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

John H. Grabber

USDA-Agricultural Research Service

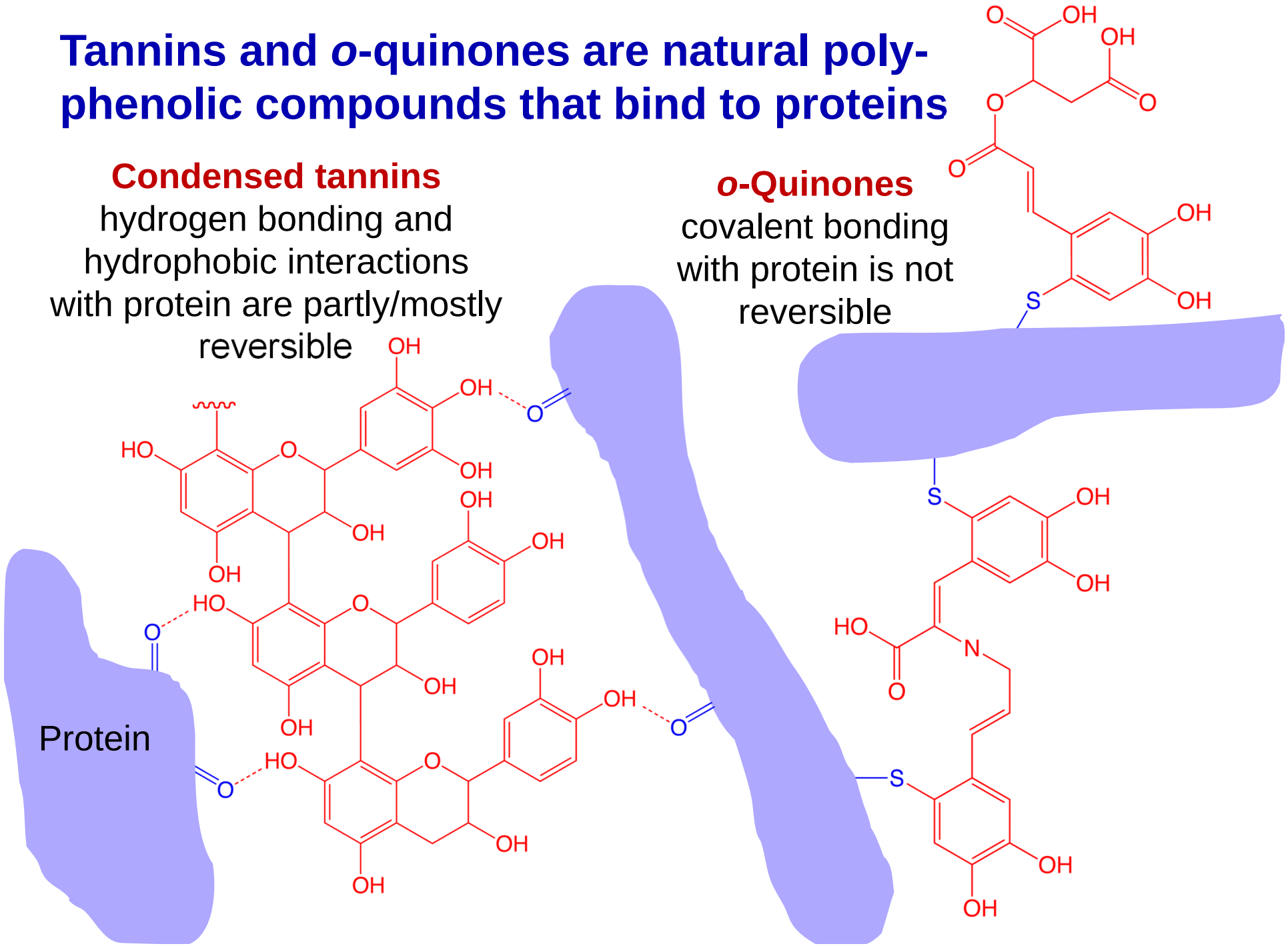
U.S. Dairy Forage Research Center, Madison, Wisconsin

What are *o*-quinones and condensed tannins and why are they important?

Tannins and *o*-quinones are natural poly-phenolic 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



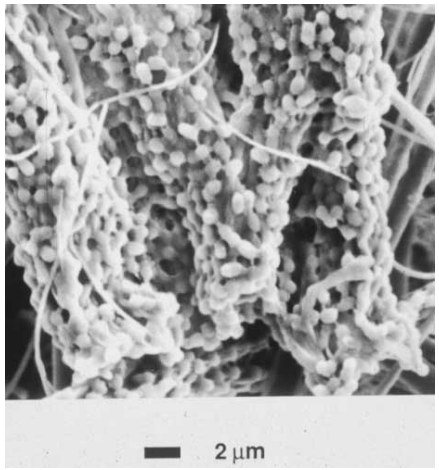
Why are *o*-quinones and condensed tannins needed in alfalfa forage?

