

Nutrient Boost for Alfalfa Silage

STEPHEN AUSMUS (K10657-1)



Molecular biologist Michael Sullivan places small-scale experimental “silos” in a water bath for fermentation. Behind him, technician Ursula Hymes-Fecht will analyze the contents for protein degradation.

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A row of wilted, chopped, green alfalfa is collected into a wagon before being taken to the silo.

It's a familiar scene in the country—a tractor chugging its way across a field, mowing down swath after swath of green alfalfa. Many farmers store and ferment these alfalfa clippings in silos. By doing so, they turn the forage into silage, the cow's equivalent of sauerkraut.

As a forage crop, alfalfa has many benefits. It fixes nitrogen in the soil—meaning there's no need to add nitrogen fertilizer—and it's a good scavenger of excess soil nitrate left by overfertilized row crops. And because it's high in protein, it's great for livestock, such as dairy cattle. Unfortunately, when alfalfa is processed into silage, up to 85 percent of its protein breaks down into nonprotein nitrogen (NPN) through a process known as proteolysis. Cows use NPN much less efficiently than protein.

Now, researchers at ARS's U.S. Dairy Forage Research Center in Madison, Wisconsin, and ARS's Plant Science Research Unit in St. Paul, Minnesota, have found an environmentally friendly way to reduce protein degradation in ensiled crops such as alfalfa. ARS has filed a patent application on the discovery, which could save farmers more than \$100 million per year.

“Right now, no practical techniques are available to farmers who want to reduce protein breakdown in alfalfa silage,” says plant physiologist Ronald D. Hatfield, of the Madison center. Research has shown that applying formic acid or using heat treatments can reduce protein degradation by 12 to 28 percent, but these methods are either too caustic or too expensive for farmers to use profitably. Formic acid, for example, must be handled with care and can be hard on some equipment.

But Hatfield and two other scientists at the center—agricultural engineer Richard E. Muck and molecular biologist Michael L. Sullivan—along with Deborah A. Samac, a plant pathologist in the St. Paul unit, have discovered a way to reduce protein loss by using

ingredients extracted from potato skins and red clover. Research leading up to their invention began more than 10 years ago.

Clues in the Clover

In the early 1990s, Muck and Hatfield were helping Beth Jones, a graduate student at the University of Wisconsin, research red clover and alfalfa silage. They found that although red clover and alfalfa have similar protein levels, the protein in red clover does not degrade during ensiling nearly as dramatically as the protein in alfalfa. In fact, red clover silage preserves 65 to 80 percent of its protein as true protein.

The researchers wanted to know why red clover, which outwardly seems so similar to alfalfa, made such excellent silage. “We looked to see whether there were different types of proteins in the two plants or differences in their protease activity,” says Muck. (Proteases are the enzymes responsible for breaking down proteins.) They didn't find anything at first.

But later, a clue emerged. Alfalfa clippings would remain green for a while after being cut, but red clover clippings would turn brown right away. Further studies revealed that red clover contains large amounts of polyphenol oxidase (PPO), the same enzyme that turns cut surfaces brown in apples, bananas, potatoes, and many other fruits and vegetables. Alfalfa has insignificant amounts of PPO.

For PPO to cause the browning reaction, it needs something to act on—a substrate—as well as exposure to oxygen. The substrates of choice for PPO are *O*-diphenols. They include compounds such as caffeic acid and related compounds, or conjugates, such as chlorogenic acid. In addition to containing high levels of PPO, red clover contains high amounts of caffeic acid and its conjugates. Alfalfa doesn't.

Hatfield explains how red clover safeguards its protein. “When the clover

is chopped up, its cells release PPO,” he says. “Once the PPO is exposed to oxygen, it reacts with the plant’s caffeic acid and forms a very reactive molecule known as an *o*-quinone. Quinones bind themselves to the proteases and keep them from degrading red clover’s protein.”

Since making these discoveries, Sullivan has been able to extract the PPO gene from red clover, and Samac has inserted it into an alfalfa plant. They recently conducted an experiment in which they chopped some transgenic alfalfa plants into 2-centimeter pieces, treated them with a bacterial inoculant, applied caffeic acid to about half of them, and let them sit for 2 weeks. Bacterial inoculants are the principal silage additives in the United States; they ensure fast and efficient fermentation in the silo.

The alfalfa plants treated with caffeic acid had 15 percent less protein degradation than untreated plants. The scientists believe they can preserve even more alfalfa protein if they improve their processing technique and grind the plant into smaller pieces.

A Potato Mash Alternative

Caffeic acid is present in high concentrations in a variety of fruits and vegetables, most notably potato skins—a common agricultural waste product. The scientists are currently working with different potato-processing plants to determine how easy it would be to extract large amounts of caffeic acid from leftover skins.

They are also looking at ways to insert the PPO gene into a bacterial inoculant. Such inoculants would excrete the protective PPO enzyme and enhance fermentation of the silage. Farmers could apply the inoculant and the potato-derived caffeic acid to their alfalfa crop. In this way, they could achieve results similar to the ones reached with transgenic alfalfa without having to grow a transgenic plant.

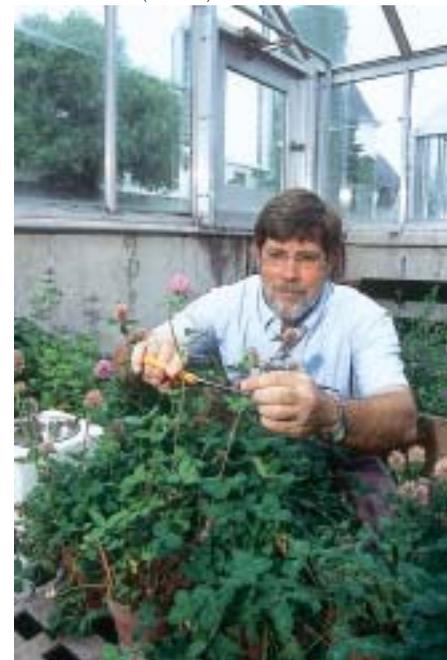
This technology should work on other ensiled crops as well, including corn and rye grass.—By **Amy Spillman**, formerly with ARS.

This work is part of Food Animal Production (#101) and Rangeland, Pasture, and Forages (#205), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

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In hopes of identifying possible ways to improve alfalfa silage, plant physiologist Ronald Hatfield harvests red clover from the greenhouse to study why it undergoes limited protein degradation in the silo.

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While a front-end loader works to fill this bunker silo, agricultural engineer Richard Muck takes forage samples that will be analyzed later for nutritive value and moisture content.