Hartnell et. al., 2005. Proc. Southwest Nutr. Conf.: 156-172.

Potential Benefits of Plant Modification of Alfalfa and Corn Silage to Dairy Diets

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Summary

Corn silage and alfalfa are the main forages fed to dairy cattle in the United States. Determining the attributes of ideal corn for silage and alfalfa for silage or hay should be done via a holistic approach encompassing the entire dairy enterprise. Attributes of ideal forage may be different for individual dairy farms depending on factors such as production level of the dairy herd; what crops if any are produced as part of the enterprise; cost, availability and quality of feed ingredients; feed storage; equipment; and management capabilities. Ideal attributes for plant modification may include those that increase milk potential per acre and/or per ton, enhance digestible NDF, improve starch quality and content, improve protein content and amino acid balance, improve agronomic traits for insect protection (safer forage supply), herbicide tolerance, virus resistance, drought tolerance, cold tolerance, improved mineral availability and enhanced yield. Progress in attaining these attributes has been slow using traditional plant breeding but will accelerate with the use of biotechnology. Dairy enterprises will benefit through forages that are less prone to contain mycotoxins or toxic weeds, or to induce bloat; have improved nutrient utilization for milk and meat production; and produce less animal wastes resulting in improved efficiency, profitability, and a better environment.

Introduction

Forages are the foundation upon which good dairy nutritional programs are built. The intake and digestibility of forage by dairy cattle directly affect their meat and milk production as well as rumen function and animal health. The fiber (cell wall) component of forage represents a major source of energy; however, less than 50% of this fraction is readily digested and utilized by the animal (Hatfield et al., 1999). If a 10% increase in cell wall digestion was obtained, the dairy industry could realize a benefit of $380 million in milk and meat sales while reducing manure solids by 2.3 million metric tonnes and grain input into dairy rations by 3.0 million metric tonnes (Hatfield et al., 1999). Corn silage and alfalfa are the main forages providing, energy, protein, digestible and effective fiber, minerals and vitamins to dairy cattle.

In determining what plant modifications are best to supply dietary ingredients for any one dairy enterprise, a holistic approach should be followed. Factors to be considered include whether forage production is part of the dairy enterprise; costs and availability of ingredients; quality, quantity, and consistency of ingredients; forage processing, feed mechanization and storage; animal nutrient requirements; and management. For example, feeding finely chopped, highly digestible processed corn silage could be problematic when fed as the only forage, but may be ideal when blended with other forages.

Plants can be modified by a variety of means. Traditional plant breeding modifies plants by selecting parental lines for a desired trait and cross-fertilizing them to produce offspring with the more desirable agronomic and/or nutritional value. But in doing so, thousands of genes are mixed, requiring many attempts and years to remove the unwanted traits and enhance the desired traits. Novel traits produced through traditional breeding may have resulted from mutations (natural and induced) as well. Alternatively, biotechnology can be used as a more predictable, precise and faster way to select specific native plant or exogenous genes that provide the plant with new genetic capabilities to tolerate herbicides, protect against insects and viruses, and enhance nutritional and health components. Precision plant breeding is a specific type of biotechnology that uses molecular genetic techniques to modify single plant genes (no foreign DNA is inserted) in the parental lines. This paper will discuss modifications of corn silage and alfalfa that have been made in the past, are currently under investigation, and may be considered in the future.

Corn Silage

Current Role of Corn Silage in Dairy Diets

Corn silage has been a part of dairy rations in many parts of the United States since the discovery of silos. In the past, it was often used as a replacement forage crop when the yields of other forages were inadequate. Typically, corn was planted for grain, but if drought or poor weather conditions resulted in poor grain yields or insufficient yields of other forages, the corn was harvested for silage. Unfortunately, corn silage produced under these circumstances was relatively poor in quality, which led some to conclude that it was a second-rate forage. However, corn silage has both nutritional and agronomic characteristics that make it a desirable component of dairy rations and it appears that its use is increasing.

Agronomically, corn silage is relatively easy to produce and manage because it is an annual crop that is harvested only once compared to perennial forages that have to be managed for survivability and harvested several times annually. It also generates the greatest annual yield of digestible energy per acre than any other forage. The advent of roller processing during chopping is ensuring that most of the energy in corn kernels is readily digested by dairy cows. Nutritionally, corn silage is palatable when ensiled properly and high in energy because it typically contains about 50% corn grain. Nutritionists like corn silage because it adds moisture and consistency to mixed dairy rations. Well-preserved corn silage is palatable and seems to improve the intake of mixed rations.

Although the corn silage in a given silo is relatively consistent, this does not mean that all corn silage is alike. The dairy NRC (NRC, 2001) provides chemical composition data for three qualities of corn silage, but they do not encompass the full range in silage qualities that can occur. Compositions provided in **Table 1** were derived from relationships among chemical components in a diverse set of corn silages (Ferreira and Mertens, 2001). Note that corn silage is described in terms of fiber level, which is the inverse of starch concentration. However, starch concentration and grain yield per acre are not as closely related. It is possible to have stunted plants with small ears that result in low grain yields per acre, but the concentration of starch in the silage is equal to that of corn with large plants and large ears that have high grain yields. Thus, it is difficult to describe the nutritional quality of drought corn because plant size and ear development can vary independently with weather variation during the crop season.

Table 1. Typical composition (% of Dry Matter) of corn silages varying in fiber content (adapted from Mertens, 2002).

