Lower Lignin Alfalfa: Updates on Agronomy and Feeding

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Summary

Alfalfa is commonly known as the "queen of forages", which is fitting for the 2nd most prevalent forage used on U.S. dairy farms. Among its disadvantages, however, is the relatively poor digestibility of alfalfa fiber. Several strategies have been used to generate alfalfa varieties with lower lignin content in recent years, including conventional breeding programs and genetic engineering. Recent research has demonstrated that these varieties do offer improved in vitro NDF digestibility **(NDFD)** if alfalfa is harvested at typical cutting intervals, or standard NDFD when harvested at longer intervals. An initial dairy cattle feeding study demonstrated that a mixed-source lower lignin alfalfa hay resulted in increased dry matter **(DM)** intake but decreased milk fat percentage compared to conventional alfalfa hay, when fed in high-energy diets to mid-lactation cows. Research to date suggests that dietary utilization of lower lignin alfalfa sources should be approached in a similar manner to brown mid-rib corn silage, anticipating shorter ruminal residence time and less physical effectiveness in the rumen as a result.

Introduction

Alfalfa has significant nutritional value to dairy cattle as a source of protein and ruminally effective fiber. Alfalfa has long been recognized in crop rotations for its ability to provide N for subsequent crops, thus reducing nitrogen fertilizer purchases, a trait that is extremely valuable now when fertilizer prices are skyrocketing. In addition, alfalfa rotations are increasingly valuable as means to sequester soil carbon, improve soil health, and protect water quality (Russell et al., 2005; Syswerda and Robertson, 2014). However, alfalfa growers face a biological dilemma because the nutritive value of the forage declines rapidly as the crop matures and increases in yield (Figure 1). In order to produce dairy quality alfalfa, growers must sacrifice yield by harvesting no later than late bud stage of maturity which typically occurs around 28 days of regrowth in the Upper Midwest. This is 7 to 10 days earlier than the traditional rule of thumb of cutting at 10% bloom which is the point where alfalfa root reserves are completely replenished after cutting and the plant can be harvested again without harm (Undersander et al., 2015). Therefore, repeated harvest at bud stage also sacrifices long-term stand life by depleting root energy reserves needed to survive winter, contributing to premature plant death and shortened stand life that further reduces total alfalfa yield and profitability over a typical 4-year production cycle.

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Development of More Highly Digestible Alfalfa Varieties

In order to solve this conundrum, for decades breeders have striven to develop alfalfa varieties with improved forage quality that would reduce the nutritional cost of harvesting near maximum yield. Conventional breeding approaches have included selecting for greater leaf:stem ratio, reduced cell wall components such as NDF and lignin, or greater NDF and overall digestibility. Seed companies differ in their naming designations for conventionally bred high-quality varieties, which may be identified in the variety name as HQ or simply Q (high quality), HD or D (high digestibility), or Hi-Gest®. Genetic modification provides an alternative approach to improving alfalfa quality. The Consortium for Alfalfa Improvement (Monsanto, Samuel Roberts Noble Foundation, and US Dairy Forage Research Center) developed a genetic modified organism **(GMO)** alfalfa trait that reduces lignin concentration. Lignin is a structural cell-wall component that lends rigidity to maturing plant stems but has no nutritional value and physically interferes with digestibility of more nutritious plant components. The reduced lignin trait was developed by knocking out a naturally existing lignin synthesis pathway (Shadle et al., 2007; Nakashima et al., 2008) so that the plant simply makes less lignin. Nothing was added to the alfalfa genetics. The reduced lignin trait was trademarked as HarvXtra® **(HVX)** by Forage Genetics International, licensed to multiple seed companies, and varieties containing the trait became widely available for purchase beginning in 2017. Because of complexities related to production of GMO alfalfa seed without cross-pollinating conventional seed fields, all HVX varieties are also stacked with the other alfalfa GMO trait, Roundup Ready®.

