Getting More from Forages

Targeted plant modifications:
Redesigning forages

Improving protein utilization by ruminants with protein-binding polyphenols

John Grabber and Ronald Hatfield
An Overview of Research on Protein-Binding Polyphenols in Dairy-Forage Systems

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Why incorporate forages with protein-binding polyphenols into dairy-forage systems?

Alfalfa proteins undergo excessive degradation (~80%) during ensiling and rumen fermentation

- Residual membrane proteins are of inferior nutritional value
Although proteolytic products are converted to rumen microbial proteins of high nutritional value, up to one-third of alfalfa protein is ultimately excreted as urea by dairy cattle

- Urea excretion–metabolic cost
- 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
Binding of polyphenols to protein restricts proteolysis in some forages

Condensed tannins: hydrogen bonding and hydrophobic interactions with protein are partly/mostly reversible

$o$-Quinones: covalent bonding with protein is not reversible
More plant protein flowing to the gastrointestinal tract

Condensed tannins and o-quinones can enhance forage protein utilization

Less ruminal protein breakdown

Increased intestinal absorption of amino acids
Expression of condensed tannins or o-quinones in alfalfa should enhance protein utilization and profitability and lessen environmental impacts.
Comprehensive research on forage polyphenols

- Improve methods for measuring polyphenols and protein degradability
- Assess plant maturity, growth environmental, and conservation method impacts on polyphenols and protein degradability
- Identify optimal concentrations and types of polyphenols for protecting protein while permitting extensive intestinal amino acids absorption
- Evaluate forage polyphenol impacts on milk production by dairy cattle
- Characterize excretion and loss of nitrogen in manure derived from polyphenol-containing diets
- Examine crop use of nitrogen from manure and forage residues containing polyphenols
- Model whole-farm impacts of growing and feeding polyphenol containing forages
- Develop alfalfa with protein-protecting polyphenols (Ron Hatfield’s talk!)

Primary goal: Identify optimal characteristics & production systems for polyphenol-containing alfalfa
Forages used in polyphenol studies

- Conventional alfalfa essentially free of protein-binding polyphenols
- Birdsfoot trefoil with 0.5 to 40 g/kg total condensed tannins
- Red clover with and without polyphenol oxidase and o-diphenol derivatives of caffeic acid
- Condensed-tannin containing big trefoil, sainfoin, crownvetch
- Polyphenol-containing alfalfa (when sufficient quantities available)
Examples of forage polyphenol research
Polyphenol assays
Butanol-HCl assay for condensed tannins

- Adding acetone increases anthocyanidin yield from tissue
Ruminal protein degradability
Rumen degradable protein:
In situ kinetic vs. calculated (g/kg crude protein)

- Good overall relationship with no bias, but...
- Relationship differs between each type of conserved forage
- Adjust degradation rates for B2 and B3 proteins?

In situ ruminal kinetic estimate

\[ y = 0.985x + 1.65 \]
\[ R^2 = 0.86 \]
Rumen degradable protein:
In situ kinetic vs. protease (g/kg crude protein)

- Poor overall relationship, especially for macerated forages
Plant maturity and environmental influences
Rumen degradable protein in red clover (% dry matter)

- Cut 1 Vegetative
- Cut 2 Early bud
- Cut 3 Late bud

Mean stage weight

1st cut
2nd cut
3rd cut
Forage conditioning and conservation effects
### In situ rumen degradable protein

(0.06 per h passage rate)

<table>
<thead>
<tr>
<th></th>
<th>Alfalfa</th>
<th>Low tannin trefoil</th>
<th>High tannin trefoil</th>
<th>Red clover</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hay</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rolled</td>
<td>781&lt;sup&gt;a&lt;/sup&gt;</td>
<td>696&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>635&lt;sup&gt;b&lt;/sup&gt;</td>
<td>650&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Macerated</td>
<td>648&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>675&lt;sup&gt;a&lt;/sup&gt;</td>
<td>589&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>534&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Silage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rolled</td>
<td>879&lt;sup&gt;a&lt;/sup&gt;</td>
<td>861&lt;sup&gt;a&lt;/sup&gt;</td>
<td>817&lt;sup&gt;b&lt;/sup&gt;</td>
<td>736&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Macerated</td>
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<td>808&lt;sup&gt;a&lt;/sup&gt;</td>
<td>749&lt;sup&gt;b&lt;/sup&gt;</td>
<td>707&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within rows with unlike letters differ ($P < 0.05$).