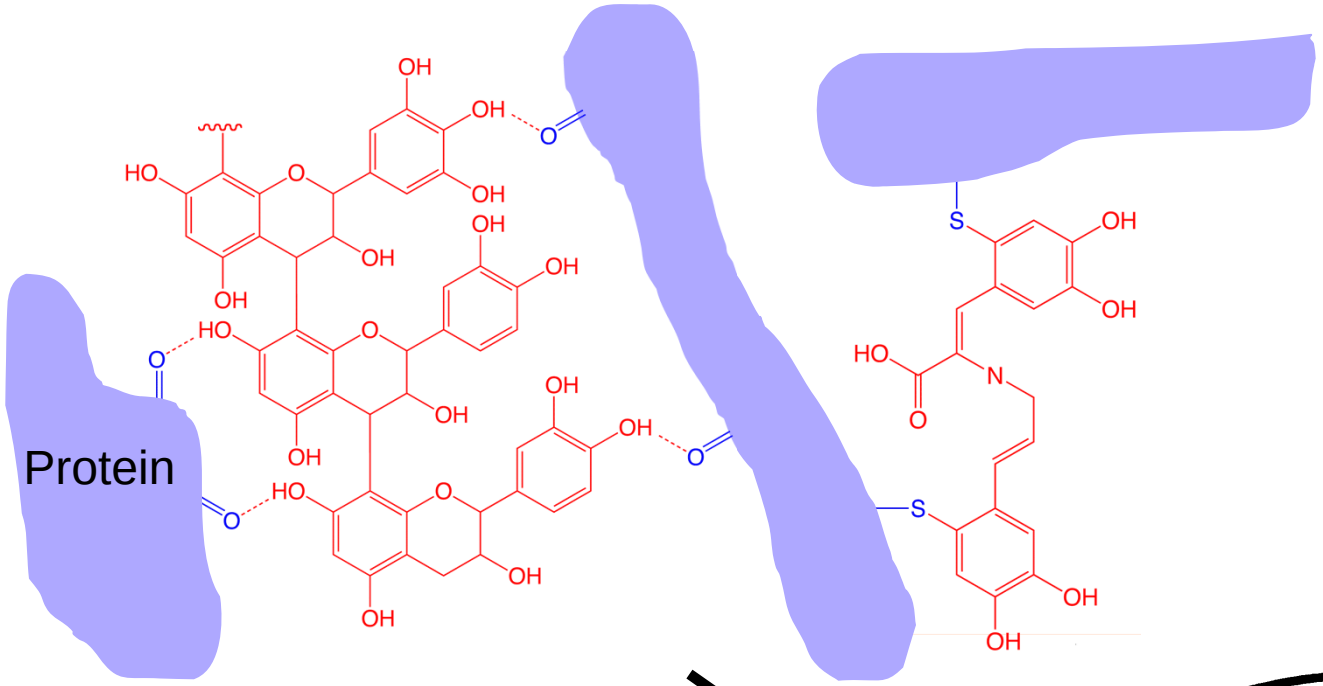
Alfalfa forage proteins undergo excessive proteolysis during ensiling and rumen fermentation (~80% of protein can be degraded)

- Residual proteins are of inferior nutritional value

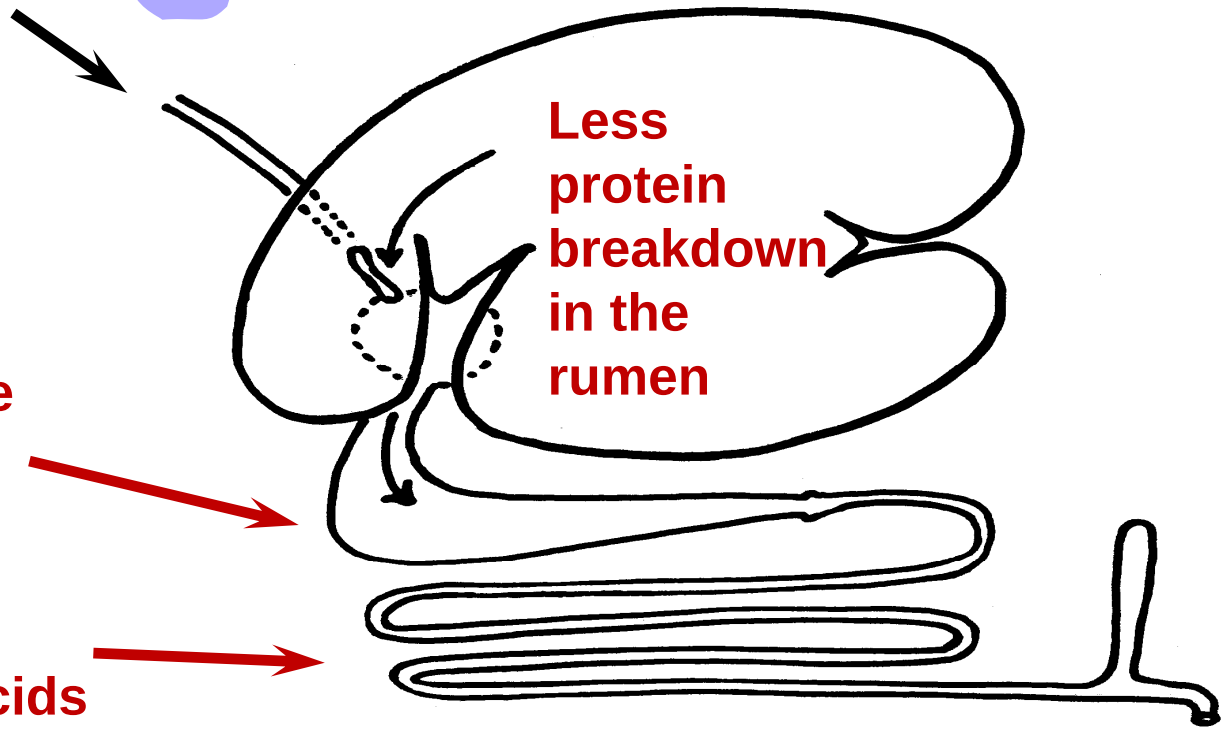
Degraded proteins are converted to rumen microbial proteins of high nutritional value, **BUT** up to one-third of alfalfa protein is ultimately excreted in urine as urea by dairy cattle

