Making Milk with Forage: Preserving the Quality of Silage Through Improved Aerobic Stability  
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Dairy Nutrition & Silage Fermentation Lab

Factors Affecting the Quality Silage
- Maturity at harvest
- Type of fermentation
- Aerobic stability during storage and feed out

Ideal Fermentation and Good Storage Conditions

<table>
<thead>
<tr>
<th>Front end fermentation</th>
<th>Storage or Feed out</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Air sugars</td>
<td>No Air Stable, high quality</td>
</tr>
<tr>
<td>lactic acid</td>
<td>acetic acid</td>
</tr>
<tr>
<td>pH</td>
<td></td>
</tr>
</tbody>
</table>

Days of Ensiling  
~90 – 120 F

Example of an Ideal Fermentation – but Poor Aerobic Stability

<table>
<thead>
<tr>
<th>Fermentation</th>
<th>Storage or Feed out</th>
</tr>
</thead>
<tbody>
<tr>
<td>No air sugars</td>
<td>Exposure to Air</td>
</tr>
<tr>
<td>lactic acid</td>
<td>acetic acid</td>
</tr>
<tr>
<td>pH</td>
<td></td>
</tr>
</tbody>
</table>

Days of Ensiling  
>140 F
What is Aerobic Stability?

- Definition of aerobic stability: The amount of time a silage remains stable (and does not spoil) after it is exposed to air under defined conditions.

  - The longer a silage stays stable after exposure to air, the better

What Causes Aerobic Spoilage?
Air and Bad Yeasts!

- Silage is exposed to air
  - Yeasts ‘wake up’ and degrade lactic acid
  - Numbers of yeasts increase
    - Highly degradable nutrients are destroyed
  - Heat is produced
    - pH increases
      - Molds/bacteria ‘wake up’ causing further spoilage
  - More heating
    - Massive spoilage

But I pack my silo well...I don’t need worry ... right?

- Air penetrates into the face of a well packed silo as much as 3 ft!
- This means...
  - If you remove 6 inches a day...silage you see has been exposed to air for 6 days
  - If you remove 12 inches a day...silage you see has been exposed to air for 3 days
Molds are NOT responsible initiating aerobic spoilage!!!!

Kung, 2004

All Types of Yeasts in Silages are Undesirable

Fermenters –
• Glucose -> ethanol + CO2
• Saccharomyces sp.
• Large DM losses

Lactate Utilizers –
Lactic acid -> CO2 + H2O
• Candida sp.
• Hansenula sp.
• Pichia sp.
• Aerobically spoils silages – low intakes, low nutritive value

Lactate Utilizing Yeasts Primarily Initiate Aerobic Spoilage

- Most common initiating spoilage microbe:  
  Lactating utilizing yeasts –
  Lactic acid -> carbon dioxide and water

- Spoilage microbe sometimes found in corn silages:  
  Acetobacteria –
  Lactic acid -> acetic acid -> carbon dioxide and water

Why Should We Care About the Aerobic Stability of Silages?

- Silage can undergo a perfect fermentation but...if followed by exposure to air, can result in poor quality feed

- Aerobic spoilage may account for more than 50% of total DM losses in a silo
Why Should We Care About the Aerobic Stability of Silages?

- Spoilage can occur during storage and feedout
- Spoiled silage can result in:
  - Production of undesirable end products
  - Depress nutrient intake and production
  - Reduce farm income

What are the Main Factors Affecting Aerobic Stability

- Air - Porosity (density) of the silage
- Numbers of lactate utilizing yeasts
- Ambient temperature

Ambient Temperature Affects Rate of Spoilage of a TMR

How do initial populations of yeasts affect time to spoilage?

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time to Spoilage (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 F</td>
<td>12 h</td>
</tr>
<tr>
<td>85 F</td>
<td>60 h</td>
</tr>
</tbody>
</table>
Relationship Between Numbers of Yeasts in Corn Silage and Aerobic Stability

- Number of hours before the silage mass increases 2°C above baseline after exposure to air.

Aerobically Spoiling Silage Often Reaches Temps above 130-140 F....

