The influence of Tylosin supplementation on pathogen populations and liver abscess prevalence in feedlot cattle

Katlyn Holzer and Carla Weissend
Background

Antibiotic use and the potential for antibiotic resistance is a HOT topic today.

Public and private pressure to reduce or even eliminate antibiotic use in animal production.
• Certain classes of antibiotics used in animal production are also used in humans.

• Tylosin is:
  • A macrolide
  • Used for the reduction and prevention of liver abscesses
  • Used in approximately 71.2% of cattle in 1000+ head feedlots

• Are there unintended consequences regarding Tylosin?
Objective

• A blinded, randomized, controlled field trial was conducted to evaluate the effect of pre-harvest feeding strategies on feedlot cattle on:
  • Presence of *Salmonella enterica* in subiliac lymph nodes
  • Fecal microbial populations (*Salmonella enterica, Escherichia coli, Enterococcus*)
  • The prevalence of liver abscesses
Experimental Design

• Commercial steers (N = 2752 hd) were placed at a feedyard in the panhandle of Texas

• Cattle were fed from Spring to Fall of 2016 and harvested at a local commercial processing facility

• Treatments (n = 10 pens/trt)
  • 1) Finishing ration with Tylosin (90mg/hd/d) fed until harvest (Tyl)
  • 2) Finishing ration without Tylosin fed until harvest (NTyl)
Experimental Design

- Pen-Composited Feces: N = 20
- Subiliac Lymph Nodes: N = 300
- Abscessed Livers: N = 2739

Image: Nottingham Veterinary School
Presence and characteristics of *Salmonella enterica* recovered from subiliac lymph nodes of beef feedlot cattle in a randomized clinical trial of dietary additives

Katlyn L. Holzer, Carla J. Weissend, Kate L. Huebner, Jessica L. Metcalf, Ifigenia Geornaras, Ketih E. Belk Paul S. Morley, and Jennifer N. Martin
What is a Lymph Node?

• Lymph nodes are an important part of the immune system
• Lymph nodes act as a filter for the body
• Lymph (body fluid) enters the lymph node so that the immune cells can recognize and kill pathogens
Lymphatic System of Bovine Animals

That’s a lot of lymph nodes!
Salmonella in Lymph Nodes

- *Salmonella* has the ability to get into host cells and be able to survive in an intracellular environment. *Salmonella* is able to survive inside the immune cells of the lymph node (macrophages) that are trying to destroy it.

http://www.webmd.com/food-recipes/food-poisoning/tc/salmonellosis-topic-overview#1
Food Safety Implications of *Salmonella* in LNs

- Peripheral LNs are found in fat and can potentially be included into beef trimmings that can be used for ground beef.

- *Salmonella* has been found in LNs, which means that *Salmonella* can be found in beef trimmings.

- Antimicrobial carcass sprays ineffective on LN *Salmonella*.
Does Tylosin have an effect on *Salmonella* in Lymph Nodes?

- Antibiotics have an effect on the microbial community of a host.
- It is unknown if an alternation of the microbial community can have a limited effect on *Salmonella* and subsequently its distribution within the LNs.

Alternative??

Macrolide ∨ Gram Negative
Materials and Methods

• A total of 15 subiliac lymph nodes were taken from each pen.

• 15 LN x 20 pens = 300 SLN
Materials and Methods
Materials and Methods

- Rappaport-Vassiliadis
- XLT4
- BG S

http://wiki.ubc.ca/Course:PATH417:2015W1/Case_2/Student_8
Results

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Prevalence (%)(^1)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tylosin</td>
<td>86.0</td>
<td>(0.73-0.98)</td>
</tr>
<tr>
<td>No Tylosin</td>
<td>83.3</td>
<td>(0.71-0.96)</td>
</tr>
</tbody>
</table>

\(^1\) P = 0.77

- **84.6% of all SLNs were positive for *Salmonella enterica***.
- The prevalence of *Salmonella enterica* in SLNs did not differ among Tylosin exposure groups (P > 0.05).
- Range of prevalence by pen for *Salmonella enterica*: 33%-100%
- Median prevalence: 90%

- This data is part of a larger study
Results

• This is in agreement with previous research of *Salmonella* in the lymph nodes of cattle fed in the southern region of the United States:
  • 76.5% from a feedlot in Mexico
  • Approximately 30% in beef feedlot cattle in the southern U.S. during the fall
  • different feedyards with a range of 0% - 88.2%

• Feedlot cattle *Salmonella* prevalence is affected by region and season.
  • The southern region of the U.S. has a higher prevalence of *Salmonella* in LN.

Gragg et al 2013 and Haneklaus et al 2012
Ongoing work

• Characterization and antibiotic susceptibility of *Salmonella* isolates

• Microbiome analysis using 16S

More data!

Presence and characteristics of *Salmonella enterica*, *E. coli*, and *Enterococcus* recovered from feces and liver abscesses of beef cattle enrolled in a randomized clinical trial of dietary additives.
Liver Abscesses in Feedlot Cattle

- Largely originate from aggressive feeding of grains
  - Acidosis
  - Rumenitis
  - Bacteria travel to liver

- Consequences:
  - Reduced Feed Intake
  - Reduced ADG
  - Reduced Feed Efficiency

2011 BQA Average Liver Abscess Rate: 20.1%

Sources:
- Nagaraja and Chengappa, 1998
- Nottingham Veterinary School
- McKeith et al., 2012
Liver Abscesses in Feedlot Cattle

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Liver Abscesses in Feedlot Cattle

Liver abscesses cost the beef industry an estimated $15.8 million each year!