- 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





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

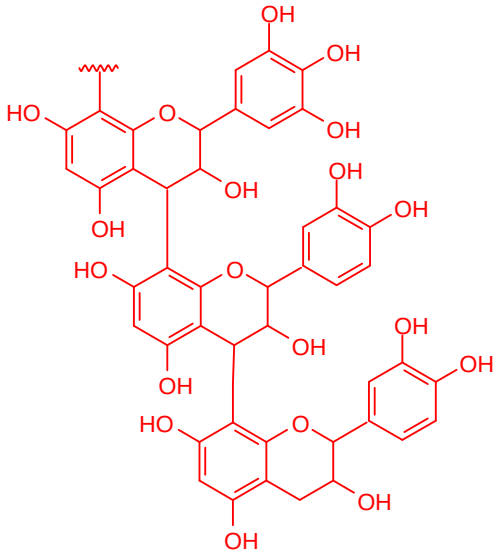


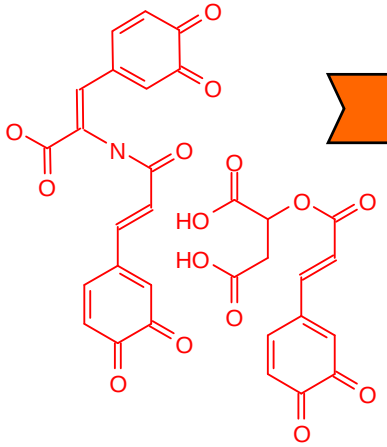
Less protein breakdown in the rumen

More plant protein flowing to the gastrointestinal tract

Increased intestinal absorption of amino acids

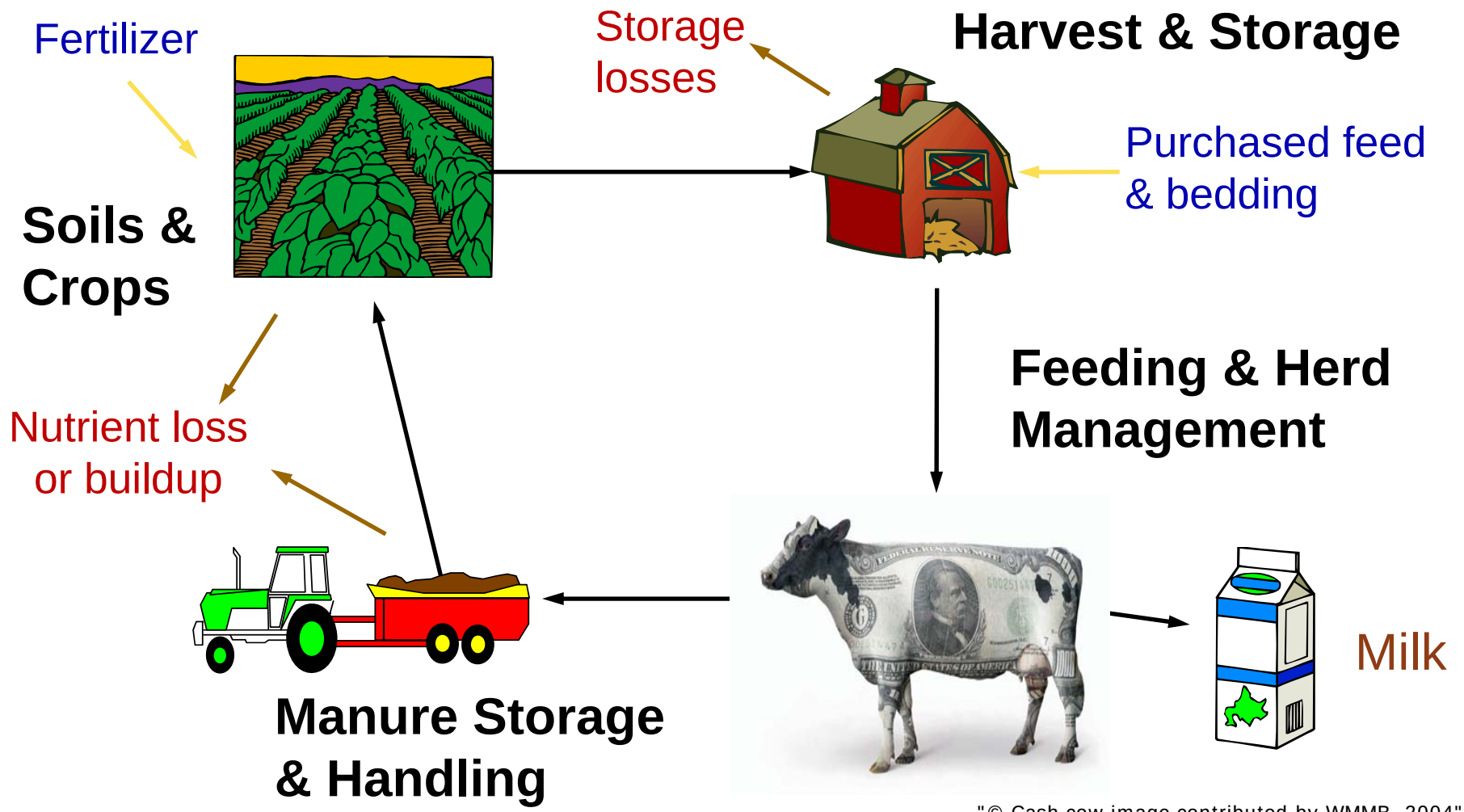
Production of condensed tannins or *o*-quinones in alfalfa forage should also improve farm profitability and lessen environmental impacts





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

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

Grabber and Rotz (2004)

Are projected benefits supported by real data?

