LOW LIGNIN ALFALFA: REDEFINING THE YIELD/QUALITY TRADEFOFF

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ABSTRACT

We determined the change in forage quality of two reduced lignin transgenic alfalfa (*Medicago sativa* L.) populations, compared to appropriate controls and grown in diverse environments. Harvests were taken beginning at late vegetative stage and continued at 5 day intervals for 5 total harvests. Forage samples were analyzed for crude protein, neutral detergent fiber, acid detergent lignin, and neutral detergent fiber digestibility (NDFD). In this study, late harvested COMT and CCOMT lines had the same NDFD as the nulls or commercial check harvested 8 to 12 days earlier. Producers using this trait may be able to delay harvest while maintaining forage quality, potentially eliminating one or more annual harvests and while increasing yield by 20 to 30%.

INTRODUCTION

Lignin provides strength to plants and allows the plant vascular system to transport water in the plant without leakage. Lignin increases with advanced maturity in alfalfa. However lignin is indigestible and reduces fiber digestibility in ruminants. Thus reducing lignin content should increase fiber digestibility at any maturity stage.

Lignin is composed of three monomers, each a carbon ring with differing methoxy group configuration and a 3-carbon tail: *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (Figure 1). These subunits polymerize into lignin in the form of the phenylpropanoids: *p*-hydroxyphenyl (H), guaiacyl (G), and syringal (S), respectively (see fig 2). This lignin molecule fills the spaces between cellulose, hemi-cellulose and pectins as the plant ages and binds with the hemicellulose. Lignin coating the cellulose allows water to move up the plant stem without leakage but also reduces digestion of the cellulose in the rumen.

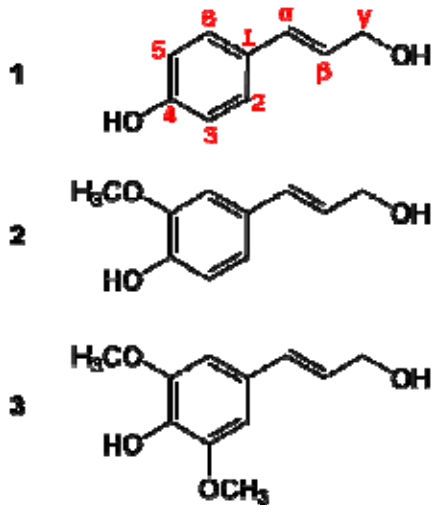

Fig 1. Three Lignin Precursors

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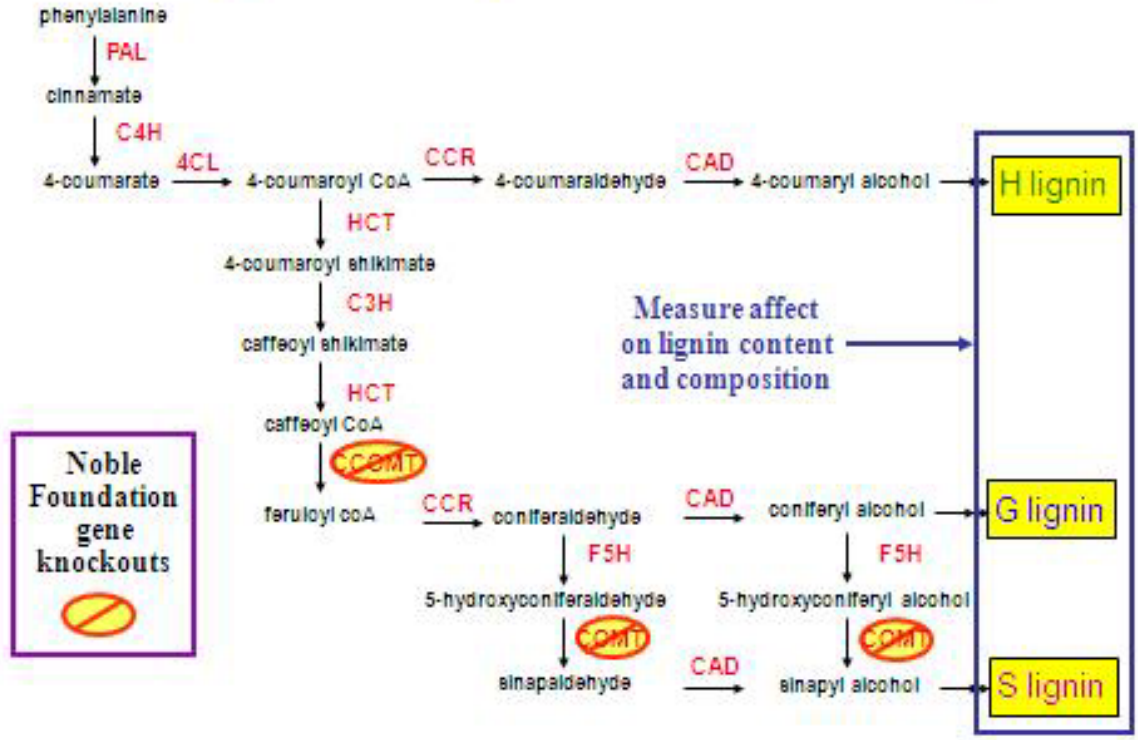
The proportions of the three lignin subunits varies among plant species. Legumes also tend to have higher lignin content than grasses and it is possible that lignin content can be reduced while maintaining the desired agronomic characteristics.

