Progress Report on Reduced-Lignin Alfalfa: Part II, Animal Digestibility Trials

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The May 2009 issue of Forage Focus featured Part I of a progress report on efforts to reduce the lignin content of alfalfa. The article outlined the reasons why this is being done and documented progress in plant modification. Part II looks at how these genetically modified alfalfas perform in animal feeding trials.

The combined efforts of the Consortium for Alfalfa Improvement* has led to the development and evaluation of alfalfa with down-regulation of two specific enzymes in the lignin biosynthesis pathway – COMT (caffeic acid 3-O-methyltransferase) and CCOMT (caffeoyl CoA 3-O-methyltransferase). Early studies show that lignin-modified cultivars are commercially feasible.

Because alfalfa is a major forage in the diet of dairy cows in the U.S., it is important to see how cows respond to reduced-lignin alfalfa. However, evaluating new alfalfas using dairy cows requires tons of feed which is difficult to obtain and is very expensive with an experimental forage. This is especially true for genetically modified plants that have to be grown under strict regulations. So researchers at the U.S. Dairy Forage Research Center developed a research protocol in which young weanling lambs are used instead of cows to evaluate the digestibility of these genetically modified alfalfas. Why lambs? Because they consume much less feed but still eat forage at high levels of intake that mimic those of dairy cows.

The purpose of the study was two-fold: to test the intake and digestibility of the reduced lignin alfalfa; and to test the use of lambs to mimic dairy cows in the early stages of developing modified plants for livestock feed.

The trial. In the lamb feeding trials, hay genotypes with down-regulated COMT or down-regulated CCOMT were compared to their respective nulls (same genotype without genetic modification) to investigate the impact of reducing lignin on digestibility of alfalfa by lambs. Objectives:

- determine impact of down-regulating specific enzymes in lignin biosynthesis pathway on digestibility of alfalfas by lambs;
- investigate the impact of intake level on digestibility; and
- evaluate effectiveness of using in vitro (test tube) and chemical methods for predicting in vivo (live animal) digestibility.

Four cuttings (first, second, early maturity third, and later maturity third) of modified alfalfas and their nulls were generated by Forage Genetics International in Nampa, ID. This alfalfa was developed for research use only (following all the necessary regulations and permits) and is not commercially available. Hays varied from 18.0-21.7% crude protein and 36.8-42.1% amylase-treated NDF organic matter (aNDFom), but were similar in protein and fiber among treatments within each cutting. The lignin concentration of COMT was typically 0.5 %-units less than its null and CCOMT was 0.7 %-units less than its null.

A 35-day digestion trial protocol was developed that measured:

- ad libitum (free-choice) intake and digestibility of a standard reference alfalfa hay;
- free choice intake and digestibility of four treatment hays within a cutting; and
- digestibility of treatment hays measured at restricted intakes that approximated maintenance levels of intake (about 2% of body weight/day).

Three to four weaning lambs (average body weight of 64-86 lbs) were used per treatment. The animal trials were conducted at the University of Wisconsin-Madison, and the protocol was reviewed and approved by the UW's Research Animal Resources Center for proper care and welfare of the lambs.

RESULTS

Intake. Free choice intake was measured at refusal levels of 9-15% of feed offered. These intakes (percentage of body weight/day) were not different among treated hays and averaged 3.43-4.05 for first cut, 3.51- 4.08 for second cut, 3.92-4.21 early maturity third cut, and 3.90 -4.06 later maturity third cut. Restricted intake varied from 1.8-2.3 for all cuttings.

Digestibility. Digestibility of aNDFom was greater for COMT than its null for all cuttings ($P<.01$) averaging .562 vs .452 and .535 vs .449 for free choice and restricted intakes, respectively. For CCOMT down-related alfalfa, digestibility of aNDFom was higher than its null for all cuttings ($P<.01$) at restricted intakes (.494 vs .435), but was only statistically significant for second cut and early maturity third cut with an average of .494 vs .448 for all cuttings at free choice intakes. Digestibility was slightly lower for free choice compared to restricted intakes.

The effect of intake level was not as large as expected. This may be related to the observation that lambs fed free choice refused

stems that were consumed when they were restricted-fed. Digestibility measured at free choice intake may be more appropriate for indicating lactating cow digestibility, but digestibility measured at restricted intake may be a better indicator of the intrinsic digestibility of the hays. Digestibility of DM and organic matter were proportional to changes in digestibility of aNDFom. It is interesting that improved fiber digestibility in alfalfa increases intake which is similar to the effect observed for brown midrib corn silage that also improves fiber digestibility.

Eating and ruminating times/kg of NDF intake were not different among down-regulated and null alfalfas. In a feeding trial, average daily gain was 10% higher for lambs fed down-regulated alfalfas compared to their nulls, but this was not statistically significant as the study used only two pens of 3-4 lambs/treatment. Preliminary results indicate that laboratory measures of in vitro (test tube) digestibility of aNDFom were positively correlated with in vivo (live animal) results, but additional analysis of this data is needed.

Summary. Down-regulating one enzyme (COMT) improved fiber digestion by the lambs twice as much as down-regulating the other enzyme (CCOMT). The increase in fiber digestibility was consistent with a 10% improvement in daily gain of lambs. For the first time, tools were available for testing "proof-of-concept" that specific changes in lignin, in plants with the same genetic background and growing environment, affect animal performance. Not only can alfalfa be modified to develop experimental genotypes very rapidly, but researchers have gained knowledge about how specific changes in lignin modify alfalfa. This knowledge will help in developing new varieties of alfalfa that have greater value.

*The Consortium for Alfalfa Improvement consists of Forage Genetics International, Pioneer Hi-Bred International, The Noble Foundation, and the U.S. Dairy Forage Research Center (USDA-Agricultural Research Service).