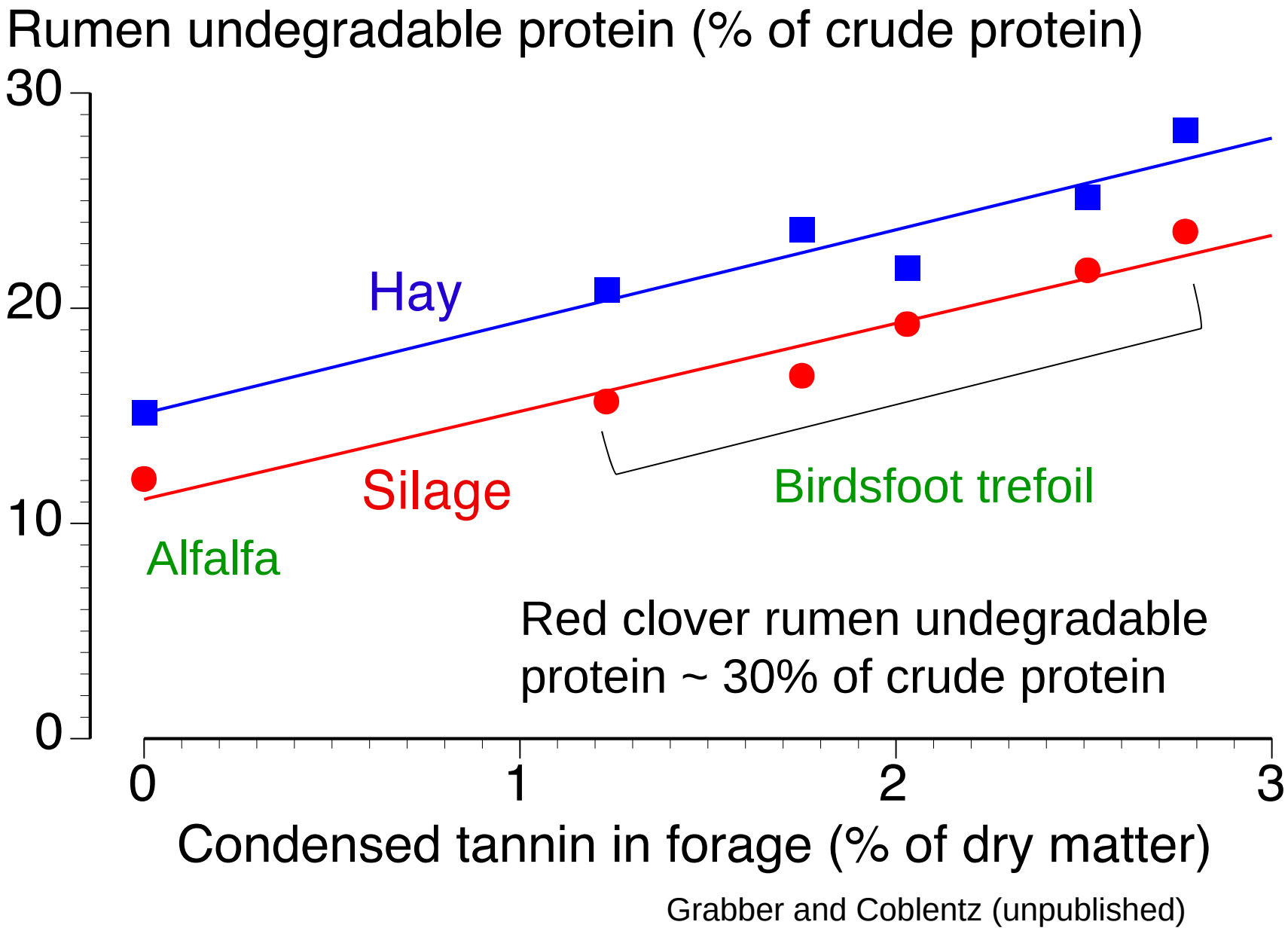
Can study whole-farm impacts of polyphenols with existing forage legumes

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






Polyphenols increase rumen undegradable protein



Grabber and Coblentz (unpublished)

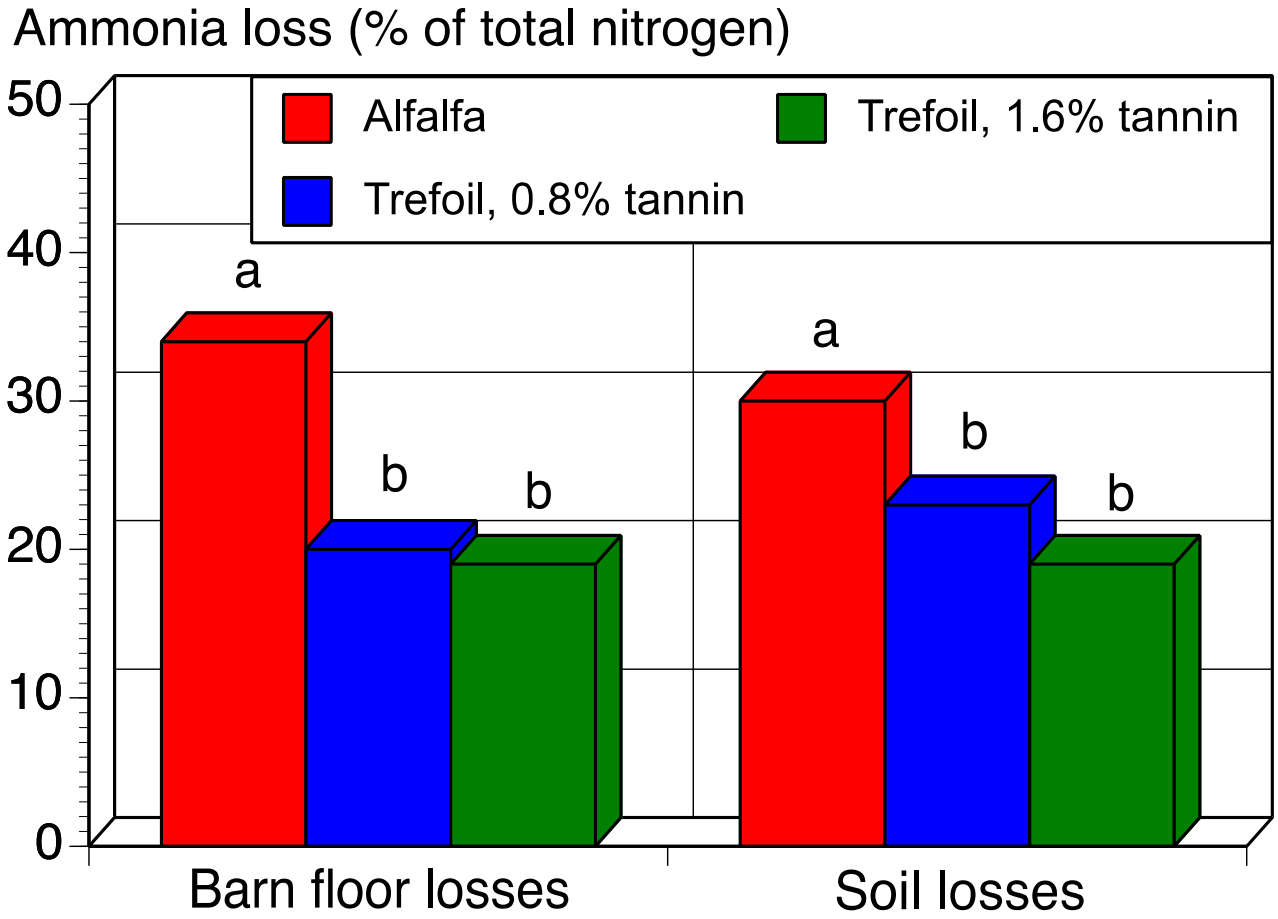
Forage polyphenols can enhance milk yields

