Title: Evaluating Progress in Alfalfa Forage Quality Improvement

Craig C. Sheaffer.
University of Minnesota, 1991 Upper Buford circle, 411 Borlaug Hall, St. Paul, MN 55108
Email: sheaf001@umn.edu

Jerome H. Cherney
Cornell University, 503 Bradfield Hall, Ithaca NY
Phone: 607-255-0945
Email: jhc5@cornell.edu

Abstract

Over the past several decades, alfalfa variety improvement in forage yield and stand persistence has occurred through both plant breeding and genetic modification. Today, many varieties are marketed as high quality (HQ). However, there is a lack of research evaluating differences among varieties in forage quality. Therefore, this study evaluated forage quality and yield of three groups of alfalfa varieties: those marketed as high quality (HQ), those developed through genetic modification to reduce lignin content (RL), and those serving as standard, reference control. Research was conducted in the seeding year and the following year in New York and Minnesota with replicated plots sampled over multiple maturities in each harvest season. Annual yield was not different among alfalfa varieties and averaged 4.4 and 6.6 ton/acre in New York and Minnesota, respectively. Over locations and several maturities, RL varieties had lower forage lignin concentration and higher NDFD concentration than other varieties, but were not consistently different in CP or NDF concentration from other alfalfa marketed as HQ. Control varieties and those marketed as high quality did not differ in lignin and NDF digestibility. For all varieties, forage lignin increased with maturity from vegetative to flowering while NDF digestibility decreased; however, the RL alfalfas maintained greater forage NDF digestibility than other varieties over a range of alfalfa maturities.

Introduction

New alfalfa varieties with improved forage quality are available for producers. However, there is lack of information for growers on direct comparison of these alfalfa varieties. We propose to answer the question, “How do the alfalfa varieties marketed as high quality compare with transformed, reduced-lignin types”? We conducted research to measure the forage quality and forage yield of a diversity of new alfalfa varieties over a range of harvest maturities used by growers.

There have long been efforts to improve alfalfa forage quality through conventional plant breeding. Until recently, differences in variety forage quality have been inconsistent over locations and greatly influenced by factors such as growth condition and crop maturity (Lamb et al., 2006; Sheaffer et al., 1998). A genetically engineered, reduced-lignin alfalfas, are now being marketed (http://www.foragegenetics.com/). In field trials in Minnesota, we found that compared to conventional alfalfa cultivars, ‘54HVX41’ a reduced lignin alfalfa, had an average of 10% less acid detergent lignin (ADL) and 10% greater neutral detergent fiber digestibility

(NDFD) compared to non-transgenic reference varieties (Grev et al., 2017). Dairy cow feeding trials with transgenic reduced-lignin alfalfa forage as a portion of the ration showed increased milk production of 2.6 lb/head/day compared to forage from conventional alfalfa controls (Undersander et al., 2009). A large number of conventionally developed varieties marketed as high quality have not been directly compared to HarvXtra branded varieties in trials spanning the northern region.

Use of alfalfa varieties with improved forage quality has potential to be advantageous to growers. At any given maturity stage, reduced-lignin alfalfa varieties will have greater feeding value than standard varieties. In addition, improved forage quality provides growers management flexibility to delay harvest to a later stage of maturity. This could allow for a wider optimal harvest window, making it possible for alfalfa growers to achieve higher yields by delaying alfalfa harvest while still maintaining higher forage nutritive values. For example, in Minnesota we found that, '54HVX41', a reduced lignin alfalfa, harvested on a 35-day harvest interval showed a 21% gain in yield and only a 3% reduction in RFQ while reference varieties had similar gain in yield but greater loss in RFQ (Grev et al., 2017). We do not know if the pattern of quality decline within increased maturity and time observed with the '54HVX41' will be similar for all the new "high quality" varieties and therefore sampling over a range of maturities is important.