Aerobically Spoiling Silage
Aerobically Spoiled Silage Stored for Months

Feeding Aerobically Spoiled Silages Depresses Intakes and Reduces Digestion in Steers

- Spoiled Silage, % of DM -

<table>
<thead>
<tr>
<th>Item</th>
<th>0</th>
<th>5.4</th>
<th>10.7</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td>17.6</td>
<td>16.3</td>
<td>15.4</td>
<td>14.7</td>
</tr>
<tr>
<td>NDF dig., %</td>
<td>63.2</td>
<td>56.0</td>
<td>52.5</td>
<td>52.3</td>
</tr>
</tbody>
</table>

Whitlock and Bolsen, 2001 KSU

Effect of Feeding a Spoiling TMR to Heifers

- Control vs Spoiling TMR fed to heifers
- Fed during the winter
- Spoiling TMR ranged from 35 to 54°C at feeding

Nutrient Analysis of TMR

<table>
<thead>
<tr>
<th>Item</th>
<th>Fresh TMR</th>
<th>Spoiling TMR</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>48.89</td>
<td>49.37</td>
<td>0.59</td>
</tr>
<tr>
<td>CP, %</td>
<td>10.57</td>
<td>11.19</td>
<td>0.27</td>
</tr>
<tr>
<td>Soluble protein, % CP</td>
<td>42.49</td>
<td>38.80</td>
<td>0.11</td>
</tr>
<tr>
<td>ADF, %</td>
<td>24.87</td>
<td>24.03</td>
<td>0.23</td>
</tr>
<tr>
<td>NDF, %</td>
<td>41.27</td>
<td>40.66</td>
<td>0.54</td>
</tr>
<tr>
<td>In vitro 30 h NDF-D, %</td>
<td>63.65</td>
<td>61.46</td>
<td>0.49</td>
</tr>
<tr>
<td>NDF</td>
<td>26.58</td>
<td>28.22</td>
<td>0.26</td>
</tr>
<tr>
<td>Starch, %</td>
<td>80.01</td>
<td>78.69</td>
<td>0.20</td>
</tr>
</tbody>
</table>

2013 Windle and Kung
### Fermentation Analysis and Numbers of Yeasts in TMRs Fed to Heifers

<table>
<thead>
<tr>
<th>Item</th>
<th>Fresh TMR</th>
<th>Spoiling TMR</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.16</td>
<td>5.17</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>WSC, %</td>
<td>2.46</td>
<td>1.85</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lactic acid, %</td>
<td>4.17</td>
<td>2.59</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Acetic acid, %</td>
<td>0.97</td>
<td>0.64</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ethanol, %</td>
<td>5.82</td>
<td>6.07</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Yeasts, log(_{10}) cfu/g</td>
<td>5.03</td>
<td>7.82</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

2013 Windle and Kung

107,151 yeasts/g → 66,069,345 yeasts/g

### Numbers of Yeasts in Rumen Fluid

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yeasts, log(_{10}) (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh TMR</td>
<td>4.16, 416,000</td>
</tr>
<tr>
<td>Spoiled TMR</td>
<td>&gt;24,500,000</td>
</tr>
</tbody>
</table>

• Different from Fresh TMR, \(P < 0.01\)

2013 Windle and Kung

### Dry Matter Intake of Heifers Fed Fresh vs. Aerobically Spoiling TMR

<table>
<thead>
<tr>
<th>Dry Matter Intake, kg/d</th>
<th>Unspoiled TMR</th>
<th>Spoiled TMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.8 lb/d</td>
<td>23.2 lb/d</td>
<td></td>
</tr>
</tbody>
</table>

• Different from Fresh TMR, \(P < 0.01\)

2013 Windle and Kung

### In Vitro 12-hr Digestibility of NDF (% of NDF) from a TMR Incubated with a Spoilage Yeast

<table>
<thead>
<tr>
<th>NDF-D (% of NDF)</th>
<th>Control</th>
<th>Log 4.4</th>
<th>Log 6.4</th>
<th>Log 8.4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>43.9(^a)</td>
<td>42.1(^{ab})</td>
<td>40.7(^{b})</td>
<td>33.9(^{c})</td>
</tr>
</tbody>
</table>

Colonies forming units of yeasts/mL of rumen fluid

Santos et al., 2011
What Do We Really Know About These Wild Yeasts in Silages?

- When yeasts are high → low intakes, low fat tests, etc.
- High numbers of yeasts are a “marker”
- Actual reasons for observed animal effects unknown
- Could be:
  - Taste?
  - Smell?
  - Toxins?
  - Competition with rumen bugs?
  - Immunological effect?