Forage	TMR intake (lb/day)	Milk (lb/day)	Protein (%)	Fat (%)
Alfalfa	54.4	68.1^{c}	3.2	3.7
Red clover with *o*-quinones	56.4	67.9^{c}	3.1	3.7
Birdsfoot trefoil				
0.8% tannin	54.4	73.9^{b}	3.2	3.6
1.2% tannin	52.9	76.3^{ab}	3.1	3.9
1.6% tannin	55.5	78.1^{a}	3.2	3.7
Polyphenol impact	None	+0 to 10	None	None

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



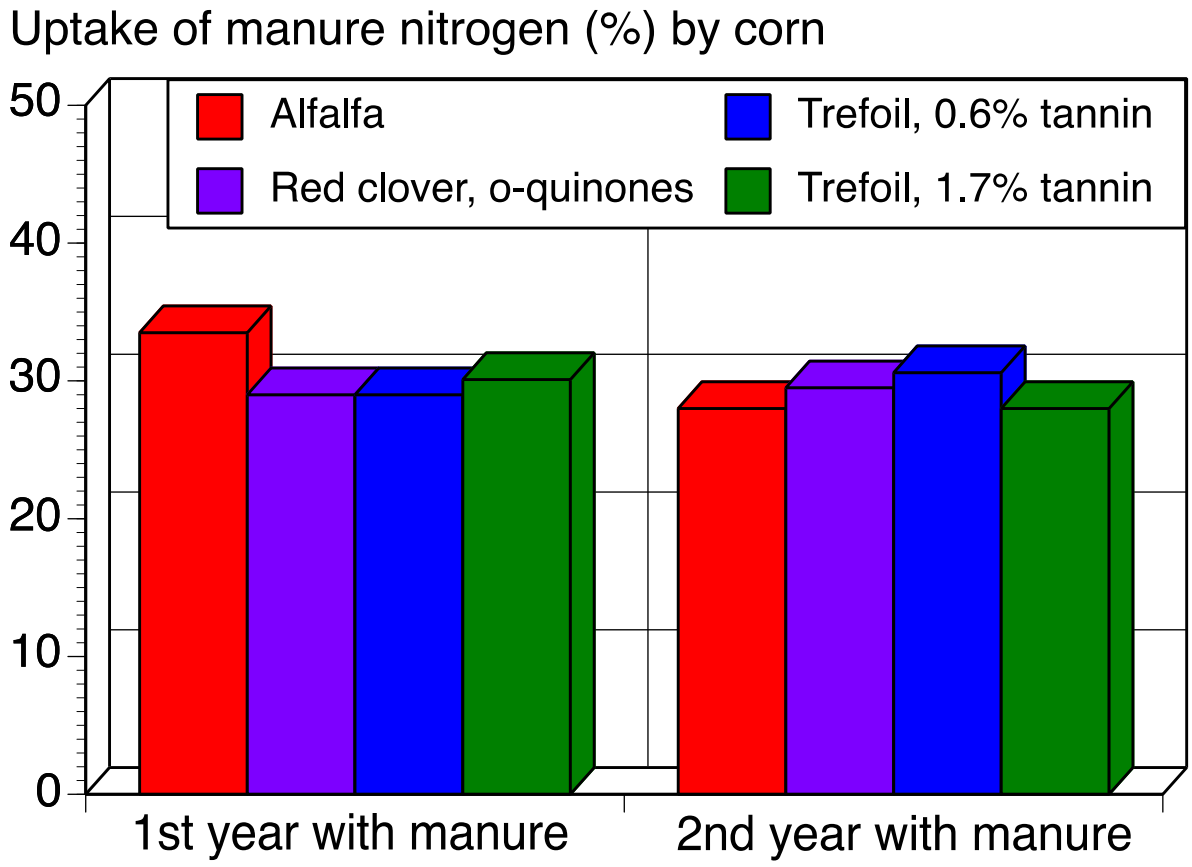
Misselbrook et al. (2005)

Means with unlike letters differ (P = 0.05)

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

Unclear whether polyphenols in manure influence nitrogen uptake by crops

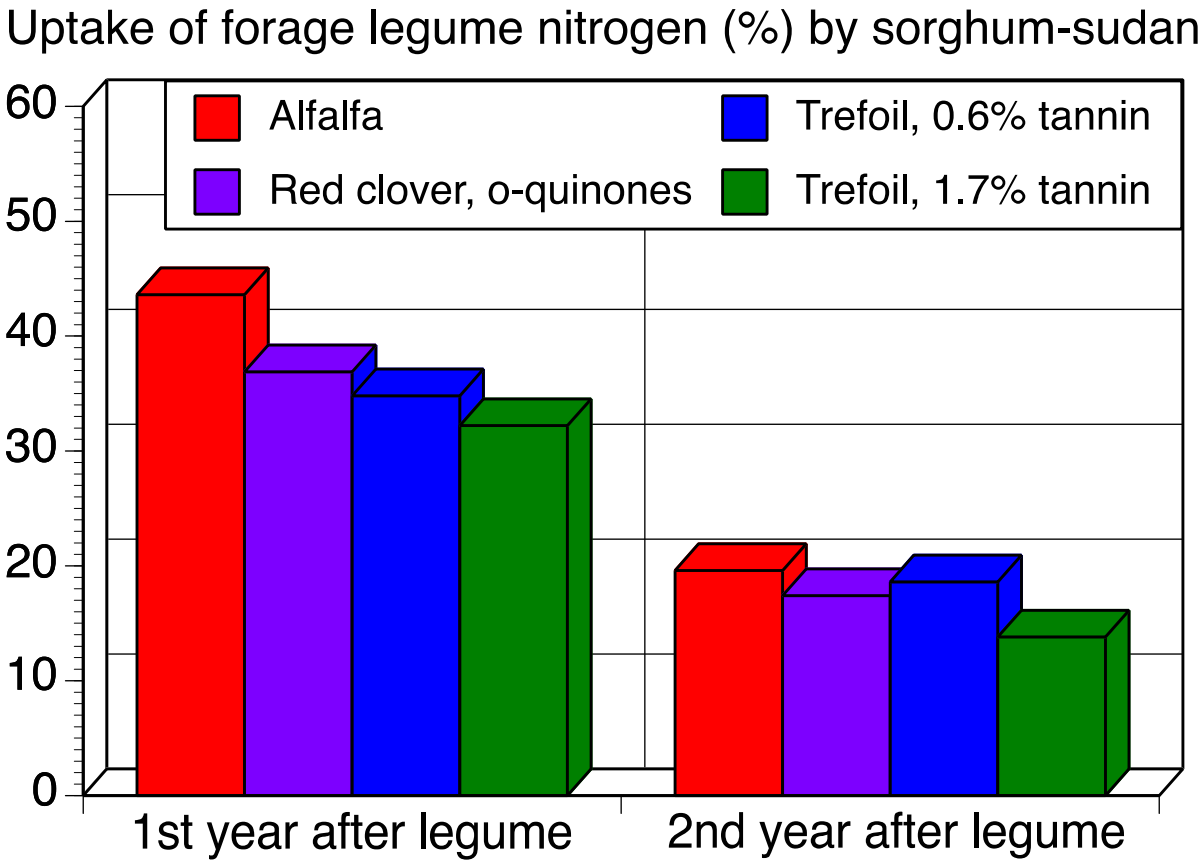
Powell & Grabber (2009)