- In both hays and silages, red clover with o-quinones ≤ High tannin trefoil < Alfalfa
- Macerated < Roll conditioned
- Polyphenol–protein interactions not enhanced by maceration
Optimal vs. excessive protein protection
Use trefoils with 15 to 40 g/kg tannin, but similar protein and fiber

- **Ruminal degradability**: In situ kinetics, single time-point in situ, in vitro protease, inhibitor in vitro with rumen bacteria
- **Intestinal degradability**: In situ mobile bag, in vitro pepsin-pancreatin, acid detergent insoluble protein
- **Intestinal availability**: protein degraded vs. available amino acids
Impacts on milk production
Forage type did not affect intake of total mixed rations containing ~17.5% crude protein and ~28% neutral detergent fiber.

As in previous trials, o-quinones in red clover did not enhance milk yields—over protection of protein?

Tannin in trefoil increased milk yields—observed in some but not all trials.

Data from Hymes-Fecht et al.

<table>
<thead>
<tr>
<th>Forage Type</th>
<th>Milk (kg/day)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa</td>
<td>30.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.16</td>
<td>3.77</td>
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<tr>
<td>Red clover with o-quinones</td>
<td>31.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.02</td>
<td>3.71</td>
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<tr>
<td>Birdsfoot trefoil</td>
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<tr>
<td>8 g/kg tannin</td>
<td>32.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.18</td>
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<td>12 g/kg tannin</td>
<td>34.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>16 g/kg tannin</td>
<td>34.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.14</td>
<td>3.62</td>
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<tr>
<td>Tannin impact</td>
<td>+1.7 to 4.4</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Means within rows with unlike letters differ (P < 0.05).
Nitrogen excretion and losses from manure
Polyphenols shift nitrogen excretion from urea in urine to more stable fecal nitrogen forms

- Ammonia emission is lower from polyphenol containing diets

Data from Misselbrook et al.
Nitrogen use and growth of crops
Yield response of annual grass crops to nitrogen from polyphenol containing forages

- Polyphenols delay the soil mineralization of nitrogen from legume tops and roots, but yields are usually not influenced.
Whole-farm impacts
Model impact of polyphenol-containing alfalfa on dairy farm (e.g. Integrated Farm System model)

Fertilizer

Soils & Crops

Nutrient loss/buildup

Storage losses

Manure Storage & Handling

Harvest & Storage

Purchased feed & bedding

Feeding & Herd Management

Milk

"© Cash cow image contributed by WMMB, 2004"
## Predicted performance if alfalfa fed as 45% of diet

<table>
<thead>
<tr>
<th></th>
<th>Net return $/cow</th>
<th>Milk yield kg/cow</th>
<th>Soybean fed kg/cow</th>
<th>Corn fed kg/cow</th>
<th>Total N loss kg/cow</th>
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</thead>
<tbody>
<tr>
<td>Normal alfalfa</td>
<td>1145</td>
<td>12330</td>
<td>1026</td>
<td>2433</td>
<td>157</td>
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<tr>
<td>Tannin alfalfa</td>
<td>1270</td>
<td>12540</td>
<td>436</td>
<td>3060</td>
<td>118</td>
</tr>
<tr>
<td>Tannin impact</td>
<td>+125</td>
<td>+210</td>
<td>-590</td>
<td>+627</td>
<td>-39</td>
</tr>
</tbody>
</table>