Silage Additives: Where Are We Headed?

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A Reminder!

- Promotion of best forage/silage management practices on farm should be our first goal
- In general, most silage additives are only “tools” that should not be used instead of good management
Where Are We Headed With Silage Additives?
Where Silage Additives Can Help

Front-end

Rapid and maximum preservation of nutrients

Improved value

Detoxification

Back-end

Stable product during storage and feedout

Aerobic stability
Use of Lactic Acid Bacteria to Improve Nutritive Value and Aerobic Stability
How Do Inoculants Affect Animal Performance?

- Kung and Muck (1997): Approximately 50% of studies reviewed reported positive effects of inoculated silage on milk production or gain, averaging 3 to 5% increase.

- But how can LAB cause such increases?
How Do Inoculants Affect Animal Performance?

Comparison of the effects of *L. plantarum* MTD/1 on silage fermentation and animal performance across studies

<table>
<thead>
<tr>
<th>Animal Performance Improved</th>
<th>Fermentation Improved</th>
<th>Digestibility Improved</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Review of Weinberg and Muck (1996)
How Do Inoculants Affect Animal Performance?

Effects on gas production from \textit{in vitro} ruminal fermentation

- Approximately 2/3 of inoculated lucerne silages (14 strains/products) produced less gas than untreated control (Muck et al. 2007)

- An \textit{L. plantarum} strain on TMR silage (Cao et al. 2010)
  - Reduced methane vs. control (9.6, 10.5 L/kg DDM)
  - Increased propionate, reduced butyrate
**How Do Inoculants Affect Animal Performance?**

Effects on microbial biomass production from *in vitro* ruminal fermentation

<table>
<thead>
<tr>
<th>Inoculant Treatment</th>
<th>VFA (mM)</th>
<th>Gas (mL/g DM)</th>
<th>MBY (mg/g TDDM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>51.7</td>
<td>158</td>
<td>354&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LP-EF</td>
<td>50.3</td>
<td>158</td>
<td>355&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LP</td>
<td>50.5</td>
<td>157</td>
<td>379&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LPe</td>
<td>52.4</td>
<td>162</td>
<td>390&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LL</td>
<td>53.3</td>
<td>162</td>
<td>380&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Contreras-Govea et al. (2011)
How Do Inoculants Affect Animal Performance?

Increased ruminal microbial biomass production *in vivo*?

<table>
<thead>
<tr>
<th>Response</th>
<th>Control</th>
<th>LP</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM Intake, kg/d</td>
<td>25.4</td>
<td>25.8</td>
<td>0.07</td>
</tr>
<tr>
<td>Milk, kg/d</td>
<td>39.6</td>
<td>40.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Milk/DMI</td>
<td>1.56</td>
<td>1.57</td>
<td>0.38</td>
</tr>
<tr>
<td>Milk Urea N, mg/dL</td>
<td>12.7</td>
<td>11.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OM truly digested in rumen, kg/d</td>
<td>14.6</td>
<td>16.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apparent OMD, %</td>
<td>44.7</td>
<td>49.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Muck et al. (2011); Muck, et al., unpublished
How Do Inoculants Affect Animal Performance?

- Still uncertain
- Evidence for improved rumen microbial activity
- But not sure why
- One possibility may be shifts in amino acid profiles in the silage
  - Maize is low in lysine for example
  - Do LAB or can LAB be used to create a better amino acid profile?
How Do Inoculants Affect Animal Performance?

Bottom line:

- Increased milk production or gain is the biggest driver of ROI for using an inoculant.
- So, there is an incentive to better understand these effects and search for strains that help livestock use their rations more efficiently.
Examples of *Inoculants* to Improve the Aerobic Stability of Silages

- *L. buchneri* – acetic acid
- MiLAB – cyclic peptides
- Biomax 5 – unknown antifungal compounds
**Lactobacillus buchneri to Improve Aerobic Stability**

*Probably the most successful inoculant developed to improve aerobic stability*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yr1</th>
<th>Yr2</th>
<th>Yr3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>75(^b)</td>
<td>97(^b)</td>
<td>75(^b)</td>
</tr>
<tr>
<td>Homolactic A</td>
<td>51(^b)</td>
<td>70(^b)</td>
<td>104(^b)</td>
</tr>
<tr>
<td><em>L. buchneri</em> 40788</td>
<td>217(^a)</td>
<td>197(^a)</td>
<td>886(^a)</td>
</tr>
</tbody>
</table>

LB applied at 400,000 cfu/g

Means in columns with unlike superscript differ $P < 0.05$. Muck, 2004
Inoculants to Improve Aerobic Stability – *The Future*?

- Identify strains of LB with faster growth rates -> improve stability earlier
- Identify mechanisms to produce more robust and stable strains of LB for production and packaging
- Identify other organisms with antifungal activity *(and ID active compounds...controversial because of regulatory issues?)*
Inoculants to Improve Aerobic Stability – *The Future*?