Means not significantly different ($P > 0.05$)

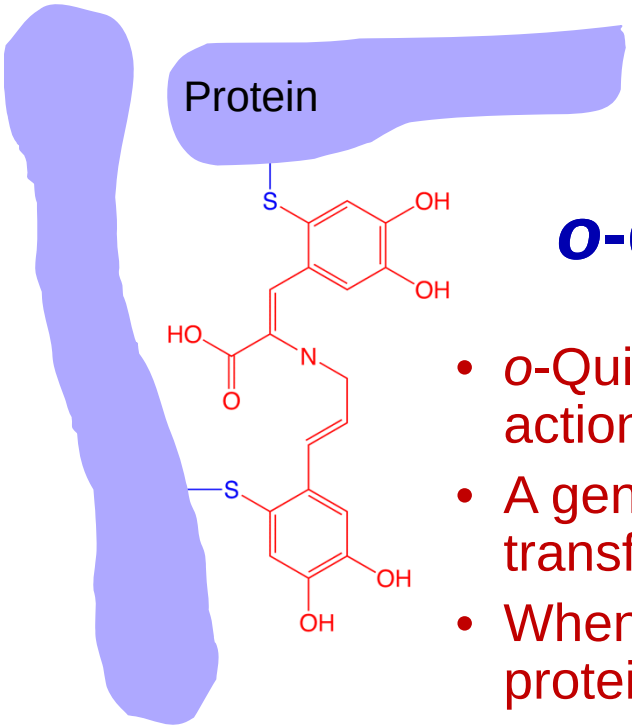
- High rates of yearly manure application (~350 lb/a total nitrogen with 180 lb/a ammonium nitrogen) may have masked polyphenol effects on nitrogen uptake.

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.



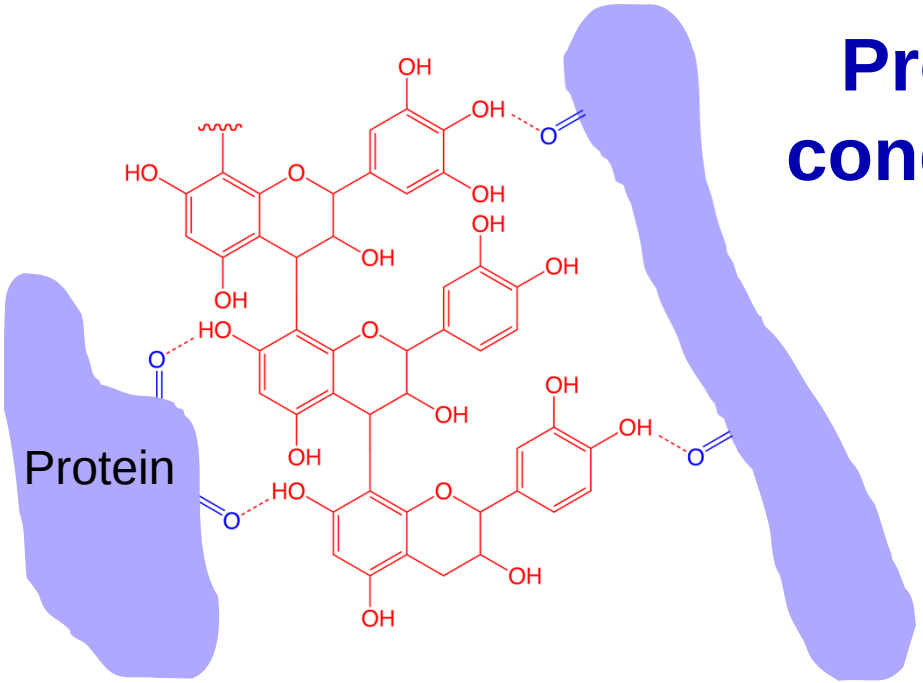
Progress in bioengineering *o*-quinone production into alfalfa

- *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*

Sullivan and Hatfield (2006)

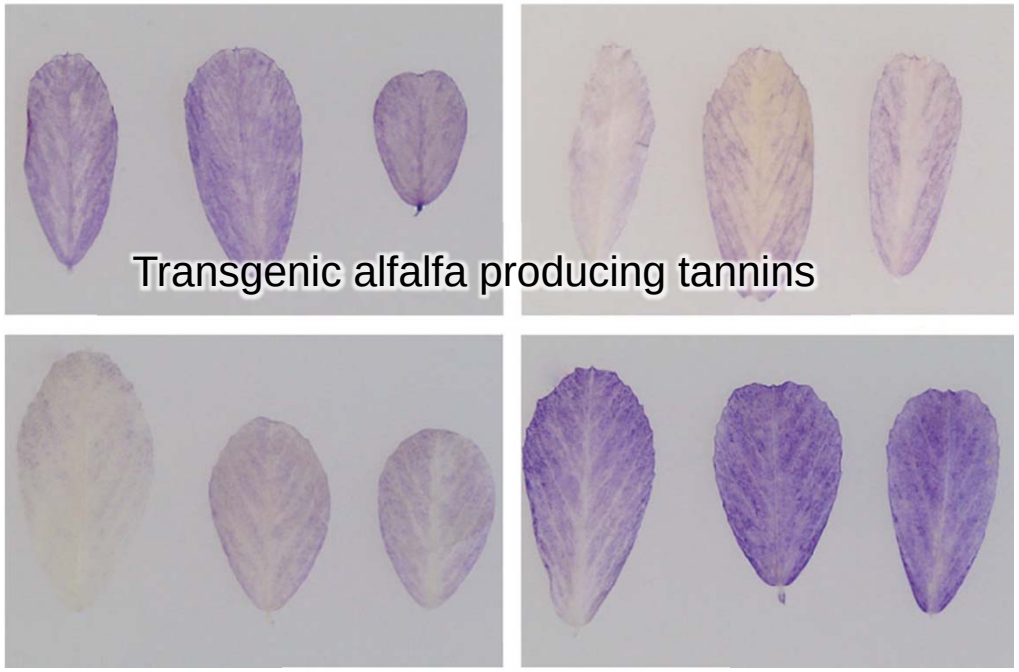
- Must finish bioengineering of *o*-diphenol production into alfalfa
- Then optimize and transfer process to elite alfalfa cultivars