How Do We Minimize Air and Spoilage Yeasts in Silages?

- Pack quickly
- Pack tightly
- Seal quickly
- Plastic and weights

Minimizing Air is Silos –

- Porosity, (not DM density) controls air in the silo
- Porosity should be < 40%
- To achieve this bulk density should be not < than 44 lb as fed/ cu ft.

- For 30% DM forage = 13.2 lb DM/cu ft
- For 40% DM forage = 17.6 lb DM/cu ft

Delayed Filling Increases Yeasts and Molds on Corn Forage

Hirsch and Kung, 1999
**Effect of Packing Density on Yeasts in Lucerne Silage (with homolactic inoculant)**

Loose pack = 9.4 lb DM cubic ft
Tight pack = 14.3 lb DM cubic ft

![Graph showing the effect of packing density on yeasts in lucerne silage with homolactic inoculant.](image)

- U-T: Untreated tight
- IN-T: Inoculated tight
- U-L: Untreated loose
- IN-L: Inoculated loose

**Oxygen Barrier Plastic Can Reduce Aerobic Losses During Storage**

<table>
<thead>
<tr>
<th></th>
<th>Single Layer PE</th>
<th>Double Layer PE</th>
<th>OB Film</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM loss, %</td>
<td>14.4</td>
<td>12.5</td>
<td>7.4</td>
</tr>
<tr>
<td>Surface mold growth, in</td>
<td>6</td>
<td>3.7</td>
<td>0.0</td>
</tr>
</tbody>
</table>

- Single layer = 4.92 mil plastic
- Double layer = 4.92 mil plastic (9.84 mil)
- OB film = Single layer 1.77 mil oxygen barrier plastic

Lynch and Kung, 2001

Wilkinson and Rimini (2002)

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**Manage the Feeding Face to Minimize Aerobic Spoilage**

- Remove sufficient silage each day to prevent spoilage ~ 12 in/d
- More in hot weather and for drier and poorly packed silages
- Keep face clean, minimize face damage
- Knock down only enough silage to feed

---

**Keep Air From Penetrating into the Silage Mass**

- Remove sufficient silage each day to prevent spoilage ~ 12 in/d
- More in hot weather and for drier and poorly packed silages
- Keep face clean, minimize face damage
- Knock down only enough silage to feed
Additives to Control Yeasts in Silages

- A low pH and lactic acid alone will not control yeasts in silages....

Mechanisms of Improving the Aerobic Stability of Silages with Additives

- Direct addition of production of (by added microbes) of organic acids with antifungal activity
- Production of other antifungal compounds (by added microbes)
  - e.g., cyclic dipeptides

Improving Aerobic Stability – Antifungal Mode of Action of Organic Acids

- Buffered propionic acid, potassium sorbate, sodium benzoate, etc.: 0.05 - 0.20 %
- Some synergistic effects of combinations
- More effective at low pH because of the undissociated form is more toxic
  - Lowering of internal cell pH
  - Possible direct effects on fermentation pathways
  - Decrease supply of ATP
  - Alter transport across cell membranes

pH Affects the Activity of Antifungal Acids

\[ p_{ka} = pH \text{ of acid at half dissociation } [A-]=[HA] \]

\[
\begin{align*}
H^+ \text{ on} & \quad \text{ionized} \\
\text{Undissociated} & \quad \text{HA} \\
R-COOH & \quad \text{pH scale} \\
\text{pka} & \quad \text{pk}_{a} \\
H^+ \text{ off} & \quad \text{ionized} \\
\text{Dissociated} & \quad A^- \\
R-COO & \quad \text{R-COO and H}^+
\end{align*}
\]
Effect of pH Gradient on Accumulation of Dissociated Organic Acid Anions (XCOO⁻)

When Are Weak Organic Acids Most and Least Effective?

When the pH is one unit less than the pKa, then about 90% is in the effective form.

When the pH is 1 unit greater than the pKa, only about 10% is in the effective form.