- Identify heat/cold tolerant strains for use in challenging environments
- Identify strains that produce beneficial substances
  - Lysine for maize silages?
  - Other bioactive beneficial end products
Killer Yeasts for Improvements in Aerobic Stability – *The Future?*

- Protein or glycopeptide toxins that kill yeasts
- Interfere with proton motive force, inhibition of cell wall synthesis, inhibition of cell division
- *Saccharomyces, Kluyveromycaces, Hansenula, Candid, Pichia*
- Use of respiratory deficient mutants that don’t assimilate lactic acid Kitamoto et al., 1993
Chemical Additives to Improve Aerobic Stability
**Chemical Additives** to Improve the Aerobic Stability of Silages

- Organic acids
  - Propionic acid
  - Potassium sorbate
  - Sodium benzoate
- Others
  - Essential oils
Chemical Additives to Improve the Aerobic Stability of Silages – The Future?

- Reduction in commercial costs of current products
- Increase effectiveness of chemical additives to allow for reduced application rates (< 0.5 to 1 L/t)
- Identification of newer formulations and/or compounds (that are safe)
Specific Additive Needs for Sugarcane Silage

- Fermentation hampered by high epiphytic numbers of yeasts, high production of ETOH (low DM recovery), poor aerobic stability
## Meta Analysis of Effects of Additives on the Fermentation of Sugarcane Silage

<table>
<thead>
<tr>
<th></th>
<th>Ethanol (% DM)</th>
<th>DM losses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>7.6</td>
<td>17.3</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>10.3*</td>
<td>18.9</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>5.9</td>
<td>21.4</td>
</tr>
<tr>
<td><em>L. buchneri</em></td>
<td>5.8</td>
<td>19.3</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>5.8</td>
<td>21.1</td>
</tr>
<tr>
<td><em>CaO</em></td>
<td>1.4*</td>
<td>10.5*</td>
</tr>
</tbody>
</table>

Marcondes et al. (2011)
An Organic Acid Blend (Safesil) Improves DMR and Aerobic Stability of Sugarcane Silage

<table>
<thead>
<tr>
<th>Treatment (T)¹</th>
<th>Ctrl</th>
<th>LB</th>
<th>CaO</th>
<th>S2</th>
<th>S3</th>
<th>S5</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>5.9\textsuperscript{Aa}</td>
<td>5.2\textsuperscript{Aa}</td>
<td>4.8\textsuperscript{Aa}</td>
<td>4.7\textsuperscript{Aa}</td>
<td>2.6\textsuperscript{Bb}</td>
<td>2.6\textsuperscript{Ab}</td>
<td>0.18</td>
</tr>
<tr>
<td>100</td>
<td>2.1\textsuperscript{Bb}</td>
<td>3.3\textsuperscript{Bab}</td>
<td>2.8\textsuperscript{Bab}</td>
<td>3.9\textsuperscript{Aa}</td>
<td>3.8\textsuperscript{Aa}</td>
<td>2.2\textsuperscript{Ab}</td>
<td>1.42</td>
</tr>
<tr>
<td>DM recovery, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>75\textsuperscript{Ac}</td>
<td>76\textsuperscript{Ac}</td>
<td>90\textsuperscript{Ab}</td>
<td>97\textsuperscript{Aa}</td>
<td>98\textsuperscript{Aa}</td>
<td>99\textsuperscript{Aa}</td>
<td>8.60</td>
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<tr>
<td>100</td>
<td>75\textsuperscript{Ac}</td>
<td>75\textsuperscript{Ac}</td>
<td>90\textsuperscript{Ab}</td>
<td>85\textsuperscript{Bb}</td>
<td>98\textsuperscript{Aa}</td>
<td>100\textsuperscript{Aa}</td>
<td></td>
</tr>
<tr>
<td>Aerobic stability, h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>51\textsuperscript{Ab}</td>
<td>58\textsuperscript{Ab}</td>
<td>63\textsuperscript{Bb}</td>
<td>124\textsuperscript{Ba}</td>
<td>165\textsuperscript{Aa}</td>
<td>165\textsuperscript{Aa}</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>38\textsuperscript{Ac}</td>
<td>39\textsuperscript{Ac}</td>
<td>138\textsuperscript{Ab}</td>
<td>192\textsuperscript{Aa}</td>
<td>179\textsuperscript{Aab}</td>
<td>179\textsuperscript{Aab}</td>
<td></td>
</tr>
</tbody>
</table>

Da Silva et al., 2015
Enzymes – Improvements in Ruminal Starch-D and Fiber
Effect of Days of Ensiling on Ruminal Starch Digestion in Corn Silage
Activity of an Acidic Protease

% Activity

pH

3.6 3.8 4.0 4.2

a b c d
Correlation Between Protein Degradation and Starch-D in Corn Silages

\[ P < 0.0001 \]
\[ R^2 = 0.33 \]

Windle et al., 2014
Effect of an Exogenous Acidic Protease On In Vitro Ruminal Starch-D

Windle et al., 2014
Enzymes – Proteases – The Future?

- Cost of proteases must be reduced before they are commercially viable
- Identify LAB with high protease activity
Enzymes – Fibrolytic Based Enzymes for Improved Fiber Digestion

- Hemicellulase and cellulase complexes
- Ferulic acid esterase (FAE)
- Predigestion of fiber in the silo is questionable
Enzymes – Fibrolytic – The Future?

