Lignin and Fiber digestibility in Caffeic Acid 3-O-Methyltransferase and Caffeoyl CoA 3-O-Methyltransferase Downregulated Alfalfa

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Alfalfa (*Medicago sativa* L.) is one of the most important forages in the United States. Increasing alfalfa fiber digestibility would improve forage management and ration formulation flexibility. Currently, growers and breeders rely on near infrared spectroscopy (NIRS) to predict forage quality traits for alfalfa. With the introduction of lignin downregulated alfalfa plants, the question arises if standard alfalfa NIRS equations are accurate at predicting forage quality traits for novel low-lignin alfalfa samples. This study reports *in vitro* neutral detergent fiber digestibility (NDFD) and lignin analysis (ADL, Klason, total thioacidolysis yield, S-lignin, G-lignin, and H-lignin) of caffeic acid 3-O-methyltransferase (COMT) and caffeoyl CoA 3-O-methyltransferase (CCOMT) downregulated alfalfa. Lower stem samples of 40 COMT, 40 CCOMT, and 20 null type alfalfa plants collected over three locations from a total of 10 harvest by location combinations (2900 field samples) were analyzed in this study. Samples were

Lower Stem Calibration Samples	NIRS Breeder Equation*		Chemical Analyses						
	ADL	NDFD	ADL	Klason Lignin	Total Thioacidolysis Yield	S-Lignin	G-Lignin	H-lignin	NDFD
	%DM	%NDF	% DM						%NDF
Null	12.5b	31.8b	10.2c	14.5c	6.1c	2.7b	3.3b	0.7a	30.6c
COMT	11.2a	35.8a	9.6b	12.9b	3.8a	0.7a	3.1b	0.7a	41.0a
CCOMT	10.9a	35.3a	7.9a	11.9a	5.0b	2.7b	2.2a	0.9b	36.2b

Significantly different from each other at $p < 0.05$
* Predicted analysis using a NIRS equation for whole plant alfalfa developed for plant breeders.

selected based on predicted ADL from NIRS equations for whole plant alfalfa developed for alfalfa breeders. Although NIRS analysis indicated lower lignin and higher digestibility for COMT and CCOMT, laboratory analysis revealed that the NIRS breeder's equation had large positive bias in predicting ADL for CCOMT and large negative bias in predicting NDFD for COMT transgenics. This study also reveals that relative values among downregulated alfalfa differed for thioacidolysis measured lignin compared to ADL and Klason lignin. Total thioacidolysis yield was the best predictor of NDFD. In particular S-lignin levels were highly correlated with NDFD among all types of alfalfa samples in this study. New NIRS equations were developed successfully for all laboratory constituents evaluated in this study. NIRS equations used by forage testing laboratories will need updating to accurately predict lignin and digestibility in lignin downregulated alfalfa.