Progress in bioengineering condensed tannins into alfalfa



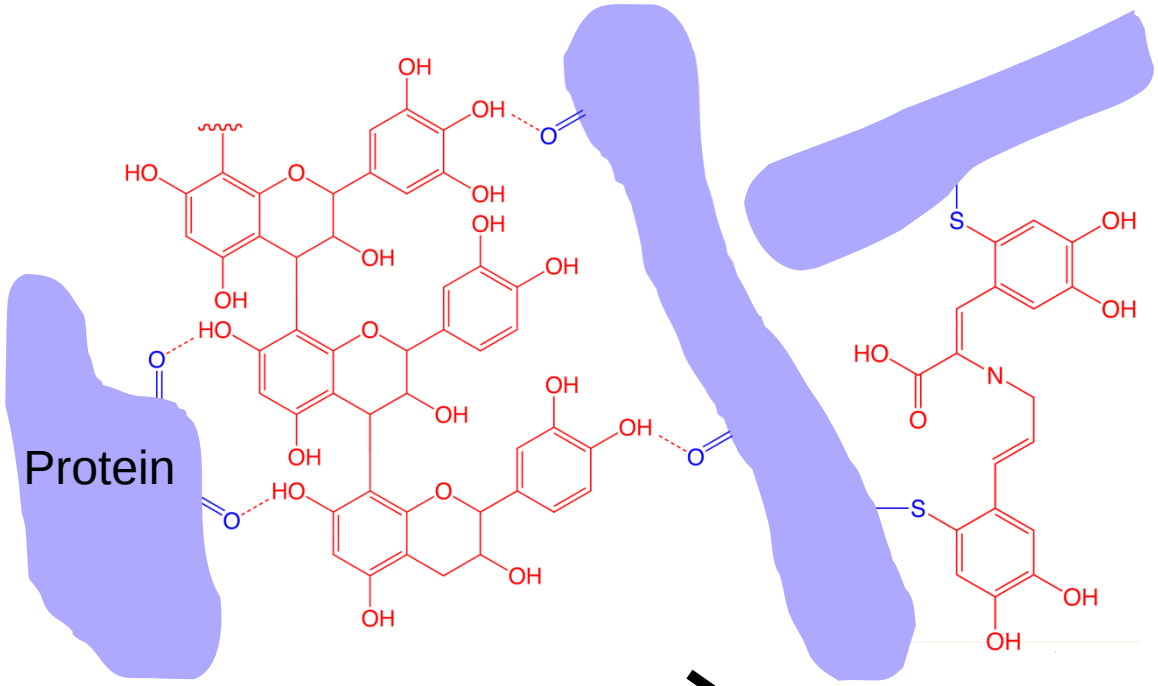
Leaves stained for the presence of condensed tannins