Agronomic practices for reduced lignin alfalfa

Reduced lignin alfalfa is marketed with two possible management strategies. The first strategy is to target harvesting the crop at the late-bud stage and take advantage of improved NDFD while enjoying a 5 to 10 day harvest buffer zone of acceptable nutritional quality in case of bad weather or other delays. The second management strategy is to extend the harvest interval 5 to 10 days to let alfalfa reach 10% bloom. This can provide greater tonnage of alfalfa with quality comparable to standard alfalfa varieties harvested at late bud. It also typically eliminates one cutting per year, which reduces harvest costs and wheel traffic over the field. Letting alfalfa reach 10% bloom and reducing wheel traffic is expected to improve stand persistence over time, although the technology has not been available long enough to give this a solid test.

What are the Nutritional Implications of Reduced Lignin Alfalfa?

Reducing lignin concentration was expected to improve NDF digestibility and this has been confirmed by several research studies (Grev et al., 2017; Arnold et al., 2019; Sulc et al., 2021). Alfalfa with the HVX trait does decline in quality with maturity, but because it starts at a better quality point than conventionally bred alfalfa, it maintains its advantage over the typical harvest range for alfalfa. When a single variety was compared across six states ranging from Pennsylvania to California, the trait performed consistently with reduced NDF (3.5 to 7.5% lower) and increased NDFD (5.3 to 7.7% greater) when compared to standard high-yield and HQ varieties harvested on the same date. On a given date, HVX alfalfa did yield 4.8 to 7.0% less tonnage than standard varieties, but when HVX harvest interval was lengthened to 35 or 38 days, HVX alfalfa yield and NDFD was similar to or better than standard varieties cut every 28 days. One early concern was whether reduced lignin content would weaken stems and increase lodging, but the research indicates HarvXtra is not more susceptible to lodging than standard alfalfa.

These field research results show that both harvest management strategies can be feasible. The next big question is how dairy cattle respond to high quality alfalfa in the diet, whether that is sourced from conventional or GMO varieties.

Evaluation of Mid-Lactation Dairy Cow Responses to Lower Lignin Alfalfa

We recently carried out a feeding study evaluating responses of mid-lactation Holstein cows to lower lignin alfalfa as a component of a multi-state collaborative project. Adjacent fields (Mead, NE) were planted with a control variety (CON; Hybriforce 3400), an engineered reduced lignin alfalfa (HarvXtra 54HVX42) and a breeding-derived lower lignin variety (HiGest 460). We utilized 2nd - 4th cutting alfalfa hay in the summer after fall planting, and the two lower lignin **(LL)** varieties were blended (50:50) for the study. The purpose of this approach was to assess the general impact of decreased lignin content of alfalfa on nutrition of dairy cattle, rather than the specific impact of particular varieties.

A randomized complete block design with 60 cows (119 ± 19 days in milk, parity 1 to 4) was conducted in tie stalls. A common diet was fed during a 2-week preliminary period, providing data to characterize all cows on a common diet, followed by 6-week treatments. We tested the replacement of conventional alfalfa hay with either 50 or 100% LL alfalfa, resulting in 3 treatments: 1) CON; 2) a 50:50 blend of CON and LL hay (BLD); or 3) LL. All diets contained 32% alfalfa to increase the impact of the tested treatments and were otherwise identical (Table 1). Ground hay was sampled throughout the study and aNDFom, lignin, and 30-h in vitro NDFD values were 45.0 ± 1.8, 7.9 ± 0.8, and 46.8 ± 4.5% for CON and 43.3 ± 2.9, 7.1 ± 0.8, and 48.8 ± 3.4% for LL.

Productivity and efficiency responses of cows on these treatments are shown in Figure 2. Cows fed the LL diet consumed 1.8 lb/day more DM than those on the CON diet ($P = 0.01$), but no change in energy-correct milk yield was observed (90.1, 91.2, and 91.3 ± 1.5 lb/day for CON, BLD, and LL, respectively; $P > 0.50$). As a result, feed efficiency tended to be decreased for BLD and LL compared to CON (Fig. 2).

