

INDUSTRY PANEL: APPROACHES TO BREEDING
FOR ALFALFA FORAGE QUALITY

INTRODUCTION

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American alfalfa breeders have been working in earnest to genetically improve the feeding value of alfalfa forage since the development of near-infrared reflectance spectroscopy (NIRS) in the 1970's. NIRS allows for relatively rapid, accurate, and inexpensive analysis of single-plant protein and fiber fractions when compared with standard analytical methods using wet chemistry.

However, the use of NIRS and other systems for making single-plant selections for forage quality is a science unto itself. Alfalfa breeders are just now finding the strengths and weaknesses in the systems and are developing strategies to increase the efficiencies of their respective breeding programs. Some breeders are questioning the accuracy of NIRS when compared to other methods of making single-plant selections for improved quality. Animal nutritionists are questioning the very concept of "high quality" as it relates to increased animal performance. There are also questions regarding correlations between traits regarded as being related to high quality and traits regarded as being undesirable. Some agronomists even question the wisdom of increasing quality in a forage that is already very high in quality.

One fact that is not questioned is that there is much to learn regarding breeding for increased forage quality in alfalfa. Presented in the following pages are summaries of answers to four important questions asked of five industry leaders. Their answers, hopefully, represent an overview of the current breeding methods and general philosophies in the industry, as well as the future direction of the industry in development of high quality alfalfa varieties.

Breeding for Forage Quality

Rodney W. Hintz

- 1) **In general, what is your company's philosophy regarding methods of breeding for forage quality?**

Our company's philosophy is to select for traits that affect the actual nutritive value of alfalfa and that will result in increased animal productivity. We do not breed for the multi-leaf trait or dark green color in our varieties because we view these traits as simply being marketing gimmicks. University tests have demonstrated that increasing the number of leaflets on an alfalfa plant does not necessarily translate into greater leaf percentage or improved feeding value and research at our station has shown no correlation between plant color and nutritive value. It is important to remember that cows do not have a requirement for a given number of alfalfa leaflets or for chlorophyll, they have requirements for nutrients such as energy and protein, so that is what we breed for. Research at the University of Wisconsin has shown that our quality selection program based on decreasing fiber and increasing protein and digestibility is capable of producing alfalfa varieties that support higher levels of milk production than competitive varieties. To maintain this emphasis on selecting for traits related to improved animal performance, W-L Research has a Ph.D. Animal Nutritionist on staff and has constructed a wet chemistry laboratory. Although NIR is still used in the breeding program, the wet lab provides breeders with the most accurate data and up-to-date techniques for breeding new varieties and evaluating current ones.

- 2) **Some alfalfa scientists have recently said that some of the new varieties actually have too little fiber for optimum dairy production. Do you believe it could be possible to breed alfalfa varieties that have adequate fiber but greater digestibility?**

Although it is rare, high quality varieties harvested under intensive management strategies may indeed be low enough in fiber to cause problems in formulating rations for lactating dairy cattle. This is a problem that can be easily corrected by simply increasing the number of days between cuttings to allow adequate fiber to be deposited in the plant. This has the added advantage of increasing the yield per cutting (each day the field is left uncut, yield increases) and may over the course of the growing season require fewer harvests, thereby reducing harvesting costs. Our research has shown that the quality-selected varieties already on the market are more digestible than unselected varieties when compared at similar fiber levels. This demonstrates that there are differences in the digestibility of fiber among varieties and that whole-plant digestibility can be increased while holding fiber level constant. The difficulty in breeding for improved fiber digestibility is that near infra-red technology (NIR) has not proven effective in screening breeding populations. This means that more costly and time consuming *in situ* or *in vitro* techniques will have to be used by breeders if they are to be successful. There is also a tendency when selecting for increased digestibility to see a decrease in fiber concentration. Care will need to be taken to insure that adequate fiber levels are maintained as digestibility is increased.

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3) Do you think that high quality (high TDN, RFV) may be negatively correlated with other important agronomic traits such as persistence or even pest resistances?

