USDA

United States Department of Agriculture

U.S. Dairy Forage Research Center

Integrated Dairy Systems: Forage, Nutrition, Environment

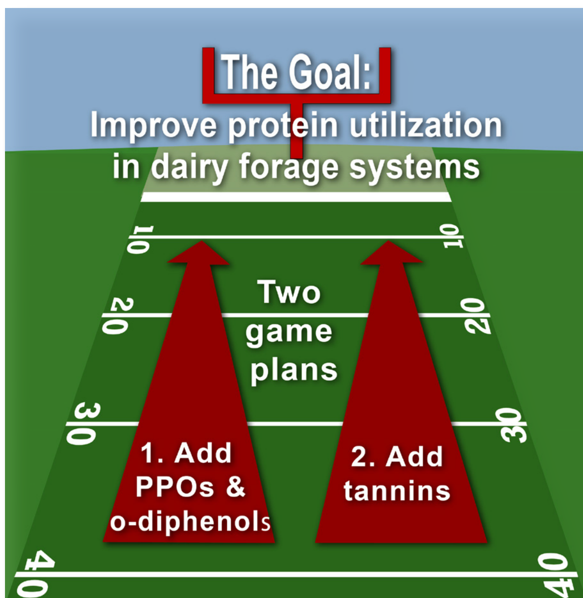
Redesigning alfalfa:

Capturing more protein for economic and environmental sustainability

Alfalfa is the "Queen of Forages" due to its relatively good digestibility, high protein, and ability to readily fix nitrogen. As a result of fixing nitrogen, the protein produced by alfalfa should have a high economic return in dairy systems because minimal outside inputs are required to produce it, and there is less risk of nitrogen runoff or leaching.

Researchers at the U.S. Dairy Forage Research Center and elsewhere are taking two different approaches to redesign alfalfa so more of its protein is utilized by the cow. One approach is to add PPOs and *o*-diphenols, the other is to add tannins. Both are very long-term research projects that could yield a substantial economic and environmental payback.

However, this assumes that the dairy cow can efficiently capture the protein produced by alfalfa. Vegetative proteins of forages like alfalfa are rapidly degraded in the rumen of dairy cows and are not efficiently utilized to capture all of their potential nutritional and economic value. Protein fragments (partially degraded protein molecules) are even less efficiently utilized . . . resulting in a need for additional protein additives in the diet . . . resulting in additional nitrogen waste in manure. It is critical that a large amount of the forage protein is preserved as "true protein" as it goes into the dairy diet. This becomes a challenge in the more humid U.S. agricultural producing regions that ensile alfalfa forage.



Because up to 75% of protein can be lost during ensiling and in the cow's rumen, the USDFRC is looking for ways to redesign forage plants so that more protein is captured to make milk and meat and less is excreted in urine and feces. Researchers have two game plans to make this happen.

How substantial? A redesigned alfalfa with a 25% decrease in protein degradation during ensiling would save an estimated \$100 million per year for the U.S. dairy industry, or more than \$10 per cow, by reducing the amount of protein supplements purchased (based on 35 million tons of silage at harvest, and soybean meal at \$314 per ton). In addition, with more protein utilized by the cow there would be substantial reductions in manure nitrogen excretions and subsequent nitrogen losses as ammonia, nitrous oxide (the most potent agricultural greenhouse gas) and nitrate. And fewer acres would be needed per unit of milk produced.

Why? Because during the ensiling process, plant proteases are released and they begin degrading the vegetative proteins even before they are fed. This is one reason ensiling practices emphasize the need to rapidly decrease silage pH to a point that it limits protease activity and stops protein degradation. Variable and changing environmental conditions often lead to the production of less than optimal silage; protein degradation may be as high as 70 to 75% of the true protein.

Red clover with PPOs and o-diphenols

While alfalfa and other forages, including corn silage, can lose a lot of protein during ensiling, red clover does not. Even under poor ensiling conditions it typically has minimal protein degradation, maintaining 70-80% of its protein intact which consequently provides more true protein in the diet and results in higher protein use efficiencies.