- 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

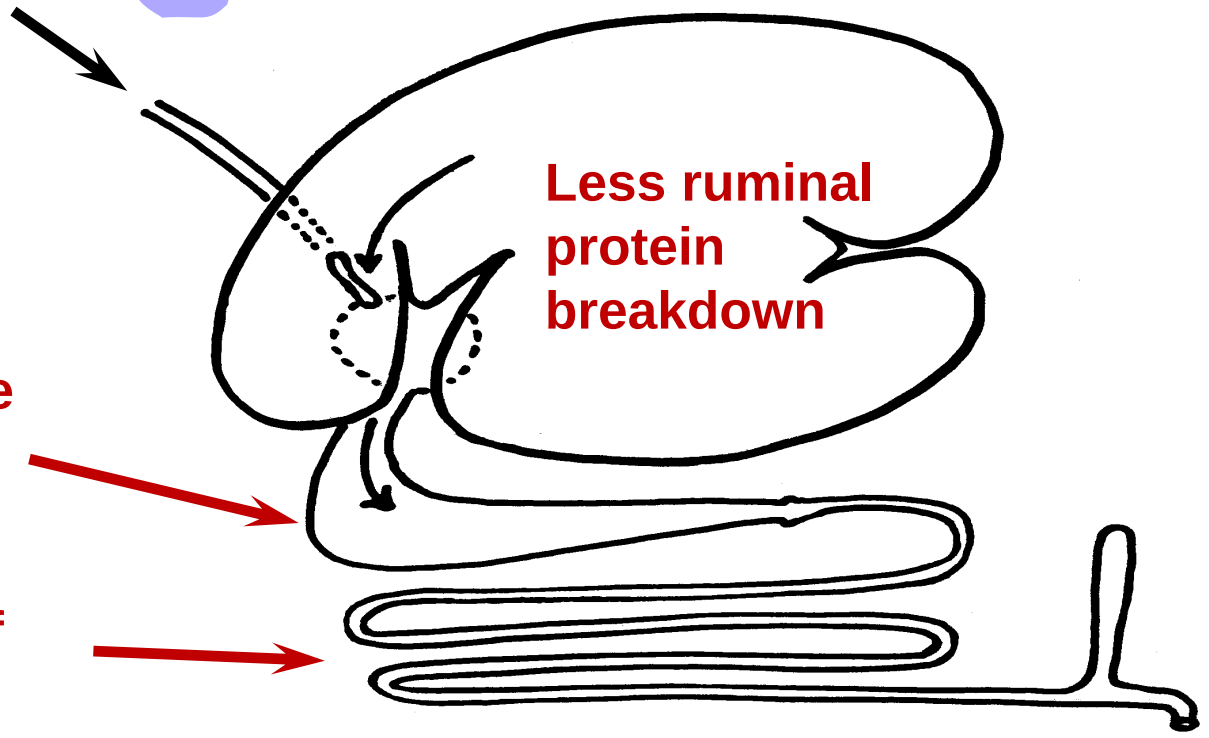


Hancock et al. 2014

Ongoing research needs



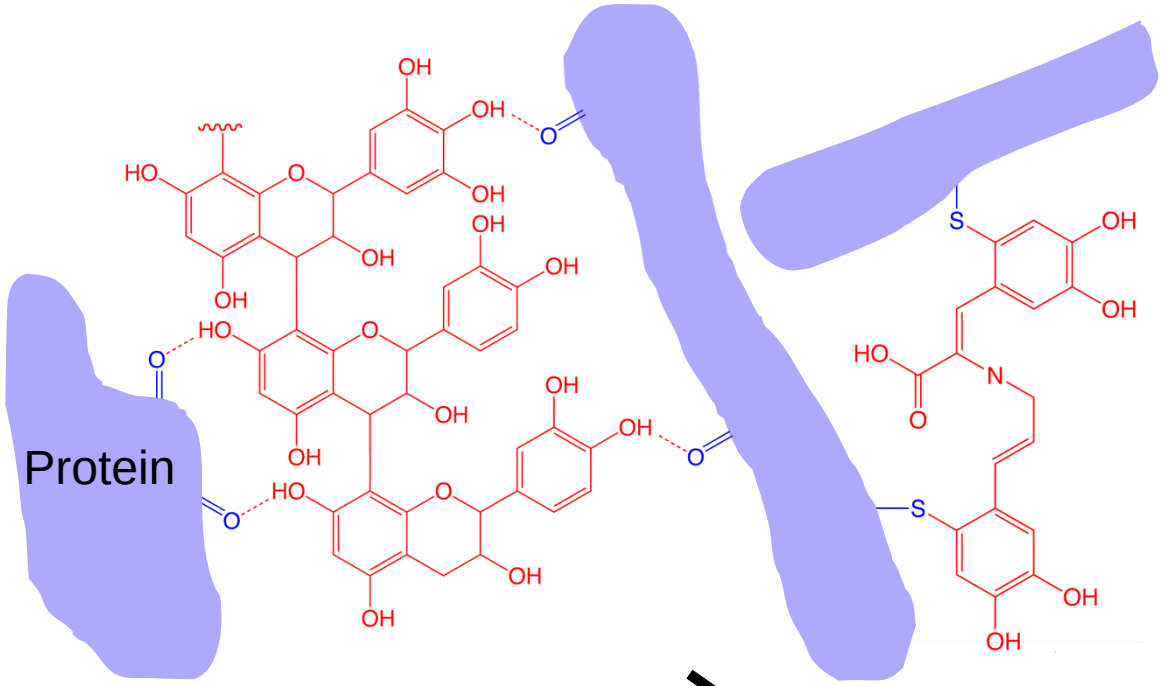
For optimal protein protection, what types and quantities of condensed tannins or *o*-quinones are needed?



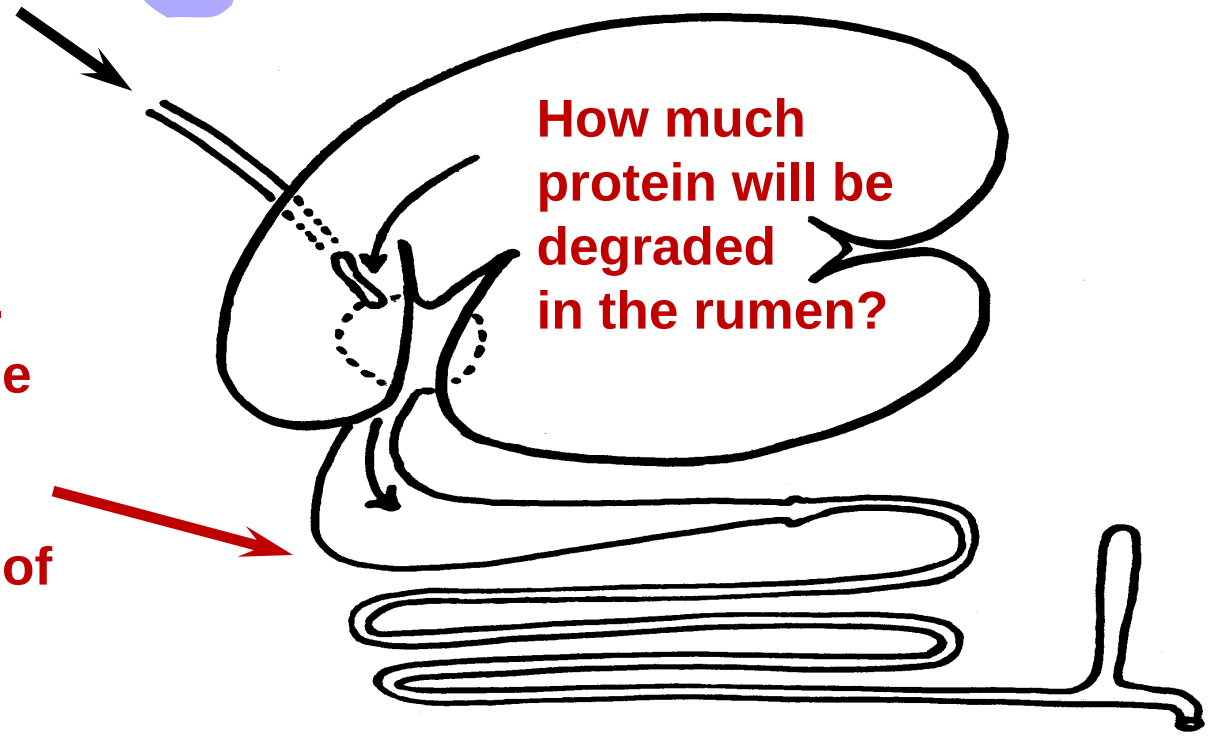
Less ruminal protein breakdown

More plant protein flowing to the gastrointestinal tract

Increased intestinal digestion absorption of amino acids



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**