- Improving accessibility of fiber
- More research on FAE, arabinofuranosidase?
- Insertion of cellulase/xylanase/amylase genes into LAB?
  - Need for high level of expression and strain persistence
  - GMO status
Effect of 11CFT (*L. buchneri* Strain that Makes Ferulic Acid Esterase) on Aerobic Stability and NDF-D of Two Corn Silage Varieties

<table>
<thead>
<tr>
<th>Item</th>
<th>Hybrid 1</th>
<th>Hybrid 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>11CFT</td>
</tr>
<tr>
<td>NDF-D, % 48 h</td>
<td>56.5</td>
<td>58.1</td>
</tr>
<tr>
<td>Aerobic stability, h</td>
<td>58.7</td>
<td>116.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Kang et al., 2009
Control of Microbial Populations in Silage with Phages and Bacteriocins
Undesirable Microbial Populations in Silage May be Controlled with Phages and Bacteriocins

- **Phages**
  - Addition of phages to control undesirable bacteria, e.g. clostridia
  - Control of natural phage populations

- **Bacteriocins**
  - From:
    - *L. plantarum*
    - Propionibacteria
    - *L. buchneri*
    - Pediococcus
    - Enterococcus
Effects of *Streptococcus bovis* HC5 on the fermentation and dry matter recovery of elephant grass silage

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HC5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacteria, log cfu/g</td>
<td>5.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Yeasts and molds, log cfu/g</td>
<td>4.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.57&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DM recovery, %</td>
<td>81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Ferreira et al. (2013)
Inhibiting Clostridia

Future approach:

■ Selection of LAB that inhibit clostridia
  ■ General selection for inhibition
    ■ E.g., Saarisalo et al. (2007)
  ■ Specific inhibition by production of bacteriocins
    ■ E.g., Flythe and Russell (2004); Marcinakova and Laukova (2004)
Reducing Proteolysis in Grass and Legume Silages
Reducing Proteolysis in Grass and Legume Silages

Albrecht and Muck (1991)
Reducing Proteolysis in Grass and Legume Silages

Problem:

- Soluble nonprotein nitrogen in silages can make it challenging to create rations where N is used efficiently by ruminants.
Legume silages fed at 60% of ration DM with diets of similar CP, NDF content to mid-lactation Holsteins

<table>
<thead>
<tr>
<th>Item</th>
<th>Alfalfa</th>
<th>Red Clover</th>
<th>Birdsfoot Trefoil</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM Intake, kg/d</td>
<td>25.4</td>
<td>25.8</td>
<td>24.0</td>
<td>0.69</td>
</tr>
<tr>
<td>Energy Corrected Milk, kg/d</td>
<td>30.0</td>
<td>29.3</td>
<td>33.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ECM/DMI</td>
<td>1.23</td>
<td>1.18</td>
<td>1.43</td>
<td>0.02</td>
</tr>
<tr>
<td>Milk Urea N, mg/dL</td>
<td>11.4</td>
<td>11.0</td>
<td>9.7</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Hymes-Fecht et al. (2013)
Reducing Proteolysis in Grass and Legume Silages

Tannins

- What type(s) of tannin are best?
- How much tannin?
- Can it be applied as a silage additive?
- Can alfalfa or other forages be genetically modified to produce tannins?
Reducing Proteolysis in Grass and Legume Silages

Red Clover System

- Polyphenol oxidase (PPO) plus o-diphenol substrates produce quinones that bind to proteins
- Will we see a commercial GMO alfalfa with PPO?
- Will we see a GMO alfalfa with both PPO and an o-diphenol?
- Possible in PPO-containing grasses that o-diphenols may become silage additives.
Controlling Mycotoxins
Controlling Mycotoxins

Problem

- Mycotoxins are produced by various filamentous fungi
  - Typically by Fusarium, Penicillium and Aspergillus species in silages
- Associated with various health issues in livestock and farm workers
- May be on the crop at ensiling or produced in the silo if there is sufficient oxygen
# Effect of Damaging the Ear on Mycotoxins in Fresh Chopped Corn Plants

<table>
<thead>
<tr>
<th>Item</th>
<th>Undamaged</th>
<th>Damaged 9 d before harvest</th>
<th>Damaged 27 d before harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>DON, ppb</td>
<td>902</td>
<td>1,670</td>
<td>20,628</td>
</tr>
<tr>
<td>Fumonisin, ppm</td>
<td>2</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Zearlenone, ppb</td>
<td>101</td>
<td>75</td>
<td>2644</td>
</tr>
</tbody>
</table>

Teller et al., 2012
Controlling Mycotoxins

- Normally little change in concentration during ensiling if the silo is managed well
- Can the silo be used to reduce mycotoxin concentrations?
  - Using agents fed to livestock already?
  - LAB that could detoxify?
  - Enzymes effective at breaking down?
Conclusions

- Silage additives are powerful tools that can be used to maintain and possibly improve forage quality

- Where we are specifically headed is unknown....

- But....
The challenge to you....

Tell us what the future holds!