Redesigning alfalfa for improved protein utilization: o-quinones vs. condensed tannins

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What are o-quinones and condensed tannins and why are they important?
Tannins and o-quinones are natural polyphenolic compounds that bind to proteins.

**Condensed tannins**
- Hydrogen bonding and hydrophobic interactions with protein are partly/mostly reversible.

**o-Quinones**
- Covalent bonding with protein is not reversible.
Why are o-quinones and condensed tannins needed in alfalfa forage?
Alfalfa forage proteins undergo excessive proteolysis during ensiling and rumen fermentation (~80% of protein can be degraded)

- Residual proteins are of inferior nutritional value
Degraded proteins are converted to rumen microbial proteins of high nutritional value, **BUT** up to one-third of alfalfa protein is ultimately excreted in urine as urea by dairy cattle

- Urea excretion—metabolic cost to the animal
- Ammonia from urea readily lost to the atmosphere
- Farmers often substitute alfalfa with protein sources derived from row crops or feed protein supplements at extra cost
More plant protein flowing to the gastrointestinal tract

Condensed tannins or o-quinones could enhance the utilization of alfalfa protein

Less protein breakdown in the rumen

Increased intestinal absorption of amino acids
Production of **condensed tannins or o-quinones** in alfalfa forage should also improve farm profitability and lessen environmental impacts.
Big claims, any evidence?
Can predict whole farm impacts of polyphenol-containing alfalfa on dairy farms using the Integrated Farm System model.
### Predicted performance if alfalfa silage comprised ~25% of the diet for lactating cows

<table>
<thead>
<tr>
<th></th>
<th>Net return $/cow</th>
<th>Milk yield lb/cow</th>
<th>Soybean fed lb/cow</th>
<th>Corn fed lb/cow</th>
<th>Total N loss lb/cow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal alfalfa</td>
<td>1,185</td>
<td>27,460</td>
<td>2,660</td>
<td>4,820</td>
<td>98</td>
</tr>
<tr>
<td>Tannin alfalfa</td>
<td>1,283</td>
<td>27,830</td>
<td>1,620</td>
<td>5,940</td>
<td>75</td>
</tr>
<tr>
<td>Tannin impact</td>
<td>+98</td>
<td>+210</td>
<td>-1,040</td>
<td>+1,120</td>
<td>-23</td>
</tr>
</tbody>
</table>

Grabber and Rotz (2004)
Are projected benefits supported by real data?
Can study whole-farm impacts of polyphenols with existing forage legumes

- Conventional alfalfa essentially free of protein-binding polyphenols
- Birdsfoot trefoil with 0.5 to 4% condensed tannins
- Red clover with \( \text{o-quinones} \) produced by polyphenol oxidase action on \( \text{o-diphenols} \)
- Other tannin containing forages like big trefoil, sainfoin, crownvetch
- Polyphenol-containing alfalfa (when sufficient quantities available)
Polyphenols increase rumen undegradable protein

Rumen undegradable protein (% of crude protein)

Red clover rumen undegradable protein ~ 30% of crude protein

Condensed tannin in forage (% of dry matter)
Forage polyphenols can enhance milk yields

<table>
<thead>
<tr>
<th>Forage</th>
<th>TMR intake (lb/day)</th>
<th>Milk (lb/day)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>54.4</td>
<td>68.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.2</td>
<td>3.7</td>
</tr>
<tr>
<td>Red clover with o-quinones</td>
<td>56.4</td>
<td>67.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.1</td>
<td>3.7</td>
</tr>
<tr>
<td>Birdsfoot trefoil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8% tannin</td>
<td>54.4</td>
<td>73.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2</td>
<td>3.6</td>
</tr>
<tr>
<td>1.2% tannin</td>
<td>52.9</td>
<td>76.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.1</td>
<td>3.9</td>
</tr>
<tr>
<td>1.6% tannin</td>
<td>55.5</td>
<td>78.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.2</td>
<td>3.7</td>
</tr>
<tr>
<td>Polyphenol impact</td>
<td>None</td>
<td>+0 to 10</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Means within columns with unlike letters differ ($P = 0.05$).

Hymes-Feldt et al. (2013)

- Fed TMRs with 50 to 60% forage averaging 17% crude protein and 28% neutral detergent fiber
Feeding forage legumes with polyphenols reduces ammonia emissions from manure slurry

- Forage polyphenols shift nitrogen excretion from urea in urine to more stable fecal nitrogen forms

Misselbrook et al. (2005)

Means with unlike letters differ ($P = 0.05$)
Unclear whether polyphenols in manure influence nitrogen uptake by crops

- High rates of yearly manure application (~350 lb/a total nitrogen with 180 lb/a ammonium nitrogen) may have masked polyphenol effects on nitrogen uptake.
Growing forage legumes with polyphenols may reduce recovery of legume nitrogen by a subsequent crop. Grabber (unpublished). Stats not run, but significant differences likely.

- Effects on nitrogen uptake and crop growth were eliminated by applying nitrogen fertilizer.
Progress in bioengineering o-quinone production into alfalfa

- o-Quinones for protecting protein are produced by the action of polyphenol oxidase (PPO) on o-diphenols
- A gene for polyphenol oxidase has been successfully transferred from red clover to alfalfa
- When ensiled this alfalfa undergoes browning and less protein breakdown if o-diphenols are added

- Must finish bioengineering of o-diphenol production into alfalfa
- Then optimize and transfer process to elite alfalfa cultivars

Sullivan and Hatfield (2006)
Progress in bioengineering condensed tannins into alfalfa

- Tannins are naturally produced in alfalfa seeds, but the pathway must be turned on in leaves.
- Concentrations of tannins in leaves of bioengineered alfalfa appear to be low (?)
- Process must be optimized and transferred to elite alfalfa cultivars

Hancock et al. 2014
Ongoing research needs
For optimal protein protection, what types and quantities of condensed tannins or o-quinones are needed?

More plant protein flowing to the gastrointestinal tract

Increased intestinal digestion absorption of amino acids

Less ruminal protein breakdown
For proper feeding, need improved and validated laboratory assays for measuring polyphenols and their impact on protein digestion.

How much protein will be degraded in the rumen?

How much polyphenol-protected protein will be digested in the gastrointestinal tract? What is the availability of amino acids?
Once alfalfa is bioengineered to produce \( o \)-quinones or condensed tannins...

- Develop high-yielding alfalfa cultivars that consistently produce desired types and quantities of \( o \)-quinones or condensed tannins for protecting protein in leaves
- Assess plant maturity, growth environment, and conservation (hay/silage) effects on alfalfa forage quality to optimize forage management practices
- Evaluate and optimize diets for these new alfalfas to enhance protein utilization and milk production
- Characterize nitrogen cycling from manure and terminated alfalfa stands to corn and other crops to optimize crop production practices and minimize nitrogen loss to the environment

✓ Primary goal: Identify optimal characteristics & production systems for polyphenol-containing alfalfa