Materials and methods

Research evaluating the forage quality of 24 alfalfa varieties was conducted at Ithaca, NY, and at St. Paul, MN. Six replicates of the cultivars were established at each location. Three of the replicates were used for forage quality sampling and three of the replicates were used for forage yield determination. For the forage quality evaluation, the experimental design was a randomized complete block with treatments in a split-plot arrangement. Whole plot treatments were alfalfa varieties that were completely randomized and that for statistical purposes were grouped into three categories (**Control, widely grown varieties not marketed as high quality; HQ varieties developed through conventional plant breeding and marketed as high quality; and RL, reduced lignin transgenetic varieties**) based on information we had received from alfalfa seed companies (Table 1). Sub plot treatments were 5-6 sequential sampling dates that resulted in forage of increasing maturity. Sampling events occurred on three separate times: in the summer of establishment year and in spring and summer of the year following seeding. For forage yield measurement, the experimental design was a randomized complete block.

Weather during the sampling periods was near normal at each location (NOAA Northeast Regional Climate Center (ITHACA CORNELL UNIV, Ithaca, NY). And (UNIV OF MINN ST PAUL-ID: 218450 without precipitation deficits. Overall, precipitation was lower in Minnesota compared to Cornell, and there was a larger range of monthly average temperatures in Minnesota in both years (Table 2).

Alfalfa varieties were planted by drilling seed into a prepared seedbed in May 2017 at Ithaca and St. Paul. Plots measured 1.8 by 6.1 meters. Before seeding, soil was tested for pH, P, K, and S and fertility amendments were made to meet recommendations for alfalfa hay production. Annual weeds at each site were controlled after establishment by application of a post-emergent herbicide, imazamox (Raptor). Potato leafhopper (*Empoasca fabae*) were controlled by application of insecticide (Arctic 3.2 EC) when necessary.

In the seeding year (2017), forage quality was evaluated by sampling the regrowth following the first cutting after establishment beginning on 21 August at Ithaca and 22 August at St. Paul. In 2018, quality was evaluated in the spring beginning on 29 May at Ithaca and 21 May at St. Paul and in the summer beginning on 3 July at Ithaca and 6 August at St. Paul. Forage quality was measured by hand-harvesting three random 0.3m^2 areas in each plot to a 5 cm height and the sampled forage was then dried for 48 hours at 60°C. Dried samples were ground and scanned using near infrared reflectance spectroscopy (NIRS) using a Perten NIRS (Model DA 7200; Perten Instruments, Springfield, IL) to estimate crude protein (CP), neutral detergent fiber (NDF), acid detergent lignin (ADL), and neutral detergent fiber digestibility (NDFD).

Alfalfa plant maturity was determined using the mean stage count (MSC) method of Kalu and Fick (1981) by hand-harvested a 0.1 m^2 areas from each plot and placing stems in groups: vegetative growth includes stages 0 through 2, budding plants includes stages 3 and 4, and flowering plants includes stages 5 and 6.

Yield was measured in 2018, the year after seeding, when alfalfa reached the bud-early flowering stage, resulting in four harvests at both locations. Harvest dates were: 30May, 2July, 10August, and 12 September at New York; and 4June, 27June, 4August and 13September at Minnesota. We mechanically harvested a 0.9 by 3 meter strip from plots adjoining the forage quality plots using a flail harvester set to leave a 5 cm stubble. Harvested forage was weighed, and dry matter content determined by drying a 500 gram sample for 48 hours at 65°C and adjusting yield to a dry matter basis.

Data was analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). Treatments were tested as fixed effects while blocks nested within locations will be tested as random effects. Statistical significance was set at $P \leq 0.05$. We tested for location by variety by maturity interactions. Means separations were performed on significant effects using Tukey's HSD test. To assess the relationship between plant maturity and forage nutritive values, we regressed forage nutritive values against alfalfa plant maturity.

Objectives:

a. **To evaluate the forage quality of new alfalfa varieties sampled in two environments over a range of maturities.**
   Varieties developed for reduced lignin content (HarvXtra) had consistently lower lignin and higher NDF digestibility than control varieties and other conventional varieties marketed as high quality. Control varieties and those marketed as high quality did not differ in lignin and NDF digestibility. Differences in crude protein, neutral detergent, and acid detergent fiber were not consistent among varieties over locations. For all varieties, forage lignin increased with maturity while NDF digestibility decreased.

b. **Measure forage yield of new alfalfa varieties marketed for improved forage quality.**

The alfalfa varieties within the three variety groups did not differ in forage yield in the year following seeding. Yields averaged 4.4 and 6.6 ton/acre in New York and Minnesota, respectively.