PROCEDURES

Transgenic alfalfa lines down-regulated for two lignin biosynthetic genes (caffeic acid 3-O-methyltransferase (COMT) or caffeoyl CoA 3-O-methyltransferase (CCOMT) have been developed (Marita et al., 2003). The lignin synthesis pathway and affected enzymes are shown in figure 2. Alfalfa populations for this study consisted of transgenic alfalfa lines down-regulated for one of two lignin biosynthetic genes (COMT and CCOMT), their null isogenic lines, and a check variety (LegenDairy 5.0). Seed was produced in December 2005 at Forage Genetics International, West Salem, WI. Crosses were made of five COMT events and five CCOMT events to elite FD4 conventional clones from 2001 and 2002 Wisconsin breeding nurseries. The seed was shipped in February 2006 to FGI Nampa, ID and planted in the greenhouse. Plants were tested for the CP-4 protein using CP4-EPSPS lateral flow strips and divided into four groups: COMT, COMT Null, CCOMT and CCOMT Null. Regulated seeds were increased on four experimental populations (two test populations, two control (Null) populations).

Fig 2 Lignin Biosynthetic Pathway



Replicated studies were conducted at Becker, MN; Arlington, WI; and West Salem, WI. The experimental design was a split plot with harvest dates as whole plots and entries as sub plots. Plants were started in the greenhouse and then transplanted into the field in spring 2008 into

rows spaced 30 cm apart with 30 cm between rows. Each plot consisted of three rows of 9 plants. The middle 7 plants of the middle row were harvested for yield and quality in summer 2008. Harvests were taken beginning at late vegetative stage and continued at 5 day intervals for 5 total harvests.

Forage samples were analyzed for crude protein (CP), neutral detergent fiber (NDF), acid detergent lignin (ADL), and neutral detergent fiber digestibility (NDFD) (in vitro 48 hr) and Relative Forage Quality (RFQ) was calculated.

RESULTS

Proof of concept trials were conducted (separately from this study) where lignin transgenic alfalfa hay fed to rapidly growing lambs (table 1) or in total mixed diets with corn silage to lactating dairy cows measured increased fiber digestibility and (table 2). In the lamb trial, while NDF was not significantly different, both intake and NDFD of the COMT line was increased over its null (active) control. CCOMT did not show any significant responses. In the study with dairy cattle, Digestible dry matter of COMT alfalfa increased 3.5% fat corrected milk over it's null (active) line by 2.6 lb/hd/day (table 2).

Table 1. Digestibility of low lignin alfalfa types and controls fed to lambs, diet was 100% alfalfa hay fed ad libitum.

100% alfalfa hay diet	aNDF % DM	ADL % DM	NDFD % NDF	DMD % DM
COMT Inactive	38.2	5.3	57.5*	67.5*
COMT Active (Control)	39.0	5.8	49.1	64.5
CCOMT Inactive	39.4	5.2	50.1	65.3
CCOMT Active (Control)	39.4	5.9	46.4	63.7
*Significant, P < 0.05				
SOURCE: Mertens et al. 2008. J. Dairy Sci. Supple. 1				