How should we integrate these new results with previous research on increased fiber digestibility in forages? A summary of studies evaluating cow responses to greater NDFD in forages, based heavily on work comparing brown mid-rib and conventional corn silage, demonstrated that increased DM intake is among the most consistent responses (Oba and Allen, 2005). This summary of the literature showed that a 1-unit increase in 30-hr in vitro NDFD was associated with approximately 0.3 lb/day increased DM intake. Our study utilized alfalfa hay sources that differed by just 2 units of 30-hr ivNDFD, and the 1.8 lb/day DM intake response was therefore greater than expected from the existing literature. This may point to a greater effect of these specific varieties on intake; however, a single study is inadequate to make this conclusion, and studies with the individual varieties are certainly warranted.

More surprising was the lack of any apparent benefit for energy-corrected milk yield despite the increased DM intake. One of the reasons for this was that LL alfalfa resulted in decreased milk fat content. Milk fat declined from

3.57 to 3.49 and 3.40 ± 0.04% in CON, BLD, and LL treatments, respectively ($P < 0.001$ for LL vs. CON). Given the relatively low total dietary aNDFom concentrations in this diet (Table 1), the increased fiber digestibility in the LL alfalfa may have caused the physically effective fiber supply to drop to a point where ruminal function was impaired, potentially resulting in decreased fibrolytic bacteria populations and mild impairment of milk fat synthesis (although milk fat yield was not significantly altered due to slightly greater milk volume). Although these results do not align with the expected ~0.5 lb/day increase in fat-corrected milk yield per unit of 30-hr NDFD (Oba and Allen, 2005), there is precedent in the literature for responses like this. Holt and colleagues (2010) reported that replacing conventional corn silage with brown mid-rib corn silage created a more extreme milk fat depression and decreased feed efficiency in a scenario where rumen function was apparently disrupted. As with corn silage, feeding alfalfa with improved fiber digestibility will likely decrease the physical effectiveness of fiber.

Nutritional Strategies for Utilizing Lower Lignin Alfalfa

Based on the limited information available so far on dairy cow responses to novel lower lignin alfalfa varieties, there is no reason to expect responses to deviate from responses to conventional alfalfa with high NDFD. However, there is room for many farms to utilize their most digestible alfalfa sources more effectively.

Targeted feeding

A number of past studies have shown that more productive cows, with greater demand for nutrients, show the greatest increase in DM intake when offered more digestible forages (Oba and Allen, 1999). Similar responses were found in our recent study of lower lignin alfalfa hay. The overall response to the LL diet vs. CON was a 1.8 lb/day increase in DM intake, but this varied across the cows on the study. Cows with the least intake on the common preliminary diet showed no increase in intake with the LL diet, whereas cows with the greatest preliminary intake increased DM intake by 4.5 lb/day on the LL diet. Why does this occur? We believe that cows with very high nutrient demands are often constrained by ruminal fill. In this scenario, consuming forages that degrade and leave the rumen more rapidly decreases the space taken by previously consumed forage, allowing cows to consume their next meal more quickly and thereby increase daily feed intake.

In most scenarios, enabling highly productive cows in the herd to consume more feed will enable them to produce more milk, as well. On farms with the capability to group cows by production level or stage of lactation, targeting higher-quality alfalfa (as well as more digestible corn silage) to the more productive cows is likely to improve whole-farm productivity and feed efficiency. Even with only conventional alfalfa sources, simply targeting the best cuttings to peak lactation cows may pay dividends. Of course, with wet forages, this strategy makes sense only if two sources can be fed at sufficient rates to keep silage faces fresh. However, many farms can justify this type of strategy and are leaving substantial dollars on the table by feeding all post-fresh lactating cows a single ration (Barrientos-Blanco et al., 2020).

Higher forage diets

As noted above, shifting to more digestible forage sources without adjusting diet formulation runs the risk of impairing ruminal function. Traditional rules of thumb for dietary fiber content do not apply uniformly, and recognition of this is what has led to efforts to establish guidelines for undigested NDF supply.

