Strategies to Enhance Energy Content in Alfalfa Foliage

Deborah Samac, Melinda Dornbusch, Bruna Bucciarelli, USDA-ARS

Available energy is often the first limiting factor in high-forage rations for dairy and beef cattle. Alfalfa is a rich source of protein that is rapidly degraded in the rumen. However, resulting free amino acids cannot be completely captured if there is insufficient energy from carbohydrate fermentation. Although alfalfa stems contain large amounts of cell-wall carbohydrates (~70% of stem dry weight), the majority of the cell walls are poorly digested, since lignin in cell walls is a barrier to cell wall digestion. Dairy rations often include starch-containing feeds to partially reduce alfalfa protein wastage, but starch fermentation in the rumen can lead to acidosis and other health problems. Scientists in the USDA-ARS Plant Science Research Unit (PSRU) in St. Paul, MN, are exploring strategies to increase available energy from alfalfa by increasing fiber digestibility, amount of pectic polysaccharides, and production of lipids.

A long-term breeding and selection program by PSRU scientists focuses on identifying the developmental processes causing poor digestion in stems and selecting for stem traits resulting in greater digestibility. Starting with 4,000 alfalfa plants with highly diverse genetic backgrounds, plants were selected to represent the range of stem *in vitro* neutral detergent fiber digestibility (IVNDFD). Each plant was evaluated for dry matter disappearance and cell wall composition. Individual plants were identified 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. These studies also suggest that development of lignified stem tissues is altered in plants with increased NDF digestibility. Two cycles of selection and interbreeding for increased IVNDFD were done with the goal of increasing digestibility at later maturity stages. Multi-location field trials tested NDF digestibility of forage harvested from early bud stage through flowering and seed set. Results indicate selection has been successful, particularly for extent of NDF digestion and reduced lignin at later maturities compared to unselected varieties. Planned experiments will investigate the nature of the changes in the selected germplasm as well as developing methods to accelerate selection for improved fiber digestibility.

Pectin is a major carbohydrate component of the cell-wall matrix and is highly digestible by ruminants with a rate of digestion similar to that of alfalfa proteins. However, it is only found in the middle lamella between cells and the primary cell wall. To increase the amount of pectin in alfalfa stems, transgenic alfalfa plants were generated expressing a key gene for pectin biosynthesis, uridine diphosphate glucose dehydrogenase (UGDH). The target cells for enhancing pectin content are xylem cells, which conduct water through the stem. Xylem rapidly develops lignified secondary cell walls and becomes more indigestible with increasing plant maturity. Since xylem forms a continuous cylinder in alfalfa stems, the indigestibility of this tissue slows particle size reduction in the rumen. The rate of xylem fragmentation and clearance from the rumen should increase if pectin concentration increases in xylem cell walls. Plants with the greatest UGDH activity and vigor were selected for field testing and were evaluated for biomass yield and pectin accumulation in stems over two years. In young, rapidly growing plants, enhanced enzyme activity occurred in a number of transgenic lines. However, enhanced activity was not retained in mature field-grown plants. The moderate increase in UGDH activity in alfalfa stems led to an increase of cell wall component xylose, but not of the sugars for pectin production. It appears the pathways for cell wall sugar biosynthesis are highly interconnected and altering production of a single enzyme in this pathway will not increase the yield of such a complex end product as pectin. Alfalfa plants differing in stem pectin content have been identified and analysis of genes and gene expression in these plants may offer clues to successful modification of alfalfa for increased pectin content and rumen digestibility.

Supplementing dairy cow diets with plant or fish oil increases available energy and has the additional advantage of reducing production of enteric methane, an important greenhouse gas. Lipid supplementation also improves the fatty acid composition of meat and milk for human consumption, reducing the proportion of saturated fats.

Scientists in New Zealand demonstrated over-expression of specific genes involved in lipid biosynthesis in ryegrass is a successful strategy to increase fatty acid production, high metabolizable energy, and greater foliar biomass. Both fresh and ensiled high-metabolizable-energy ryegrass had significant reductions of methane when incubated in rumen fluid. Collaborators of scientists in the PSRU showed overexpression of two genes involved in lipid synthesis, Fat Storage-Inducing Transmembrane protein 2 (FIT2) and diacylglycerol *O*-acyltransferase 2 (DGAT2), significantly increased lipid droplet formation in leaves and seeds of several model plant species. One strategy to increase energy in alfalfa forage and reduce methane production by cattle is to increase the amount of neutral lipids produced in leaves. Transgenic alfalfa plants expressing FIT2 or DGAT2 produce more lipid droplets in leaves than unmodified plants (Figure 1). Ongoing work will determine the lipid content and composition, the effect of gene expression on other forage quality traits, and forage digestibility.

Figure 1. Visualization of lipid droplets (bright green spots) in alfalfa leaf cells. Left: untransformed plant. Right: plant expressing FIT2.