Results and Discussion:

In both Minnesota and New York, averaged for all harvests, the reduced lignin (RL) variety group was highest in average NDFD and lowest in lignin concentration (Table 3). The control and HQ variety groups did not consistently differ in these parameters. As illustrated by the spring 2018 data, forage lignin and NDF digestibility was affected by maturity. Lignin concentration increased while NDF digestibility decreased. RL varieties were always among the highest in digestibility and lowest in lignin, but the relationship with the other varieties was not consistent over the large range of maturities (Figure 1 and 2). Varieties groups did not differ in CP or NDF concentration in Minnesota, but in New York the variety groups sometimes differed in CP, and NDF. However, there were no consistent rankings. Crude protein concentration averaged 20.0, 19.6, and 23 % for the 2017 establishment year, spring 2018, and summer 2018 harvests in New York, respectively; and 21.7, 20.1, and 19.2% for these same harvest periods at Minnesota. NDF averaged 30.6, 41.1, and 32.4% for the 2017 establishment, spring 2018, and summer 2018 harvests in New York, and 35.9, 44.5 and 45.2% for these same harvest periods at Minnesota. Our values across a broad group of RL and non RL varieties confirm that results of Grev et al., 2017, in Minnesota, and Arnold et al., 2019 , over six states, who showed that new HarvXtra RL alfalfa varieties had increased NDF digestibility with similar CP, and NDF concentrations compared to two non RL varieties. Our results also indicate that marketing claims regarding enhanced forage quality of conventionally developed varieties should be carefully examined by seed purchasers.

No differences in forage yield were detected among varieties at either location, but annual yield was lower at New York for all treatment groups compared to Minnesota, likely the result of fewer growing degree days from May through August in New York. Forage yield in the year after seeding (2018) was as follows for the three alfalfa variety groups: CON 4.38, HQ 4.54, RL 4.29 ton/acre at New York and CON 6.72 , HQ 6.66 , RL 6.49 ton/acre for Minnesota. Grev et al. (2017) reported forage yield being slightly lower for RL varieties than the non RL varieties (8.0 vs. 8.3 ton/acre) while Arnold et al. (2019) reported that RL entries had 4.9 to 7.5% lower yields than non-RL varieties.

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Keywords:

Alfalfa varieties
Alfalfa forage quality
Lignin

Table 1. Alfalfa varieties evaluated for forage quality. Three groups were control entries representing varieties not selected or marketed for forage quality (CON) ; HQ (HQ) entries representing varieties that are marketed as having high forage quality, and reduced lignin (RL) varieties.

Company	Variety	Variety group	Fall Dormancy	Winter Survival
NexGrow	6472A FGI	CON	4	1
Preferred Seed	PS2010LH	CON	4	2
Dairyland	Magnum 7	CON	4	2
Dairyland	Hybriforce 2420/wet	CON	4	2
SeedWay	N-R-Gee	CON	4	2
SeedWay	AlfaMax	HQ	4.5	2
WL	WL365HQ FGI	HQ	4.9	1.1
WL	WL356HQ.RR FGI	HQ	3.8	1.6
Alforex	Hi-Gest 360	HQ	3	2
King's Agriseed	KF 425HD	HQ	4.5	2
NexGrow	6585Q	HQ	5	2
America's Alfalfa	AmeriStand 427TQ	HQ	4.3	1.8
Land O'Lakes	LegenDairy HXD	HQ	3	1
Pioneer	54Q14 JH	HQ	4	2
S & W	SW4107	HQ	4	2
S & W	SW3403	HQ	3	2
GrowMark FS	475HVXRR (2016)	RL	4	2
FGI	Hx-14376 FGI	RL	4	2
FGI	Hx-15144 FGI	RL	4	2
Channel Seed	HVX	RL	4	2
GrowMark FS	440HVX.RR	RL	4	2
WL	341HVX.RR FGI	RL	4	2.1
NexGrow	6409 HVXR FGI	RL	4	1
America's Alfalfa	480 HVXRR FGI	RL	4	2