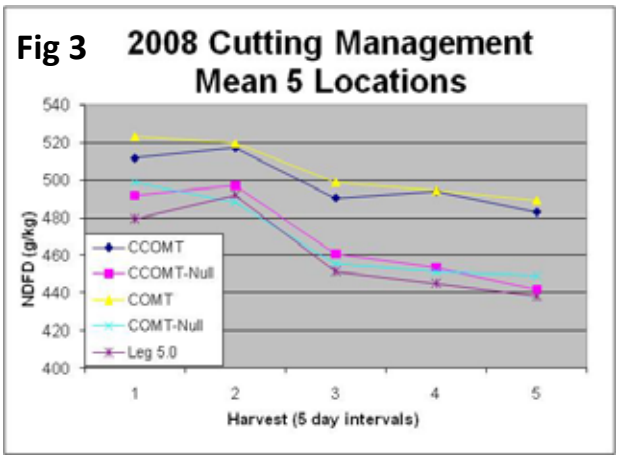
Table 2. Lactating cow responses to alfalfa hays with down-regulated lignin biosynthesis

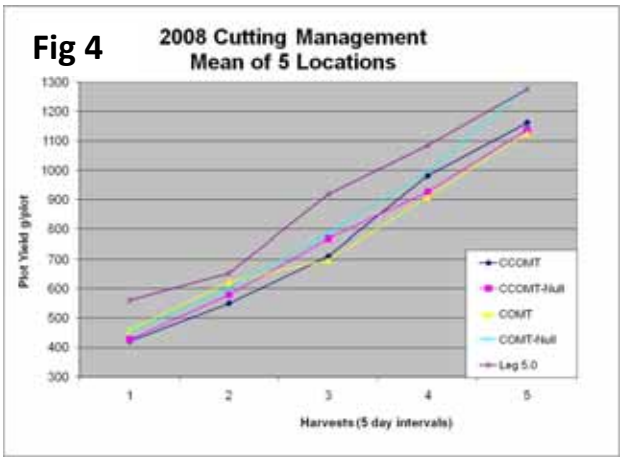
Alfalfa hay type[1]	CP % DM	NDF % DM	NDFD %NDF	Milk lb/day
COMT Inactive	18.1	31.1	53.5**	84.7*
COMT Active (Control)	18.4	29.3	42.5	82.1
CCOMT Inactive	18.1	42.5	48.6**	84.5
CCOMT Active (Control)	18.3	31.1	44.5	86.7
[1]TMR diets - 50 % alfalfa hay, 10 % corn silage, 40 % concentrate				
*Significant, P < 0.10; ** significant P <0.01 (different from control)				
SOURCE: Weakley et al. 2008. J. Dairy Sci. Supple. 1				

Alfalfa stems from reduced lignin genotypes increased sugar yield which potentially could increase ethanol > 50% compared to standard alfalfa.

In the current study, the null lines and the check variety (LegenDairy 5.0) had similar fiber digestibility which gradually declined as maturity advanced (later harvest date). Both low lignin lines had consistently higher fiber digestibility at each harvest date (figure 3). The CCOMT line averaged 12 percentage units less ADL than the null and had 10.2 percentage units more NDFD. The COMT line averaged 3.7 percentage units less ADL than the null and 14.0 percentage units more NDFD. A change in lignin composition in the COMT population is a likely explanation for the increased fiber digestibility relative to lignin content. Thus it will be possible to harvest

higher quality with either of the transgenic alfalfa lines down-regulated for lignin synthesis when harvesting occurs on a similar schedule as for non-transgenic lines.





Another way to view this data is that COMT and CCOMT lines harvested 8 to 12 days later than the nulls or commercial check had the same forage quality. Later harvesting will allow for higher yield per cutting (figure 4).

The CCOMT line stood (lack of lodging) as well in space plantings at the commercial alfalfa line. COMT is an artificially created mutation for the same gene as bm3 which is a naturally occurring reduced lignin gene in corn. We did see some standability issues with this line. However, further breeding efforts may be able to solve this problem.

CONCLUSION

When alfalfa begins to regrow after being cut, the growth per acre is low (perhaps less than 50 lbs/acre/day) but as the crop matures the growth per day increases (to 200 lb/acre/day or more at harvest). Thus, in alfalfa harvest systems where we took 3 vs 4 cuttings within the same time period, alfalfa yield was 20 to 30% greater (with the labor of one less cutting) for the 3-cut system. In the past the quality of the 3-cut system was much lower; now it may be possible to delay harvest to get the higher yield with the same forage quality as from standard alfalfa varieties cut earlier. I believe that this will be the most important aspect of this transgenic reduced lignin alfalfa, rather than higher quality forage harvested at the same date.

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