2011 BQA Average Liver Abscess Rate: 20.1%

Primary Causative Bacteria of Liver Abscesses

- Fusobacterium: 95-100%
- Trueperella: 32%
- Other: 49%

Using Tylosin

- Tylosin → Reduces Liver Abscesses
- There is some regional variability
- Public pressure to reduce use

Image: http://www.chemicalbook.com
The Impact of Tylosin Exposure

Tylosin

What are the food safety implications?
Bacteria of Interest

• Bacteria of Interest:
  • *E. coli*
  • *Salmonella enterica*
  • *Enterococcus* spp.

• How does Tylosin affect the microbial populations?
Materials and Methods

MacConkey → *E. coli*

Enterococcus → *Enterococcus*

Frozen Isolates

BGS

XLT4
Liver Abscesses in Feedlot Cattle

- **0**: Normal liver
- **A Minus**: 1-2, less than 2 cm in diameter or resolved abscess scars
- **A**: 2-4, 2cm to 4cm, well organized abscesses
- **A Plus**: more than 1 abscess > 4 cm
  or more than 4 abscesses > 2 cm

[Link to PDF]
Results

There were largely no differences in microbial populations between the Tylosin and No Tylosin groups

Fecal Populations of *E. coli* and *Enterococcus* populations (log cfu/g)

<table>
<thead>
<tr>
<th></th>
<th>Tylosin</th>
<th>No Tylosin</th>
<th>Range</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At Placement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>6.26</td>
<td>6.42</td>
<td>(5.30 – 7.09)</td>
<td>0.45</td>
</tr>
<tr>
<td><em>Enterococcus</em></td>
<td>4.23</td>
<td>4.89</td>
<td>(3.56 – 5.56)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>At Harvest</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>5.64</td>
<td>6.10</td>
<td>(4.00 - 6.54)</td>
<td>0.09</td>
</tr>
<tr>
<td><em>Enterococcus</em></td>
<td>4.23</td>
<td>4.01</td>
<td>(3.12 – 5.38)</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Data were analyzed using the PROC MIXED function in SAS
Results

- Cattle fed Tylosin are 1.5 times less likely to suffer from liver abscesses
  - **Adjusted Relative Risk Value: 1.5**
  - 95% Confidence Interval: (1.3-1.8)
  - **P-value: < 0.0001**

- Range of liver abscess prevalence by pen: 7.0% - 35.9%

**Effect of Tylosin supplementation on liver abscess prevalence**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Livers Abscessed</th>
<th>Total Number of Livers</th>
<th>Liver Abscess Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tylosin</td>
<td>212</td>
<td>1363</td>
<td>15.5%</td>
</tr>
<tr>
<td>No Tylosin</td>
<td>316</td>
<td>1376</td>
<td>23.0%</td>
</tr>
</tbody>
</table>

Data were analyzed using the PROC GENMOD function in SAS
Results

- Cattle fed Tylosin are **1.95 times less likely** to suffer from severe liver abscesses.
- Range of liver abscess prevalence by pen: **3.4% - 29.7%**

**Effect of Tylosin supplementation on liver abscess prevalence**

<table>
<thead>
<tr>
<th>Liver Score</th>
<th>Tylosin (N = 1363)</th>
<th>No Tylosin (N = 1376)</th>
<th>Odd Ratio</th>
<th>95% CI</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1151</td>
<td>1060</td>
<td>reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-</td>
<td>89</td>
<td>102</td>
<td>1.25</td>
<td>0.9 – 1.7</td>
<td>0.14</td>
</tr>
<tr>
<td>A</td>
<td>22</td>
<td>34</td>
<td>1.69</td>
<td>0.9 – 2.9</td>
<td>0.06</td>
</tr>
<tr>
<td>A+</td>
<td>101</td>
<td>180</td>
<td>1.95</td>
<td>1.5 – 2.5</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Total</td>
<td>212</td>
<td>316</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Ongoing Work

- Characterization and Susceptibility testing of *Salmonella, E.coli*, and *Enterococcus* populations

- Microbiome analysis (16S)
  - Liver abscesses
  - Carcass swabs
  - Beef trimmings
  - Arrival and Harvest Feces
Conclusions

This study suggests:

• that Tylosin inclusion has no influence on the overall prevalence of *Salmonella* in the SLNs

• that Tylosin inclusion has no influence on fecal *E.coli* and *Enterococcus* populations of fed beef cattle

• that Tylosin inclusion does serve to reduce the occurrence of liver abscesses in feedlot cattle.
Conclusions

• How Tylosin affects the microbial populations provides valuable information:
  • Identification of appropriate and effective alternatives
  • Shift in the antimicrobial susceptibility of *Salmonella*

• The importance of Tylosin for liver abscess reduction

• Investigating alternatives is increasingly important
Industry Implications

• This research will add to the body of knowledge regarding the impact feed additives have on the presence of liver abscesses and fecal and lymph node microbial populations.

• It will also contribute to understanding the microbiome shifts which may influence these outcomes.
Acknowledgements

• A big THANK YOU to...

Jennifer Martin
Geornaras
Jessica Metcalf
Graduate Students

Keith Belk
Paul Morley

Gina
CSU
Thank You!