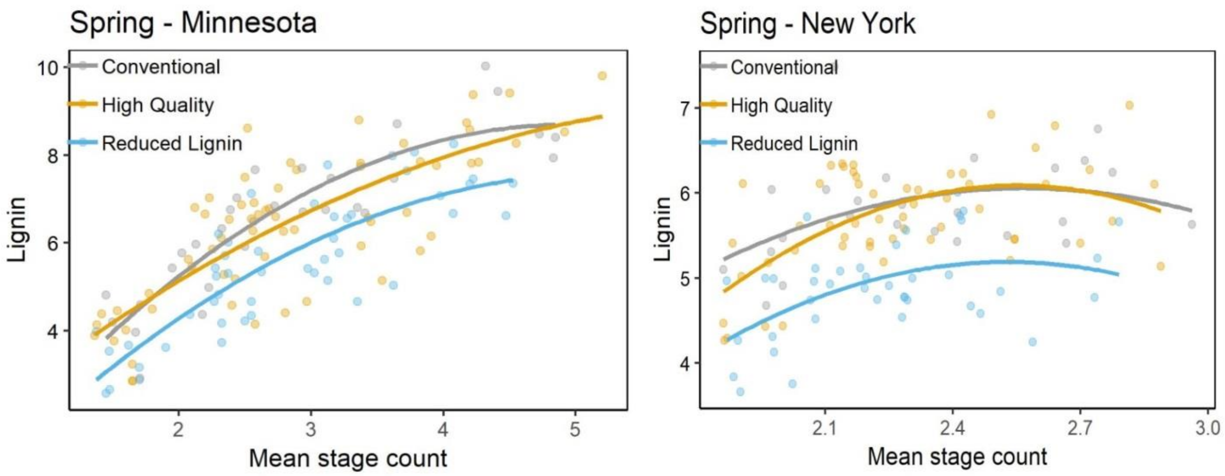
Table 2. Average monthly air temperature and precipitation and growing degree days using a base temperature of 40 F.

		2017			**2018**		
		Average Temperature, °C	**Total Precipitation, cm**	**Growing Degree Days**	**Average Temperature, °C**	**Total Precipitation, cm**	**Growing Degree Days**
	January	-1.7	6.7	0.0	-6.9	6.0	26.9
	February	0.3	5.6	38.7	-1.0	5.2	47.0
	March	-2.2	8.3	46.7	-1.9	8.2	17.4
	April	9.7	11.7	218.0	3.5	5.4	89.5
	May	12.3	11.4	284.0	15.7	9.8	383.0
	June	18.0	9.4	434.0	17.3	5.3	415.0
	July	20.4	16.9	523.0	21.4	10.0	555.0
	August	18.6	6.0	467.0	21.4	12.6	554.0
	September	16.2	5.6	383.0	17.8	13.6	430.0
	October	12.6	17.9	299.0	9.1	13.6	198.0
	November	2.9	5.2	76.2	1.2	10.5	50.2
	December	-3.7	3.4	18.9	-0.8	5.6	23.0
	SUM	**------------**	**108.0**	**2788.5**	**------------**	**105.8**	**2789.0**
	January	-7.5	2.4	0.0	-11.1	2.5	3.3
	February	-2.3	1.8	12.5	-11.9	2.8	3.8
	March	-0.7	1.5	43.0	-1.4	2.3	19.2
	April	8.4	9.3	172.0	1.0	5.0	75.8
	May	13.3	16.6	308.0	18.6	10.9	467.0
	June	20.3	8.0	505.0	21.5	15.4	539.0
	July	22.3	6.2	582.0	22.0	11.1	574.0
	August	18.6	22.6	469.0	21.1	9.9	545.0
	September	17.6	3.2	423.0	17.4	15.7	419.0
	October	9.5	12.3	210.0	6.2	7.6	132.0
	November	-0.6	1.1	28.0	-3.3	3.8	16.4
	December	-8.3	1.6	15.6	-5.0	4.7	2.7
	SUM	**------------**	**86.5**	**2768.1**	**------------**	**91.8**	**2797.2**

Table 3. Average alfalfa forage neutral detergent fiber digestibility (NDFD) and lignin concentration for control, high quality marketed (HQ) and reduced lignin (RL) varieties for three harvests in New York and Minnesota in 2017 and 2018. Values are averaged for forage harvested from vegetative to early flowering harvest stages.

