Alfalfa for Maximum Livestock Nutrition & Forage Yield

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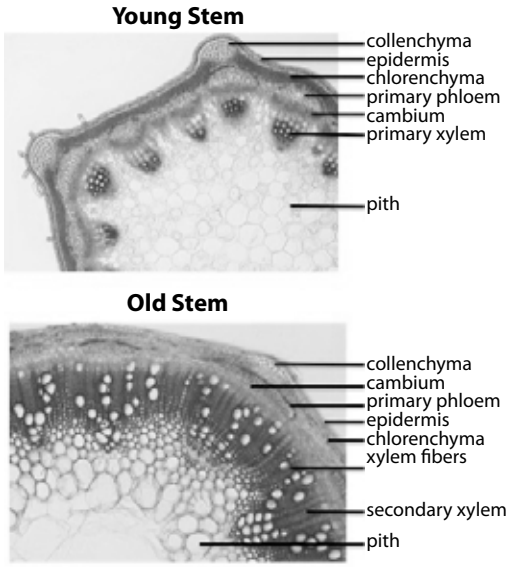
Alfalfa is a valuable forage for ruminant livestock because it is high in crude protein and energy (carbohydrates). However, maximum yield per acre is sacrificed in order to produce the highest feed quality product. In more mature alfalfa the high concentration of fiber limits the energy available to animals. How can alfalfa be modified to provide more energy at later maturity? The following is a summary of the long-term research conducted by scientists with the USDA-ARS in the Plant Science Research Unit (PSRU) in St. Paul, MN, to understand the plant characteristics that are roadblocks to producing a high energy feed and to develop alfalfa that retains digestible protein, has digestible carbohydrates, and has maximum productivity over an extended harvest period.

The ruminant digestive system is well adapted for utilization of roughages, or feeds with a large proportion of fibrous material. In forages, fiber is considered to be the plant cell walls that are digested slowly by ruminants. Microbes in the rumen secrete enzymes that breakdown starch and soluble sugars and partially breakdown the fiber and protein to support their growth. They produce volatile fatty acids, which are absorbed into the bloodstream of the animal. If there is insufficient carbohydrate digested, ammonia from the breakdown of protein is also absorbed into the blood and travels to the liver to be converted to urea and excreted in the urine. Digestion of the microbial biomass and remaining protein in the feed occurs in the abomasum (fourth stomach) and small intestine. The more rapidly feed is digested, the greater the food intake by the animal. In general, higher feed intake results in greater animal performance. Thus, even relatively small increases in forage digestibility (2-5%) have the potential to have a large impact on animal performance and costs for production. Additionally, increased fiber digestion will decrease manure production and losses of protein to urea thereby decreasing waste disposal issues.

A key to successful development of a more digestible cultivar is to focus on altering digestibility of alfalfa stems. As the alfalfa plant matures, the herbage becomes less digestible. Even when harvested at bud stage, a large amount of the forage is difficult to digest, and from 50-70% of more mature forage is poorly digested. This is due to the difference in digestibility of the cell walls. While cell walls of alfalfa leaves are easily digested, most of the cell walls in the stem are difficult for rumen microbes to digest. Previous attempts to select alfalfa simply for increased disappearance of total forage dry matter (DM) by rumen microbes led to an increase in the leaf fraction without changes in stem digestibility. The breeding and selection program by PSRU scientists focuses on identifying the developmental processes that cause poor digestion in stems and selection for stem traits that result in greater stem digestibility.

At the beginning of the project the first goal was to determine whether there is sufficient variation in stem digestibility to warrant a selection and breeding program. A wide range of alfalfa germplasm was surveyed for differences in stem digestibility. Although little variation was found in the cultivars tested at the time of the study, there was a significant amount of variation in forage quality traits among the unimproved germplasm tested, indicating that selection could be used to improve forage quality. PSRU scientists concluded that progress could be made by selecting for stems with the greatest in vitro neutral detergent fiber (NDF) digestion. The NDF fraction refers to the less digestible cell wall fraction of alfalfa, but the actual cell wall traits related to in vitro NDF digestibility were unknown.