Not at all. Looking at the varieties that are coming out of our high quality (HQ) breeding program, we see no differences in yield, persistence or pest resistance compared to varieties that have not been selected for quality traits. As long as selection for other agronomic characters, such as yield and pest resistance, is conducted simultaneously with quality selection we have seen no negatively correlated response. From a management standpoint, selection for high quality may actually increase persistence and forage yield by permitting a less severe cutting schedule. Increasing the interval between cuttings by 3 to 5 days, as we can do with quality selected varieties, increases the time available for the alfalfa plant to replenish root carbohydrates. This can dramatically improve plant vigor and in most cases extends the productive life of the stand.

4) It is likely that increasing forage quality via increasing TDN and RFV will become commonplace in the near future. What would you consider to be the next most important quality factor for breeders to manipulate?

Increasing TDN and RFV simply involves decreasing fiber concentration and as previously discussed, there are limits to how far we can reduce the fiber level of alfalfa without causing nutritional problems. Although some may argue that increased protein concentration or altered protein composition should be the next objective, from a nutritional perspective the major limitation to increased utilization of alfalfa is its low energy density. The way to increase the energy density of alfalfa is through increased digestibility, specifically fiber digestibility. The key challenge to breeders will be to maintain adequate fiber levels while increasing fiber digestibility. This challenge will be further increased by the limited ability of NIR to adequately predict fiber digestibility. To be successful, alfalfa breeders will have to resort to *in vitro* or *in situ* evaluations and will need to deal with very large population sizes to simultaneously maintain fiber and increase digestibility.

Breeding for Alfalfa Forage Quality

Jeff Rumney¹

- 1 In general, what is your company's philosophy regarding methods of breeding for forage quality?** Pioneer has tried to have a balanced, long-term approach when initiating a new selection program. Our program focuses on yield potential as the most important trait for the farmer. With this approach, we realize that we will not always be first in the market place with novel traits. Our strategy to improve forage quality is currently centered on phenotypic and genotypic selection for high RFV, in plant material that has been previously selected for agronomic merit. Our approach to releasing a variety is to select those varieties that have superior milk/acre values rather than high RFV.
- 2. Some alfalfa scientists have recently said that some of the new varieties actually have too little fiber for optimum dairy production. Do you believe it could be possible to breed alfalfa varieties that have adequate fiber but greater digestibility?** This response from a dairy nutritionist is not surprising. These scientists question our efforts to improve alfalfa forage quality, because alfalfa is a very good forage. Therefore, increasing RFV, may have little merit to dairy cows. The benefit comes in widening the harvest window for the alfalfa growers, so that an acceptable RFV can be captured. I do believe it will be possible to improve the digestibility of alfalfa, but we need a better understanding of exactly what we need to select for, and how the selection will affect plant growth.
- 3. Do you think that high quality (high TDN, RFV) may be negatively correlated with other important agronomic traits such as persistence or even pest resistances?** This is a tricky question, because the answer depends on your perspective. Realize that both answers are correct. I do know that plants with high RFV and agronomic merit exist. During the selection process, however, it is very easy to allow the agronomic merit to degrade if the breeder focuses solely on RFV (or any other single trait.)
- 4. It is likely that increasing forage quality via increasing TDN and RFV will become commonplace in the near future. What would you consider to be the next most important quality factor for breeders to manipulate?** I believe that the next step will be to select plant material at the in vitro level and to test new varieties at the animal performance level. I do question the validity of these programs however. Our seed industry can support a finite level of research effort, and still remain economically viable. The seed industry must be able to recapture the research expenditures through the sales of seeds. It is apparent that our industry is currently over extended in research expenditures. The benefit of selecting for higher forage quality, which is of dubious benefit to the farmer and to the dairy producer, might never be recovered by the seed industry.

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Lauren Johnson
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1) In General, What is your company's philosophy regarding methods of breeding for forage quality?

Our company's goal is to integrate enhanced digestibility into our varieties while maintaining and even improving yield, persistence, and multiple pest resistance. We emphasize yield in our selection programs. A variety with high yield potential and high quality potential can be managed to produce high yields of top quality hay. However, an even more digestible variety with lower yield potential can never be a top yielding variety. We also emphasize persistence under the types of management that result in high quality hay. For instance, our newer varieties are more tolerant of aggressive cutting schedules. Finally, selection for multiple pest resistance is emphasized to maintain high yields and high quality in conditions that lead to disease and insect pressure.

