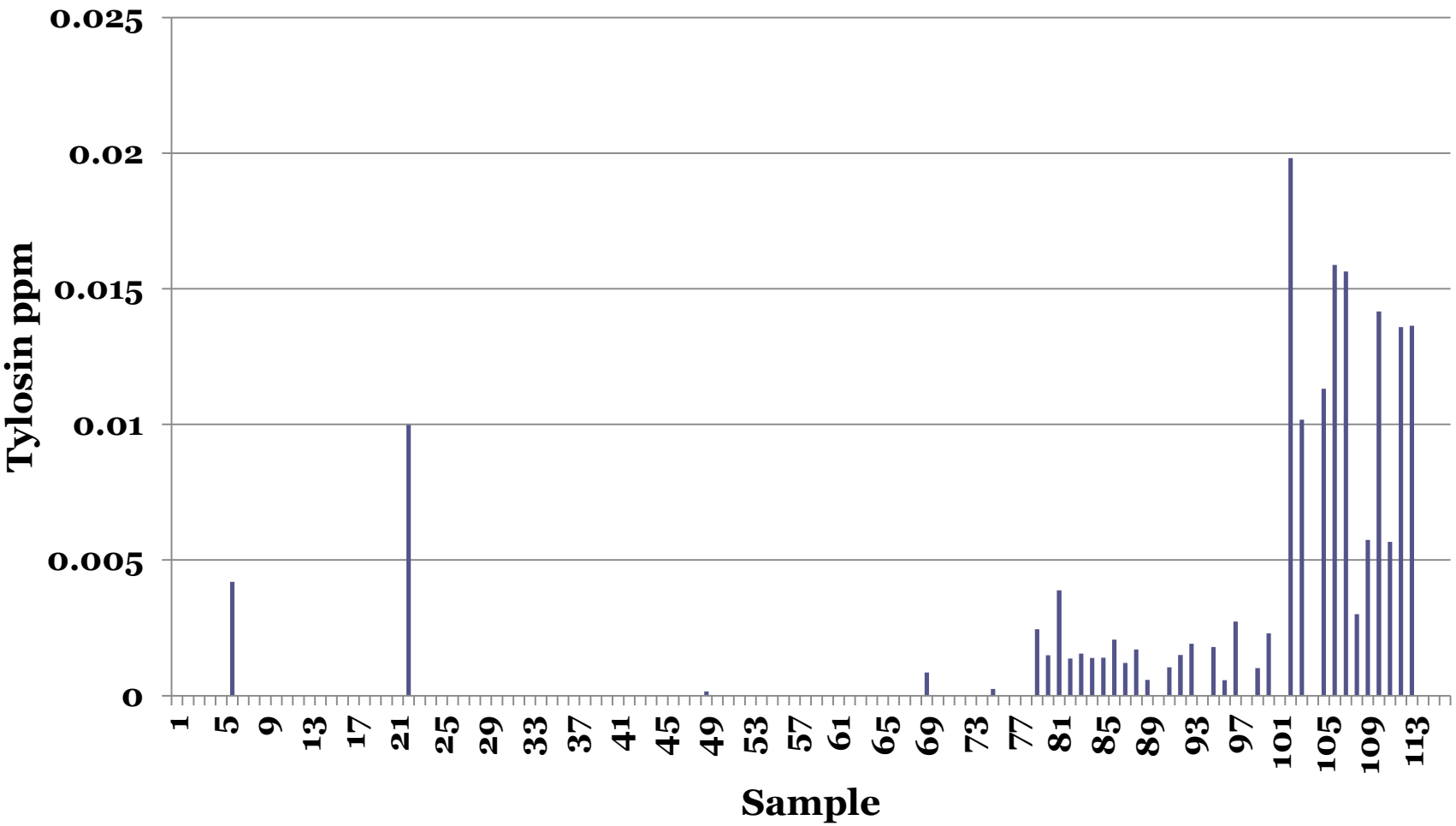


FDA approved feeding levels of tetracycline for various food animal species

Antibiotic	Species	Min (ppm)	Max (ppm)
Tetracycline	Cattle	7.5	7.5
Tetracycline	Chicken	11.0	55.1
Tetracycline*	Swine	11.0	55.1
Tetracycline	Turkey	11.0	55.1
Tetracycline	Distillers Grain Samples	0	0.007