USDFRC scientists discovered that the key factor in red clover is an enzyme called polyphenol oxidase (PPO) and an abundance of its substrate, special chemicals called *o*-diphenols. PPO converts the *o*-diphenols to *o*-quinones which are highly reactive molecules that can bind to proteins. These *o*-quinones react with the native proteases, decreasing their activity and leaving much of the protein intact during the ensiling process.

First step

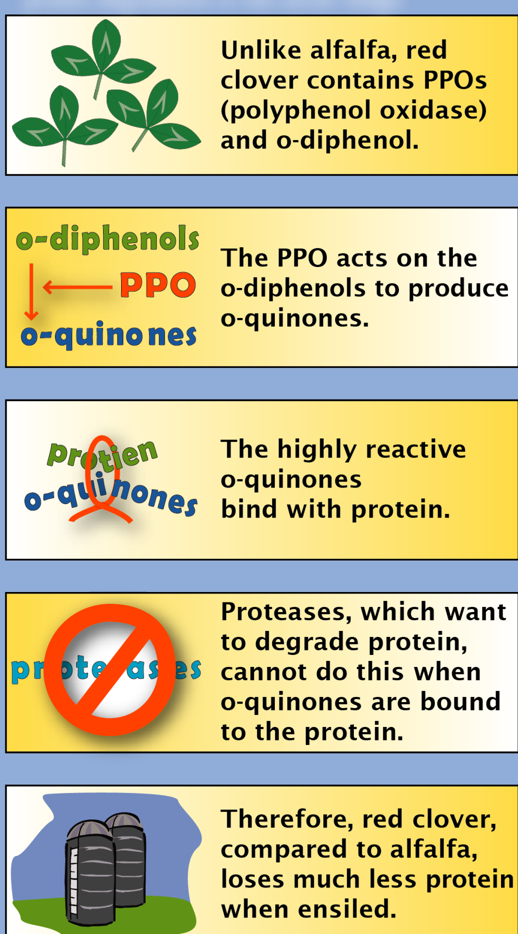
The question is, "Can the PPO system that works so well in red clover be transferred to alfalfa?" To fully develop a PPO/*o*-diphenol system in alfalfa requires two new components to be introduced into alfalfa: an active PPO enzyme and the *o*-diphenols it needs as substrate to produce the critical *o*-quinones. An extensive survey of alfalfa germplasm indicated that there were no natural variants that contained active PPO in the vegetative portions of the plant.

Therefore, a precision breeding approach was used to insert the red clover PPO gene into alfalfa. The first step of this process has been quite successful and alfalfa plants expressing the red clover gene can inhibit proteolytic activity when appropriate *o*-diphenols are added as a substrate (figure on next page).

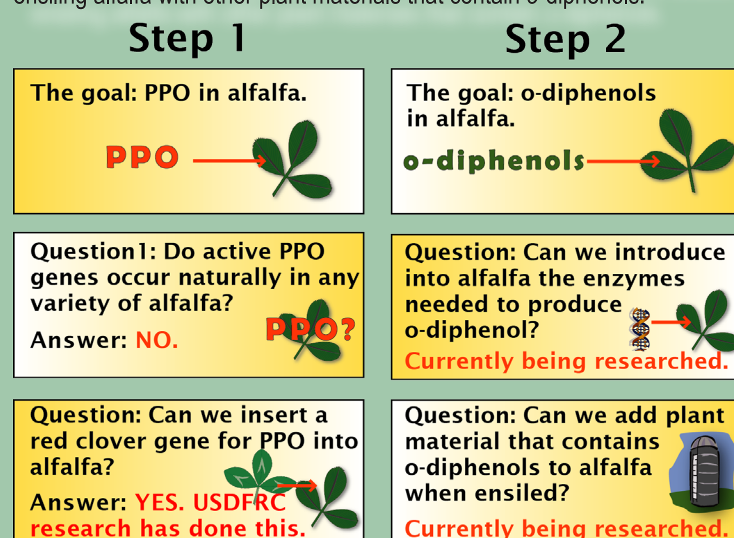
Second step

The next step of this process is to have a supply of *o*-diphenols since alfalfa currently does not produce such PPO substrates. Of course, the most desirable approach would be to have alfalfa synthesize the appropriate *o*-diphenol substrate. We are currently working on ways to introduce the necessary genes for the enzymes needed to produce a suitable *o*-diphenol. An alternative approach would be to use external sources of diphenols that are abundant in many plants such as potato peels, coffee grounds, and forages like timothy. We are also working to test the feasibility of co-ensiling PPO-forage with plant materials or plant extracts that contain *o*-diphenols to decrease proteolytic activity and preserve the greatest possible amount of the native protein as intact protein.

