The Effects of an Exogenous Protease on the Fermentation and Nutritive Value of Corn Silage Stored at Two Temperatures

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Starch Digestion is Highly Correlated with Milk Production

\[ y = 0.1285x + 20.582 \]

\[ R^2 = 0.7032 \]

Firkins et al., 2001
Accessibility of Starch in Feeds

• Starch must be accessible by bacteria in the rumen and intestine of the cow for fermentation and digestion, respectively.

• Two primary factors limit the access to starch
  – Pericarp
  – Protein/starch matrix
The Protein/Starch Matrix

- Starch is embedded in a prolamin protein matrix
  - Major amino acid: proline
  - Hydrophobic, so difficult to digest

- Acts as a barrier, hindering access to starch

- Starch-D decreases 0.86 percentage units for each unit increase in prolamin protein content (as a percent of starch)
Proteolysis of the Protein/Starch Matrix During Storage Results in Increases in Starch-D

Prior to ensiling

After 240 d of ensiling

Hoffman et al., 2011
Using a Protease to Increase Starch-D

Young et al., 2012
Effect of Temperature on Fermentation

- Protease activity is affected by pH as well as temperature
- Forages are often harvested in hot weather
- Silages heat during fermentation and retain heat during storage
- Temperature affects fermentation
  - Cool temperatures may retard fermentation
  - Warm temperatures (>40°C) may cause an altered or restricted fermentation
Objectives

- To evaluate the potential use of exogenous proteases to improve ruminal starch digestion after short periods of ensiling.

- To determine if the temperature of storage affects ruminal starch digestion with or without an exogenous protease.
Materials and Methods

• Whole plant corn was harvested at about 37.3% DM

• Forages ensiled in 5 replicated bag silos per treatment and opening

• Treatments
  – Additive: Control (C) or Protease (EZ, 2000 ppm)
  – Storage Temperatures: 22°C or 40°C

• Silages were ensiled for 0, 2, 7, 45, 90 d
Materials and Methods

• Silages analyzed for standard nutritive components and 7h *in vitro* ruminal starch digestibility (IVSD, 3 mm particle size).

• Protease activity measured using hemoglobin as a substrate
  – pH 3.6, 3.9, 4.2
  – 4°C, 22°C, 40°C

• Statistics
  – 2 × 2 × 5 factorial arrangement of treatments
  – Analyzed using the Fit Model procedure of JMP
  – Significance declared at $P \leq 0.05$
Protease Information

• The experimental metalloprotease was supplied by Novozymes, Bagsvaerd, Denmark

• Carboyhydrase activity analysis: no detectable amylase, xylanase, or endoglucanase activity
Activity of the Protease at Different pH and Temperatures

% Activity

<table>
<thead>
<tr>
<th>pH</th>
<th>Activity (°C)</th>
<th>4°C</th>
<th>22°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6</td>
<td>a</td>
<td>b</td>
<td>c</td>
<td></td>
</tr>
<tr>
<td>3.9</td>
<td>b</td>
<td>d</td>
<td>e</td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>c</td>
<td>f</td>
<td>ef</td>
<td></td>
</tr>
</tbody>
</table>

Legend:
- 4°C
- 22°C
- 40°C
### Fermentation after 90 d

<table>
<thead>
<tr>
<th></th>
<th>Cool Untreated</th>
<th>Cool Treated</th>
<th>Hot Untreated</th>
<th>Hot Treated</th>
<th>SEM</th>
<th>Temp</th>
<th>Enz</th>
<th>Temp x Enz</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.65</td>
<td>3.58</td>
<td>3.59</td>
<td>3.60</td>
<td>0.02</td>
<td>0.28</td>
<td>0.15</td>
<td>0.10</td>
</tr>
<tr>
<td>Lactic acid, %</td>
<td>5.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31</td>
<td>&lt;0.01</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>Acetic acid, %</td>
<td>0.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.73&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.05</td>
<td>&lt;0.01</td>
<td>0.15</td>
<td>0.02</td>
</tr>
<tr>
<td>WSC, %</td>
<td>0.89</td>
<td>0.89</td>
<td>2.94</td>
<td>3.38</td>
<td>0.34</td>
<td>&lt;0.01</td>
<td>0.53</td>
<td>0.53</td>
</tr>
<tr>
<td>Yeast, cfu/g</td>
<td>4.15</td>
<td>4.39</td>
<td>ND</td>
<td>1.47</td>
<td>0.23</td>
<td>&lt;0.01</td>
<td>0.08</td>
<td>0.19</td>
</tr>
</tbody>
</table>

- No effect of temperature/protease on pH
- Fermentation was altered in Hot silages:
  - Lower contents of lactic and acetic (esp. in untreated silages)
  - Higher concentrations of WSC
- Less yeasts in Hot silages
Starch Digestibility

% IVSD vs Days of Ensiling

- 40°C – C
- 22°C – EZ
- 22°C – C
- 40°C – EZ

Days of Ensiling

0 20 40 60 80 100

40 50 60 70
Conclusions

• Although temperature does affect proteolysis during ensiling (as measured by sol-N), temperature alone does not appear to affect the increase in IVSD that occurs naturally with time.

• Adding an exogenous protease to corn silage at the time of ensiling did not result in better IVSD during the very early stages of fermentation.

• However, by 45 d, protease addition resulted in increased IVSD compared to untreated silages and the effect was greatest at 40°C.
Conclusions

• By 90 d of ensiling, IVSD was higher and similar in protease treated than in untreated silage.

• Adding an exogenous protease at the time of ensiling has the potential to improve IVSD after a moderate length of ensiling.

• Further research is needed to establish whether a lower dose of enzyme that would be economical can be established.
Thank you
Effect of Days of Ensiling on Starch Digestion in Corn Silage
Effects of a Protease Added to High Moisture Corn Prior to Ensiling

- **Starch-D**
- **Prolamin Proteins, % DM**

- **Days of ensiling**
- **Days of Ensiling**

- **Untreated**
- **Treated**

Graph showing the effects of protease addition on starch-D and prolammin proteins over days of ensiling.