*Values presented as ppm for minimum and maximum allowed for swine derived from FDA clearance stated as g/ton for finishing swine consuming an average of 5.4 lbs feed/day.

Results - tylosin residue concentrations

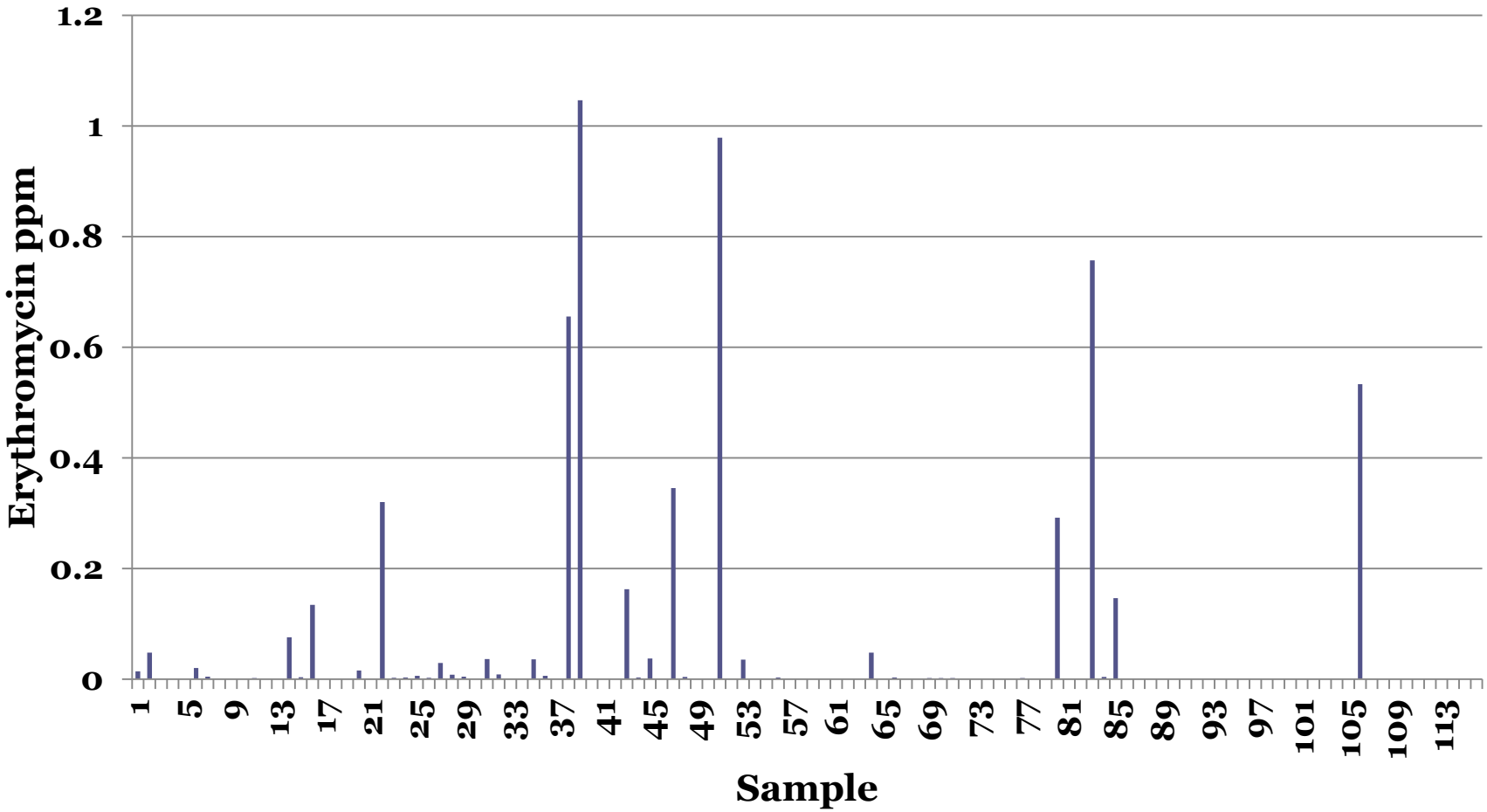


FDA approved feeding levels of tylosin for various species vs. levels detected in distillers grains samples

Antibiotic	Species	Min (ppm)	Max (ppm)
Tylosin	Cattle	8.8	11.0
Tylosin	Chicken	4.4	55.1
Tylosin	Layer Hen	22.0	55.1
Tylosin*	Swine	11.0	22.0
Tylosin	Distillers grains samples	0	0.02

*Values presented as ppm for minimum and maximum allowed for swine derived from FDA clearance stated as g/ton for finishing swine consuming an average of 5.4 lbs feed/day.