In most cases, then, at least some cows will need to be fed more forage when forage sources become more digestible.

The positive aspect of this is that cows fed high-forage diets with highly digestible forage sources are able to produce a lot of milk. In one example in the literature, cows fed a 68% forage diet based on brown mid-rib corn silage (38% NDF in the diet) produced *more* fat-corrected milk than did cows fed a 40% forage diet with conventional corn silage (Oba and Allen, 2000). Such higher-forage diets, especially in the current price environment, likely provide for lower input costs and set a farm up for greater income over feed costs if high levels of milk production can be achieved. The biggest bottleneck to this approach for many farms today is land availability. If higher-quality forage is planted and harvested, often with some sacrifice of yield, it can be very difficult to generate the tonnage needed to provide 65% forage diets to all lactating cows in a herd. However, for farms with a lot of acres available, a high-forage strategy with limited external feed inputs should be strongly considered.

Focus on agronomic benefits

Another option is to bypass the opportunity to harvest alfalfa with greater NDFD and instead take advantage of longer cutting intervals. For dairies that grow their own alfalfa but have it custom-harvested, the opportunity to reduce from 5 to 4 cuttings per year may have a meaningful impact on cost per ton of alfalfa, with minimal to no loss in yield. Furthermore, the wider harvest window provides flexibility to put up forage when weather conditions allow, reducing the risk of having very poor-quality cuttings during a season. For some farms, this is likely the most valuable application of lower lignin alfalfa varieties.

How Can We Assign an Appropriate Value to more Digestible Forages?

Recently, Dr. Bill Weiss of Ohio State proposed a relatively straightforward way to account for typical responses to forages with high NDFD when assigning economic value to these feedstuffs. This calculation utilizes the expected dry matter intake and fat-corrected milk responses to increased 30-hr in vitro NDFD, along with the value of milk and the cost of feed, to assign the additional income over feed cost to the forage. We recently posted a spreadsheet to help carry out these calculations quickly (Figure 3).

These expected responses are derived from studies where the forage under investigation comprised the majority of the dietary forage, and it's certainly the case that low inclusion rates of a forage would not be expected to have such dramatic impacts. However, these calculations are not dependent on a forage being fed at a high rate. A low-inclusion rate forage would have limited impacts on performance but would also have little impact on the calculated diet cost if the forage value is derived from these calculations.

Conclusion

Newer alfalfa varieties that are lower in lignin and offer greater in vitro NDFD have the potential to influence the productivity of dairy cattle. However, there is very little information on dairy cow responses to these varieties so far, and the initial studies have not demonstrated any increase in component-corrected milk production. These results point to the importance of carefully considering physically effective fiber supply in diets with highly digestible forages, as dropping below the lower limit to maintain ideal rumen function can hurt milk fat production. Nonetheless, through targeted feeding, higher-forage diets, and modified harvest schedules, the lower-lignin alfalfa varieties offer opportunities for improved whole-farm profitability, if managed correctly. The right approach to use these varieties – or not – depends on the conditions on each farm.

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Table 1. Ingredient and nutritional composition of treatment diets containing conventional (CON), lower lignin (LL), or a 50:50 blend (BLD) of alfalfa hay. Values are actual mean inclusion rates from feed management software.

	CON	**BLD**	**LL**
Ingredient, % of DM			
CON Alfalfa	29.3	14.9	-
LL Alfalfa	-	16.0	29.5
Corn silage (BMR)	20.3	20.4	21.2
High moisture corn	13.4	12.9	14.1
Corn grain	11.8	11.5	11.3
Protein, mineral, and vitamin supplement	8.7	8.4	8.3
Corn distillers grains	5.6	5.4	5.3
Soybean hulls (pelleted)	4.9	4.8	4.7
Soybean meal	3.3	3.2	3.1
Energizer 2 tallow	2.5	2.4	2.3
AjiPro L1	0.3	0.3	0.3
Nutrient, % of DM (unless otherwise specified)			
DM (% as-fed)	56.7	56.8	56.3
CP17.5	17.7	17.5	
ADF	19.6	19.6	19.0
aNDFom	29.2	28.7	27.7
Starch	25.0	24.7	25.7
Fatty acids	4.3	4.3	4.3
Ash	9.0	9.0	8.7