Our primary tool for further enhancement of digestibility is Near Infrared Reflectance (NIR) technology. Starting with our best germplasm, we select plants based on some combination of insect or disease resistance, individual plant yield, leafiness (usually in a multifoliate leaf background), recovery, dormancy, and persistence. Using our NIR analyzer, the selected plants are analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), acid detergent lignin (ADL), bypass protein percentage (BPP), and Phosphorus (PH). Based on these results, we put together combinations of parent plants that best combine the various traits. The resulting new variety should have increased digestibility and improved yield and persistence.

Some alfalfa scientists have criticized the NIR machine, suggesting that it is not as accurate as wet chemistry. However, we are careful to check a subset of our samples using wet chemistry, and to upgrade our calibration equations as needed. NIR technology allows us to sample large numbers of plants while minimizing human error.

The multifoliate leaf (ML) trait has been used for a number of years in the dormant varieties that are adapted to the northern areas of the United States. It has recently become more important here in the areas where the nondormant varieties are grown. Alfalfa normally has 3 leaflets per leaf. However, with the ML trait, the plant has from 3 to 9 or even 11 leaflets per leaf. The intention of selecting for this trait is to increase the leaf to stem ratio, thereby enhancing digestibility. We have found that while selection for high ML expression can be of some value, selection via NIR technology has been even more successful. Our philosophy is to combine the two types of selection.

2) Some alfalfa scientists have recently said that some of the new varieties actually have too little fiber for optimum dairy production. Do you believe it could be possible to breed alfalfa varieties that have adequate fiber but greater digestibility?

There are several different components of fiber, each of which has different digestion potential. Both cellulose and hemicellulose are of intermediate digestibility. Lignin, another component of fiber that is very poorly digested actually blocks digestion of the other fiber components. It has been suggested that some kinds of lignin decrease digestibility of other fiber components more than other kinds of lignin. Changing the ratios of the various components of the fiber, and changing the way that they are put together could result in a variety with adequate fiber and enhanced digestibility. However, large modifications in cell wall composition may have negative affects on productivity. Decreasing lignin concentrations has been associated with decreased yield and increased lodging.

3) Do you think that high quality (high TDN, RFV) may be negatively correlated with other important agronomic traits such as persistence or even pest resistance.

There has been some speculation that breeding for improved quality will decrease yield, decrease seed yield, and possibly even decrease the levels of resistance to some pests resulting in decreased persistence. Some of the early high quality varieties adapted to the northern climates had excellent leaf to stem ratios but low yield potential. In contrast, our company has emphasized selection for quality only within our highest yielding germplasm. The resulting high quality varieties have had excellent forage yield potential. At this time we have not experienced any negative correlated responses to selection for high digestibility.

4) It is likely that increasing forage quality via increasing TDN and RFV will become common place in the near future. What would you consider to be the next most important quality factor for breeders to manipulate?

Bypass protein is the part of the total protein that actually passes through the rumen to the small intestine where it can be more fully utilized by the cow. Selecting for an increased ratio of bypass protein to nonbypass protein would result in a more efficient protein source. Protein supplementation, a major production cost, could be decreased or eliminated. In theory, the bloat potential of such an alfalfa variety would also decrease.

Another possibility would be to increase the amount of phosphorus in alfalfa. Experimental alfalfa lines have already been developed that accumulate more phosphorus in their tissue. This should decrease the need for phosphorus supplements, and bring the CA:P ratio into a better balance.

BREEDING FOR FORAGE QUALITY

Donald Miller¹

QUESTIONS FOR PANEL

1.) **In general, what is your company's philosophy regarding methods of breeding for forage quality?** It is our company's philosophy that much of the high quality alfalfa that is currently being developed will not be fully utilized by the ruminant animal. We feel that in order to truly develop a high quality feed, we must increase the amount of by-pass protein in the alfalfa. Therefore we are looking at increasing the amount of stem protein as a means of increasing the level of by-pass protein.

2.) **Some alfalfa scientists have recently said that some of the new varieties actually have too little fiber for optimum dairy production. Do you believe it could be possible to breed alfalfa varieties that have adequate fiber but greater digestibility?** Yes. Much of the work done on improving alfalfa quality has been on the leaf portion of the plant. We feel that by looking at the stem portion of the plant, in our breeding efforts we should be better able to produce a quality feed that maximizes the ruminant animals performance.