Results – erythromycin residue concentrations

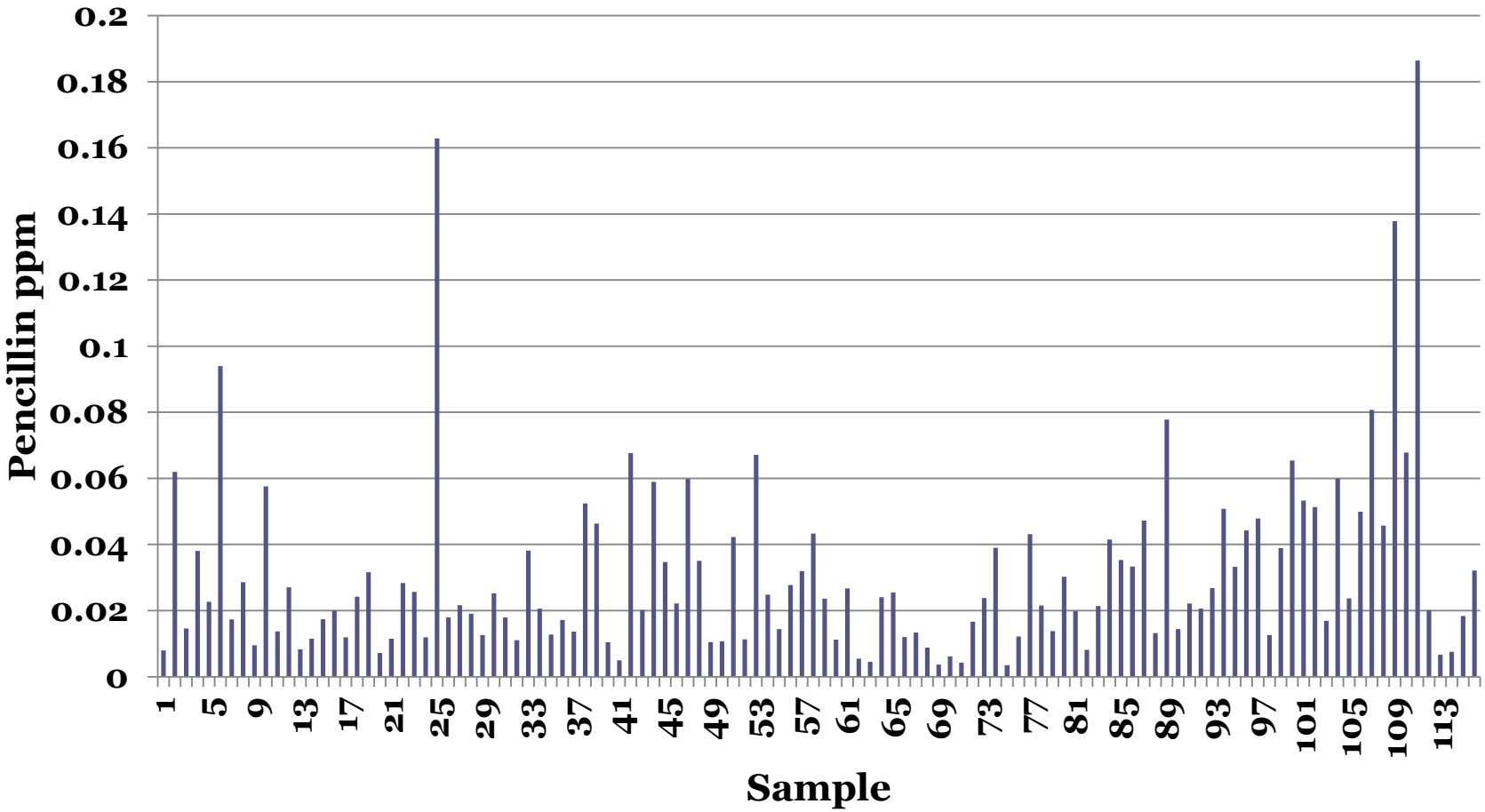


FDA approved feeding levels of erythromycin for various species vs. levels detected in distillers grains samples

Antibiotic	Species	Min (ppm)	Max (ppm)
Erythromycin	Cattle	3.7	3.7
Erythromycin	Chicken	5.1	20.4
Erythromycin	Layer	20.4	20.4
Erythromycin	Turkey	5.1	20.4
Erythromycin*	Swine	10.2	10.2
Erythromycin	Distillers grains samples	0	1.0

*Values presented as ppm for minimum and maximum allowed for swine derived from FDA clearance stated as g/ton for finishing swine consuming an average of 5.4 lbs feed/day.

Results – penicillin residue concentrations



FDA approved feeding levels of penicillin for various species vs. levels detected in distillers grains samples

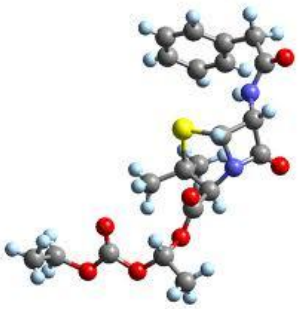
Antibiotic	Species	Min (ppm)	Max (ppm)
Penicillin G Procaine	Chicken	2.8	55.1
Penicillin G Procaine*	Swine	11.0	55.1
Penicillin G Procaine	Turkey	2.8	55.1
Penicillin G	Distillers grains samples	0.003	0.19

*Values presented as ppm for minimum and maximum allowed for swine derived from FDA clearance stated as g/ton for finishing swine consuming an average of 5.4 lbs feed/day.

Results – Do residues cause bacterial inhibition?

- To date, 116 sample extracts tested against sentinel bacteria
  - **1 sample** showed inhibition of *E. coli* ATCC 8739
    - Bacterial threshold = 10^4
  - **0 samples** showed inhibition of *L. monocytogenes* ATTC 19115