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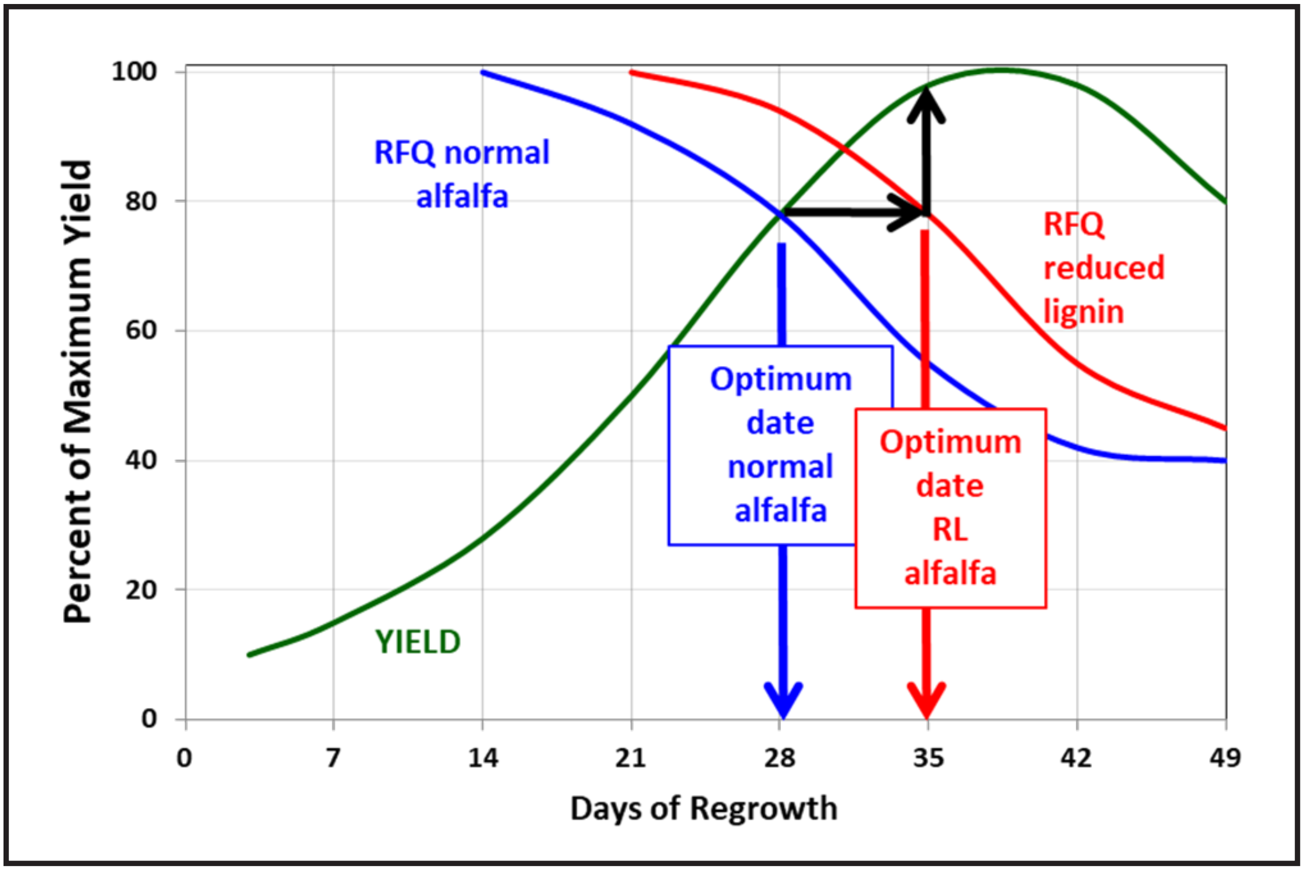

Figure 1. The ultimate goal of reduced lignin (RL) alfalfa is allowing harvest date to be delayed 5 to10 days, increasing yield with similar quality (relative forage quality: RFQ) and better persistence than normal varieties.