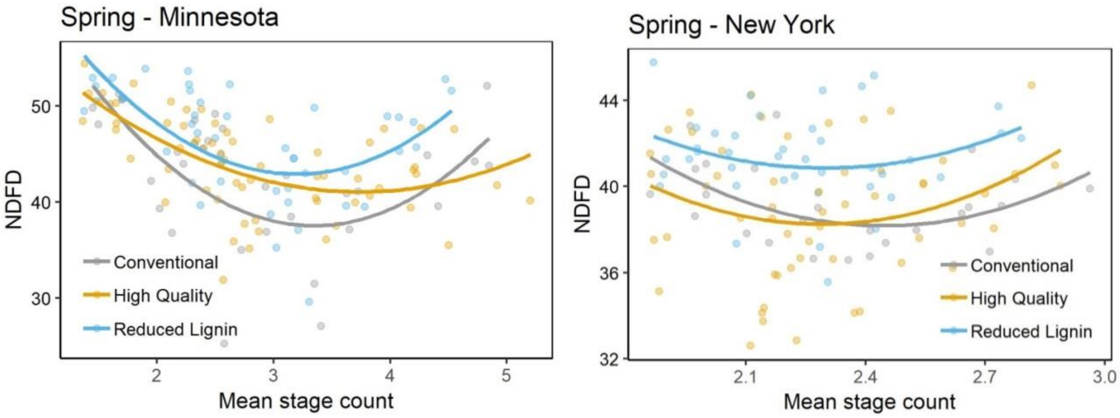
		New York			Minnesota		
Nutritive Value	**Group**	**Summer, 2017**	**Spring, 2018**	**Summer, 2018**	**Summer, 2017**	**Spring, 2018**	**Summer, 2018**
NDFD, %	CON	$40.3^{a,y}$	$39.0^{a,b,y}$	$38.0^{b,y}$	$55.1^{a,y}$	$42.5^{b,y}$	$42.8^{b,y}$
	HQ	$40.5^{a,y}$	$39.1^{b,y}$	$37.2^{c,y}$	$55.4^{a,y}$	$43.7^{b,y}$	$42.6^{b,y}$
	RL	$42.5^{a,x}$	$42.0^{a,x}$	$39.7^{b,x}$	$57.1^{a,x}$	$46.2^{b,x}$	$45.1^{a,y}$
Lignin, %	CON	$6.0^{a,x}$	$6.0^{a,x}$	$4.1^{b,x}$	$5.7^{c,x}$	$6.4^{b,x}$	$6.9^{a,y}$
	HQ	$5.9^{a,x}$	$6.0^{a,x}$	$4.3^{b,x}$	$5.5^{c,x}$	$6.3^{b,x}$	$6.7^{b,y}$
	RL	$5.4^{a,y}$	$5.2^{a,y}$	$3.7^{b,y}$	$4.9^{c,y}$	$5.4^{b,y}$	$5.9^{b,y}$

† Means followed by similar superscripts a, b and c indicate similar values within rows using Tukey comparisons of estimated marginal means across harvest seasons. Means followed by similar superscripts x and y indicate similar values within columns using Tukey comparisons of estimated marginal means across variety groups.


Spring - Minnesota

Spring - New York

Figure 1. Change in alfalfa variety forage lignin concentration when alfalfa was harvested at several maturity stages in spring of the year following seeding.
2= late vegetative, 3= early bud, 4=late bud, 5=early flower


Spring - Minnesota

Spring - New York

Figure 2. Change in alfalfa variety neutral detergent fiber digestibility when alfalfa was harvested at several maturity stages in spring. Stages were: 2= late vegetative, 3= early bud, 4=late bud, 5=early flower