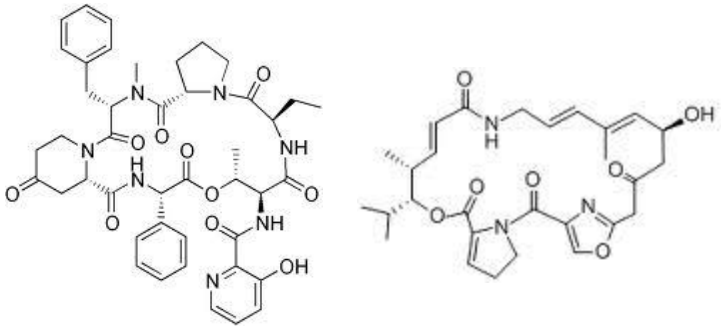
Penicillin G inactivation



- Poor stability below pH 5, most stable at pH 6.0 to 6.4
- Sharply inactivated at all pH levels (4.8, 4.5, 4.2, 4.0, 3.8) and faster at 35° C than at 25° C
  - Islam et al. (1998)
- Half life of 14 days when in solution at 24° C
- Easily inactivated by primary alcohols and some sugars
- At pH of 4.5 or 9.0, rate of inactivation increases 10-fold
- At pH 3.2 or 10.5, rate of inactivation increases 100-fold
- Completely degraded at pH 3 and a temperature of 37° C for 30 min.

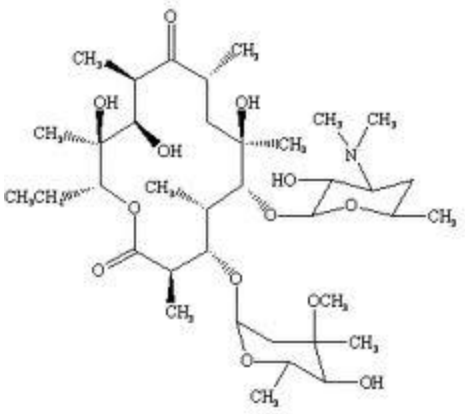
Virginiamycin inactivation in ethanol production

- Does not remain in ethanol after distillation
- Is destroyed at temperatures > 93° C
- Distillers grains dryer temperatures range from 93 to 232° C
- Inactivated during ethanol distillation
  - Hynes et al. (1997)



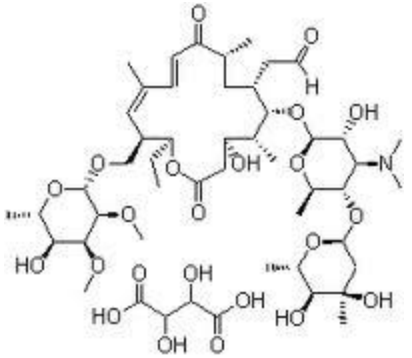
Erythromycin inactivation

- Insoluble in water, soluble in alcohol (Brisaert et al. 1996)
  - Stability decreases when alcohol content increases
- Thermally unstable especially in solutions containing water (Brisaert et al., 1996)
  - Degrades faster at higher temperatures
- Stability pH dependent (Brisaert et al., 1996)
  - Optimal pH values between 7-8
  - Stability decreases when pH decreases



Tylosin inactivation

- Most stable at pH 3.5 and 9.0 (Ter-Sarkisian et al., 1984)
  - Significant inactivation of antibiotic outside of these stability ranges
  - Inactivation increases with increased temperature level and exposure period



Summary of preliminary results for antimicrobial residues in DDGS

- % of samples with detectable residues
  - Virginiamycin - < 2%
    - < 1 ppm (GRAS limit)
  - Tetracycline -24%
  - Tylosin – 30%
  - Erythromycin – 37%
  - Penicillin – 100%

- No residues > 1 ppm, most were < 0.2 ppm
- Only 1 sample showed inhibition to *E. coli* ATTC 8739
- No samples showed inhibition to *L. monocytogenes* ATTC 19115

Conclusions

- Sources of tylosin and tetracycline residues are unknown
- Residue concentrations in distillers grains are extremely low
  - Much less than minimum approved FDA feed levels for food animals
- There is minimal concern of residues having inhibitory properties when using *E. coli* ATCC 8739 and *L. monocytogenes* ATCC 19115 as sentinel bacteria
- It is likely that the majority of antibiotic residues in distillers grains are inactivated during the distillers grains production process

Acknowledgements

- Funding provided by:
  - MN Corn Research and Promotion Council