Relationship Between pH and Form of Acids

<table>
<thead>
<tr>
<th>Preservative</th>
<th>pKa</th>
<th>% undissociated acid at pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>4.74</td>
<td>99 95 63 33</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>2.74</td>
<td>64 15 1.7 0.5</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>4.19</td>
<td>98 83 33 13</td>
</tr>
<tr>
<td>Prop acid</td>
<td>4.87</td>
<td>100 96 70 43</td>
</tr>
<tr>
<td>Sorbic acid</td>
<td>4.76</td>
<td>99 95 65 37</td>
</tr>
</tbody>
</table>

Issues with Adding Chemical Additives to Improve Aerobic Stability of Silages

- Relatively high costs
- Requires significantly more water for application (usually 1 to 2 liters/ton or more of wet forage) than microbial inoculants (as low as 40 ml/ton with low volume applicators)
Improving the Aerobic Stability of Silages with Additives

- Microbial inoculants
  - Homolactic acid inoculants (alone) can often make aerobic stability worse

**Aerobic Stability of Orchardgrass Silage**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yr 1</th>
<th>Yr 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>178b</td>
<td>198b</td>
</tr>
<tr>
<td>H-inoculant A</td>
<td>46c</td>
<td>176b</td>
</tr>
<tr>
<td>H-inoculant B</td>
<td>44c</td>
<td>203b</td>
</tr>
<tr>
<td>H-inoculant C</td>
<td>42c</td>
<td>not tested</td>
</tr>
</tbody>
</table>

- Because organic acid production is shifted primarily to lactic acid

Improving Aerobic Stability with *Lactobacillus buchneri*

- Naturally occurring bacterium that converts small amounts of lactic acid to acetic acid
  - Acetic acid is highly antifungal – decreases numbers of yeasts
  - Identified by Muck and Spoelstra

Pathway of Lactic Acid Degradation by *L. buchneri*

(Oude Elferink et al., 2001)

- Lactic acid $\rightleftharpoons$ lactaldehyde $\rightleftharpoons$ 1,2-propanediol
- Pyruvic acid $\rightleftharpoons$ CO$_2$
- Acetate CoA $\rightleftharpoons$ acetaldehyde $\rightleftharpoons$ ethanol
- Acetic acid

*production can be equivalent to 7 - 12 lb of acetic per ton of wet 35% DM silage*

*L. buchneri 40788* Field Study on Farms in the Midwest USA

- Corn silage samples
- Collected from dairy farms
- 15 farms using no inoculant
- 16 farms using an inoculant containing either *L. buchneri* (LB) 40788 alone or LB and *P. pentosaceus* (LBC)
Spoilage Yeasts in Corn Silages Untreated or Treated with *L. buchneri* 40788 From Dairy Farms in the US

<table>
<thead>
<tr>
<th>Item</th>
<th>Untreated</th>
<th>LB-40788</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spoilage yeasts,</td>
<td></td>
<td></td>
</tr>
<tr>
<td># per g of silage</td>
<td>320,000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43,000&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>P < 0.05

Mari et al., 2009

Aerobic Stability of Maize Silages Untreated or Treated with *L. buchneri* 40788 From Dairy Farms in the US

Effects of *L. buchneri* + *P. pentosaceus* on the Aerobic Stability of Maize Silage – 5 Replicated Studies from Different Locations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aerobic Stability, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>44</td>
</tr>
<tr>
<td><em>L. buchneri</em> 40788</td>
<td>267</td>
</tr>
<tr>
<td><em>L. buchneri</em> 40788 + <em>P. pentosaceus</em></td>
<td>255</td>
</tr>
</tbody>
</table>

Schmidt et al., 2006

Some (+) & (-) of Organic Acids (OA) and *L. buchneri* (LB)

(-) OA - must be undissociated to be active - less active when pH is high

(+) OA - more stable product during storage and application

(+) OA - No reliance on an organism having to compete in silage and produce antifungal compounds

(-) LB - must be alive, survive fermentation and produce adequate amts of acetic acid…

(+) LB – lower cost and volume applied than OA
Silages That Are Most Prone to Aerobic Spoilage that may Benefit from Silage Additives

- High moisture maize
- Maize and barley silage
- Silages with high DM (>40%DM)
- Silage fed during warm weather (summer, etc.)
- Silages fed out slowly
- Silage that will be moved between silos
- Silage fed from intermediate feeding piles

L. Kung, Univ. of Delaware

Summary

- In order to maintain forage quality, silages should ferment well and be aerobically stable
- Yeasts that metabolize lactic acid under aerobic conditions are the primary initiators of spoilage
- Basic silo management and various additives have the potential to minimize aerobic spoilage of silages