Process by which PPOs and o-diphenols reduce protein degradation in red clover silage.



Reducing protein degradation in alfalfa via PPOs and *o*-diphenols is a two-step process: Inserting the red clover PPO gene into alfalfa; and adding *o*-diphenols to the silage-making process, either by modifying the alfalfa or ensiling alfalfa with other plant materials that contain *o*-diphenols.



For more information about this PPO/o-diphenol research, contact:

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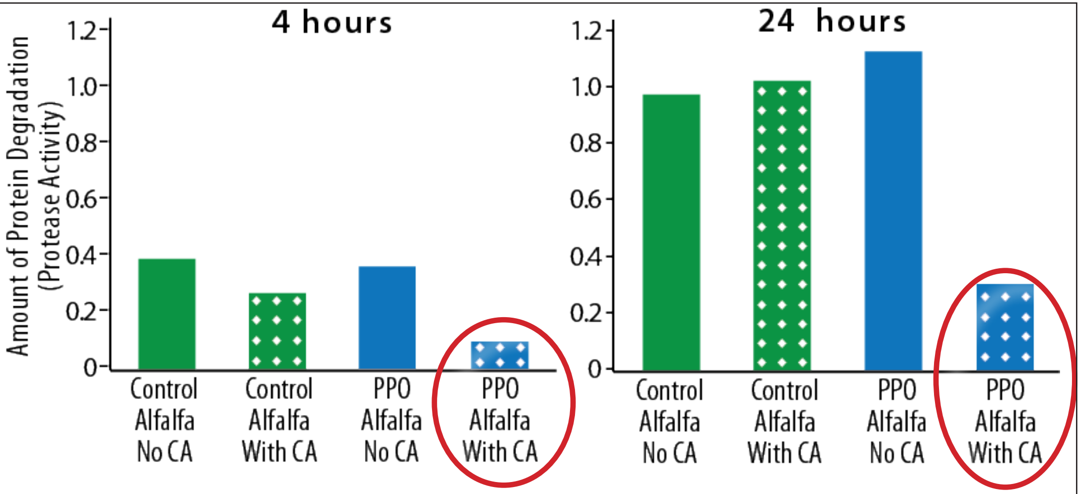
Need both PPO and *o*-diphenol

These two charts show reductions in protein degradation at both 4 and 24 hours when a PPO alfalfa is ensiled with its substrate, *o*-diphenols, in the form of caffeic acid (CA). The presence of PPO alone or CA alone did not reduce protein degradation.



CA = caffeic acid, an diphenol that acts as a PPO substrate.

This work with red clover PPO and using the gene to modify alfalfa clearly indicates a feasible strategy for decreasing excessive protein degradation during the ensiling of alfalfa. Such improvements would have direct economic benefits to the farmer as well as positive impacts upon the environment. It may be a way to put a bit more luster in the Queen's crown.

Introducing tannins

Condensed tannins are natural protein-binding substances that are found in some forages such as birdsfoot trefoil and sainfoin. Numerous studies have demonstrated two major benefits of condensed tannins in forages:

Reduced non-protein nitrogen (NPN) formation. When alfalfa protein is degraded during ensiling, NPN is formed. Although a portion of this NPN can be converted to nutritionally valuable microbial protein in the rumen, excessive levels are converted to urea and excreted in urine. The binding of condensed tannins to forage proteins helps to protect protein from degradation during the ensiling process.