3.) **Do you think that high quality (high TDN, RFV) may be negatively correlated with other important agronomic traits such as persistence or even pest resistances?** We have seen in some cases that yield may be negatively affected if you select plants solely on the basis of quality. However, if yield and quality are jointly used in the selection process, the negative effect can be overcome. This indicates that quality and yield are both complex traits. Therefore, in order to develop a high quality, high yielding alfalfa with adequate pest resistance and persistence, we must use all of these agronomic traits in the selection process. This makes the breeding effort more difficult, but not impossible.

4.) **It is likely that increasing forage quality via increasing TDN and RFV will become commonplace in the near future. What would you consider to be the next most important quality factor for breeders to manipulate?** I think the next important area may be the harvest window. If we can increase the time period that optimum quality occurs in the field, the farmer will have a better chance of scheduling his harvest to obtain high quality hay.

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Breeding For Improved Forage Quality At Forage Genetics

Bill Knipe¹

Information presented in this paper is in response to the following four questions:

1. In general, what is your company's philosophy regarding methods of breeding for forage quality?
2. Some alfalfa scientists have recently said that some of the new varieties have too little fiber for optimum dairy production. Do you believe it could be possible to breed varieties that have adequate fiber but greater digestibility?
3. Do you think that high quality (high TDN, RFV) may be negatively correlated with other agronomic traits such as persistence or pest resistance?
4. It is likely that increasing forage quality via increasing TDN and RFV will become commonplace in the near future. What would you consider to be the next most important quality factor for breeders to manipulate?

Breeding for improved forage quality and yield at Forage Genetics involves selecting for several different but related traits. We select for high multifoliolate expression, low fiber as determined by NIR technology, and improved leaf retention through good leaf disease and insect resistance (potato leafhopper and aphids). We are shifting from ADF to in vitro dry matter disappearance in our selection process as this is considered by many to be a better estimate of digestibility than ADF. Additionally, forage yield is always included as part of our selection criteria. During the selection process for low fiber we pay particular attention to NDF and ADF values at stages of maturity later than bud or 10% bloom. We are developing genotypes that retain low fiber values at later stages of maturity.

We do not believe that excessively low fiber is a general problem with the newer high quality varieties. Quality of harvested and stored alfalfa is frequently below dairy quality standards. Additionally, if fiber values are too low at the prebud or bud stage of maturity a grower can simply harvest at a later stage of maturity resulting in increased yields and improved stand persistence while maintaining desirable quality. Actually, this is one of the goals of the breeding program at Forage Genetics.

We have not experienced negative correlations of high quality with any trait other than forage yield. This correlation is significant but not 100% and can be overcome by evaluating large numbers of genotypes for both yield and quality. Identifying genotypes with both high quality and top yields is the major focus of our breeding program.

For the near term we will continue to select for low fiber and improved digestibility with particular emphasis on genotypes which maintain high digestibility at later stages of maturity. For the long term we have a strategy to improve protein utilization through genetic engineering. We believe this addresses the two most limiting factors of alfalfa as a feed: low digestibility (high fiber) compared to a grain and a low percentage of bypass protein.

A study at Montana State University by Dr. Dennis Cash and his associates documents the progress in breeding for forage quality. Results of this work are summarized in table 1.

Table 1. RELATIVE FEED VALUE OF TRIFOLIOLATE, HIGH QUALITY TRIFOLIOLATE, MULTIFOLIOLATE, AND MULTIFOLIOLATE HIGH QUALITY ALFALFA TYPES AT 8 SAMPLE DATES IN MONTANA (CASH ET AL..1994)

<u>Variety</u> <u>Type</u>	<u>Number of</u> <u>Varieties</u>	<u>Average</u> <u>RFV</u>
Trifoliolate	3	155.4
High Quality (Tri)	3	164.9
Multifoliolate	3	163.5
Multifoliolate High Quality	3	167.4
Mean		162.8
Isd (.05)		3.0

Trifoliolate=Standard trifoliolate types with no selection for improved quality.

High Quality Tri=Trifoliolate types with selection for improved quality.

Multifoliolate=Lines selected for expression of the multifoliolate trait.

Multifoliolate High Quality=Multifoliolate lines selected for both expression of the multifoliolate trait and improved quality.

Values are averages of 3 variety types over 8 sample dates.

RFV is a measure of forage quality based on ADF and NDF; larger values are best.

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