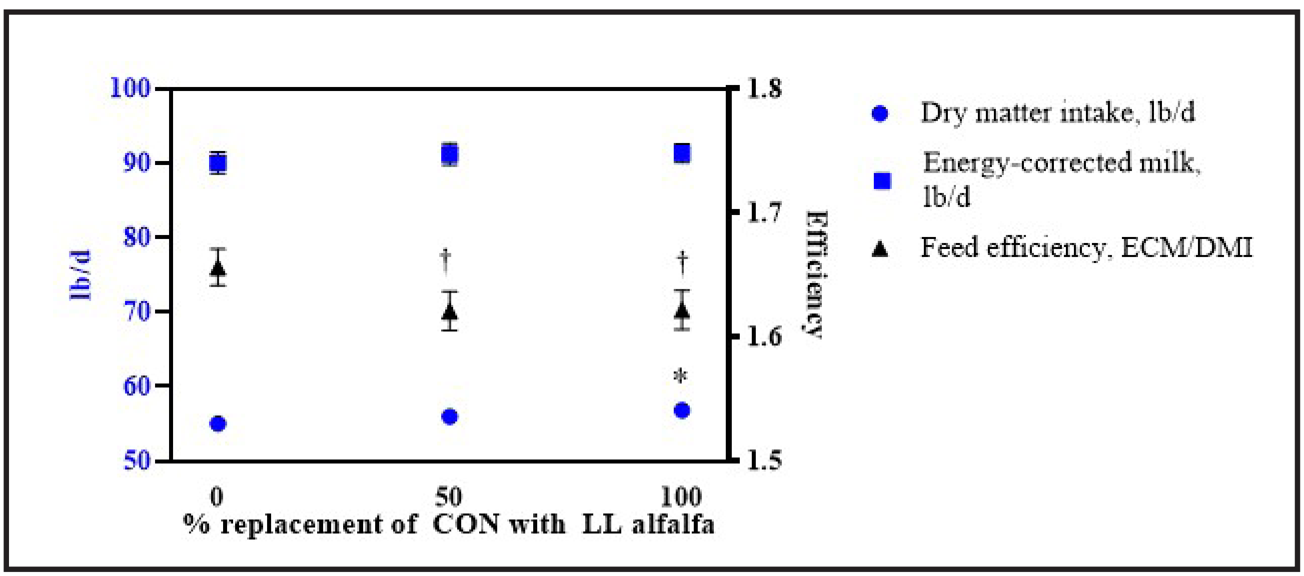

Figure 2. Responses of Holstein cows in mid-lactation fed conventional alfalfa hay (CON), a 50:50 blend, or lower-lignin (LL) alfalfa for 6 weeks. The LL hay was a 50:50 mixture of two alfalfa varieties developed through conventional breeding and genetic engineering, respectively, to improve NDF digestibility. * P < 0.05 and † P < 0.10 vs. CON.

Forage Value Adjustment for NDFD

Cells that require data input	
Current Milk $/cwt	18
Lactation ration cost, $/lb DM	0.11
Forage DM%	35%
Forage cost, $/ton as-fed	$ 40.00
Sample ivNDFD-30h, % NDF	64
Lab average ivNDFD-30h for forage type, % NDF	54.5
Change in ivNDFD-30h, %NDF	9.5
Change in forage value, $/ton as-fed	**$ 17.29**
Adjusted forage value, $/ton as-fed	**$ 57.29**

Kirby Krogstad and Barry Bradford, Michigan State University
Approach credited to Dr. Bill Weiss, Ohio State University

Figure 3. Screenshot of the decision support tool available at https://www.canr.msu.edu/dairymetabolismgroup/Decision-Tools/ for calculating forage value adjustments to account for NDFD.