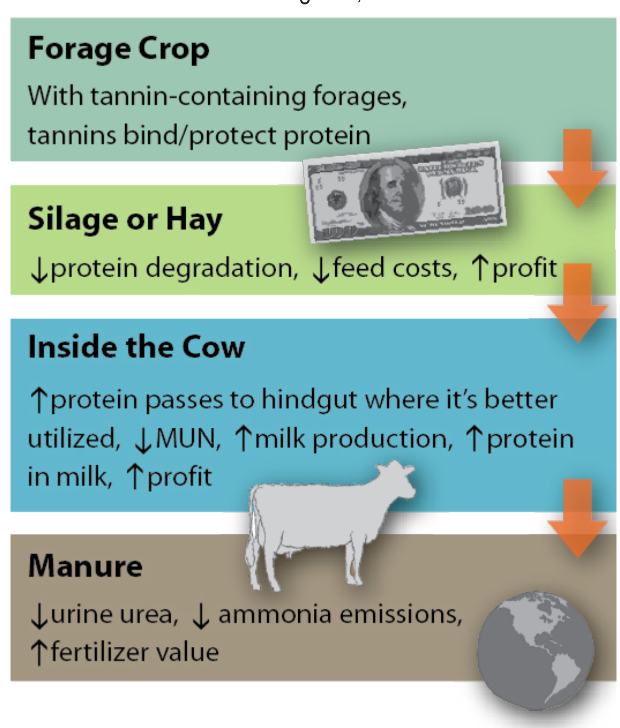
Elevated flow of rumen undegraded protein to the hindgut. Modest amounts of condensed tannins (2-3% of dry matter) in forage often increase the amount of true protein reaching the hindgut which boosts milk production and weight gain of livestock. Excessive amounts of condensed tannins can, however, adversely affect livestock production by depressing the intake of feed and the digestion of protein and other nutrients.

Although condensed tannins can enhance the productivity of livestock, further research is needed to determine the optimal characteristics of condensed tannins (i.e. concentration and chemical composition/structure) needed for maximizing livestock productivity and overall nitrogen use efficiency of dairy farms. To accomplish this, scientists at USDFRC and other institutions are working on the following projects:

1 ***Develop more accurate, routine methods for measuring condensed tannins and their effect on protein digestibility.***

One way is to improve the accuracy of the widely-used butanol-HCl assay for measuring condensed tannin concentrations in forages. Other work is aimed at

When forages contain condensed tannins, the nitrogen use efficiency improves because more protein in the silage is available to the cow for making milk, not manure.



evaluating and improving laboratory assays for predicting the impact of condensed tannins on conserving true protein during the ensiling process and in the rumen and gastrointestinal tract. Widespread use of these assays will ensure that diets are optimally formulated to make the best use of condensed tannin-containing forages.

2 ***Identify optimal condensed tannin characteristics for forages.***

Scientists have developed techniques to purify a wide variety of condensed tannins from forages such as birdsfoot trefoil, big trefoil, sainfoin, crownvetch, white clover, and lespedeza. Powerful analytical techniques such as "nuclear magnetic resonance" and "thiolysis" are being used to characterize the composition and structure of isolated condensed tannins. These condensed tannins are being used in various laboratory assays to identify the optimal characteristics of condensed tannins for limiting non-protein nitrogen formation during ensiling and ruminal digestion while permitting extensive digestion of forage protein in the gastrointestinal tract.

3 ***Reduce nitrogen loss from farms.***

Forage-containing condensed tannins, or commercial condensed tannin products added to diets of dairy cows, can reduce urea excretion in urine and inhibit the enzyme urease, which is responsible for the conversion of urea to ammonia. Scientists have found that condensed tannin-containing diets reduce ammonia loss from dairy barn floors and from soils after manure slurry application. Other studies have or will examine whether condensed tannins can enhance the utilization of nitrogen in crop rotations.

4 ***Develope or improve condensed tannin-containing forage crops.***

USDFRC scientists are working with commercial forage breeders to characterize alfalfa that has been bioengineered to produce condensed tannins in its forage. USDFRC scientists are also using traditional breeding methods to improve birdsfoot trefoil for use on conventional or organic grazing-based farms. Best management practices will be identified to help ensure that farmers can reliably grow and feed high quality condensed tannin-containing forages to livestock.

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