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

G.C. Shurson¹, D.M. Paulus¹, A. DiCostanzo¹, G.I. Crawford², F. Diez-Gonzalez³, and R.C. Fink³

¹Department of Animal Science  
²University of Minnesota Extension  
³Department of Food Science and Nutrition  
University of Minnesota, St. Paul, U.S.A.
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.

- Dairy: 40%
- Beef: 40%
- Swine: 10%
- Poultry: 9%
- Other: 10%
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.
Results from 2008 FDA multi-state distiller’s grains sampling survey - unpublished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 > 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

- **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

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Species</th>
<th>Min (ppm)</th>
<th>Max (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virginiamycin</td>
<td>Chicken</td>
<td>5.5</td>
<td>22.0</td>
</tr>
<tr>
<td>Virginiamycin*</td>
<td>Swine</td>
<td>5.5</td>
<td>11.0</td>
</tr>
<tr>
<td>Virginiamycin</td>
<td>Turkey</td>
<td>11.0</td>
<td>22.0</td>
</tr>
<tr>
<td>Virginiamycin</td>
<td>Distillers grains samples</td>
<td>0</td>
<td>0.60</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Species</th>
<th>Min (ppm)</th>
<th>Max (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>Cattle</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Chicken</td>
<td>11.0</td>
<td>55.1</td>
</tr>
<tr>
<td>Tetracycline*</td>
<td>Swine</td>
<td>11.0</td>
<td>55.1</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Turkey</td>
<td>11.0</td>
<td>55.1</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Distillers Grain Samples</td>
<td>0</td>
<td>0.007</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Species</th>
<th>Min (ppm)</th>
<th>Max (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tylosin</td>
<td>Cattle</td>
<td>8.8</td>
<td>11.0</td>
</tr>
<tr>
<td>Tylosin</td>
<td>Chicken</td>
<td>4.4</td>
<td>55.1</td>
</tr>
<tr>
<td>Tylosin</td>
<td>Layer Hen</td>
<td>22.0</td>
<td>55.1</td>
</tr>
<tr>
<td>Tylosin*</td>
<td>Swine</td>
<td>11.0</td>
<td>22.0</td>
</tr>
<tr>
<td>Tylosin</td>
<td>Distillers grains samples</td>
<td>0</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Species</th>
<th>Min (ppm)</th>
<th>Max (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>Cattle</td>
<td>3.7</td>
<td>3.7</td>
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<tr>
<td>Erythromycin</td>
<td>Chicken</td>
<td>5.1</td>
<td>20.4</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Layer</td>
<td>20.4</td>
<td>20.4</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Turkey</td>
<td>5.1</td>
<td>20.4</td>
</tr>
<tr>
<td>Erythromycin*</td>
<td>Swine</td>
<td>10.2</td>
<td>10.2</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Distillers grains samples</td>
<td>0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Species</th>
<th>Min (ppm)</th>
<th>Max (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G Procaine</td>
<td>Chicken</td>
<td>2.8</td>
<td>55.1</td>
</tr>
<tr>
<td>Penicillin G Procaine*</td>
<td>Swine</td>
<td>11.0</td>
<td>55.1</td>
</tr>
<tr>
<td>Penicillin G Procaine</td>
<td>Turkey</td>
<td>2.8</td>
<td>55.1</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>Distillers grains samples</td>
<td>0.003</td>
<td>0.19</td>
</tr>
</tbody>
</table>

*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* ATCC 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