Figure 1. Sections through young and old alfalfa stems stained to visualize lignified cell walls (dark). Older stems are primarily composed of lignified xylem cells that are never digested by rumen microbes and form most of the undigested fiber in alfalfa hay. Group 1 cells with thin unlignified cell walls, rapidly digested: chlorenchyma, cambium, secondary phloem. Group 2 cells with thin lignified walls, digested slowly: pith. Group 3 cells with thick unlignified pectin-rich walls, rapidly digested: epidermis, collenchyma, primary phloem of young stems. Group 4 cells with thick lignified walls, never digested: primary phloem of old stems, secondary xylem, xylem fibers.



To address this gap in knowledge, a series of studies were done using microscopy to identify the stem cell walls that are digested rapidly and those that were digested slowly, if at all, and then to determine the composition of both types of cells. Four groups of cells were identified based on cell wall digestion and composition: 1) thin, unlignified cell walls

Continued on page 20 ►

◄ Continued from page 19

that are rapidly digested; 2) thin, lignified walls that are digested slowly; 3) thick, unlignified, pectin-rich walls that are rapidly digested; and 4) thick, lignified walls that are only marginally digested. This latter group of cells, stained dark in Figure 1, is composed almost exclusively of the xylem tissue that conducts water and provides structural support for the stem. As stem segments become older, the xylem proliferates and forms most of the stem cell wall material at harvest. The lignin is thought to cross-link polysaccharides in the cell wall, preventing digestion by rumen microbes. The studies concluded that improving fiber digestibility should target genes that reduce total cell wall concentration, perhaps by reducing the rate of xylem formation, or that reduce the amount of lignin, and genes that increase the amount of pectin to improve the amount of easily digestible carbohydrate. Genetic modification of alfalfa to reduce the action of specific enzymes involved in lignin synthesis has been used successfully by other researchers to improve forage digestibility. Additionally, selection and breeding for increased pectin has also been accomplished by other researchers and been found to increase forage digestibility.

The PSRU scientists undertook a selection and breeding program to develop an alfalfa with increased NDF digestibility when harvested at later maturity. Although the in vitro NDF digestibility assay, which uses rumen fluid, is a very useful means to evaluate stem digestibility, it is too time-consuming for use in a breeding program in which thousands of samples need to be tested. However, near-infrared reflectance spectroscopy (NIRS) is a rapid test that is well suited for use in a breeding program once it has been calibrated for the samples being evaluated. Thus, a new calibration was needed specifically for examining alfalfa stems with a range of NFD digestibility. Starting with 4,000 alfalfa plants with highly diverse genetic backgrounds, plants were selected to represent the range of stem in vitro NDF digestibility. Each plant was evaluated for in vitro DM disappearance, cell wall composition and the near infrared reflectance data collected. The new NIRS calibration allows researchers to determine NDF digestibility of alfalfa stems rapidly and at low cost. Importantly, individual plants were identified from this study that had an increased rate and extent of NDF digestion compared to traditional alfalfa plants, and improved digestibility was stable over different years and growth environments. The most digestible plants had higher levels of pectin and the hemicellulose component fucose in stem cell walls and lower levels of lignin, suggesting that a breeding program for increased NDF digestibility could select simultaneously for these changes in stem cell walls. This suggests that development of lignified stem tissues is altered in plants with increased NDF digestibility.

The plants identified with increased NDF digestibility have been used in two cycles of selection and interbreeding with the goal of increasing digestibility at later maturity stages. With each cycle of selection, thousands of stem samples were tested by NIRS to identify those with desirable NDF digestibility and those plants were intercrossed to produce the next generation of improved plants. Multi-location field trials are being established with the latest generation of materials. The forage harvested will be tested for NDF digestibility from early bud stage through flowering and seed set. Data from the first year of evaluation indicated selection had been successful, particularly for extent of NDF digestion. Based on the final results of these trials, forage at specific growth stages will be harvested from larger plots and used in animal feeding trials in collaboration with scientists at the US Dairy Forage Research Center in Madison, WI. These experiments will provide information on the digestibility of the new alfalfa line compared to conventional alfalfa as well as a comparison of animal performance.