Forage description	CP[a]	EE[b]	Ash	NFC[c]	Star[d]	Pec[e]	aNDF[f]	ADF[g]	ADL[h]
Very low fiber	8.3	3.2	4.1	48.4	31.1	1.9	36.0	21.0	1.57
Low fiber	8.6	3.1	4.6	43.2	27.2	1.7	40.5	24.0	1.91
Normal fiber	8.8	3.0	5.1	38.1	23.2	1.5	45.0	27.0	2.25
High fiber	9.0	2.8	5.7	33.0	19.2	1.3	49.5	30.0	2.59
Very high fiber	9.3	2.7	6.2	27.8	15.3	1.1	54.0	33.0	2.93

[a] Crude protein
[b] Ether extract or crude fat
[c] Nonfiber carbohydrates calculated by difference (NFC = 100 – CP – EE – Ash – aNDF)
[d] Starch
[e] Pectin, estimated from NFC
[f] Amylase-treated neutral detergent fiber determined with sodium sulfite and amylase
[g] Acid detergent fiber
[h] Acid detergent lignin using 72% sulfuric acid

The relationship between maturity and composition is unique for corn silage among forages because it is a mixture of stover and grain. In general, the proportion of fiber and lignin in plants increases as they mature and this is true for the stover in corn. The lignin concentration of corn stover increases and its digestibility declines with maturity (Daynard and Hunter, 1975; Hunt et al., 1989); however, as the corn plant matures after silking it generates grain that dilutes the concentration and nutritional impact of the relatively mature stover often resulting in whole plant digestibilities that vary little with maturity. Typically, the dry matter digestibility (DMD) of corn silage increases with maturity as grain increases and the ratio of amylase-treated neutral detergent fiber (aNDF) to neutral detergent solubles (NDS = 100 – aNDF) decreases. Thus, the typical negative relationship between fiber concentration and maturity do not hold for corn silage, and maturity, which is indicated best by dry matter concentration in corn silage, is not highly related to its nutritional quality.

Relative to alfalfa, corn silage is low in protein, ash, and lignin. In addition, the biological value of the protein in corn silage is low because it is low in lysine and it is much lower in many trace minerals than alfalfa. Corn silage is similar to alfalfa in aNDF and higher in non-fiber carbohydrates (NFC) because it is lower in protein and ash. The predominant NFC in corn silage is starch, and corn silage contains very little pectin or neutral detergent soluble fiber (NDSF) as defined by Hall et al. (1997). Starch in corn silage can be readily fermented in the rumen if corn kernels are immature (> 25% moisture) and are adequately processed or chewed. When the starch in corn silage is digested completely, NDS contributes about 2/3 of the total digestibility of corn silage. The aNDF digestibility (NDFD) of corn silage is typically between 50 to 70% at maintenance levels of intake. However, high concentrations of rapidly fermentable starch in some dairy rations may inhibit the digestion of fiber directly or through the lowering of ruminal pH (Mertens and Loften, 1980; Grant and Mertens, 1992). Although the potential extent of digestion of aNDF in corn silage is higher, its digestion rate is much slower than alfalfa. The combination of starch inhibition and slow rates of fiber digestion may result in less than optimal digestion of fiber in corn silage.

Although corn silage is palatable, it often results in lower intake and production when it is fed as the sole forage in dairy rations even though these rations appear to be for balanced protein, fiber and minerals. It is speculated that rations containing only corn silage as forage may limit intake and production due to excess rapidly fermentable starch, low effective fiber, and/or slow rates of fiber digestion. It is recommended that corn silage comprise between 1/3 and 2/3 of the forage in dairy rations.

Attributes of Ideal Corn for Silage

An ideal corn silage could have starch with less inhibitory effect on ruminal digestion yet provides the energy needed to drive microbial efficiency; more protein with better amino acid balance; and fiber that is more rapidly digested with a higher potential extent of digestion and greater effectiveness in promoting optimal ruminal digestion. Selecting for increased protein through traditional breeding, typically decreases starch content and elevates oil content. If higher oil content is selected, protein appears to be elevated with a decrease in starch content. Thus, if higher starch is selected, both oil and protein content may negatively be affected. To optimize microbial efficiency, it seems that corn silage should provide an increased source of available energy rather than increasing protein or oil at the expense of starch.

Increasing the amount and digestibility of aNDF in corn silage and improving fiber digestibility could result in reduced quantities of other forages needed by the dairy enterprise to meet dairy cow requirements. Whole plant NDF concentration is negatively correlated with grain yield, in vitro digestibility and in vivo DM digestibility but not correlated with corn silage dry matter (DM) yield (Weiss and Wyatt, 2002). Corn silages with higher NDF concentrations could have value as a fiber source, but its value would be reduced or eliminated if the higher NDF concentration resulted in reduced digestibility and net energy of lactation (Weiss and Wyatt, 2002). Owens et al. (2002) emphasized that although direct selection for traits like greater fiber digestibility and slower grain drying may be desirable, selection for grain yield should be a more reliable and repeatable method to increase silage DM digestibility than for selection of stover digestibility. In addition, they suggest both relative yields of net energy and the optimum energy density of the diet to achieve a desired level of animal production must be considered when selecting ideal silage hybrids.

Other desirable attributes include high yields or milk production potential per acre, more digestible stover, freedom from mycotoxin, drought tolerance, slow kernel dry down (not as important if kernel processors are used on harvesters), excellent ensiling characteristics, and high intake potential. Which attributes are more desirable must be determined holistically. Corn silage appears to be most effective in rations when fed with other fiber sources that have greater rates of digestion and passage than whole corn plant fiber. If alfalfa and rapidly digestible non-forage fiber sources are available and cost-effective, then emphasis might be placed on high starch and DM yield per acre because the non-fiber component of corn silage contributes most to its energy value. A high grain corn silage would provide the ruminally available starch and net energy to compliment these other fiber sources. However, if corn silage is the sole or major fiber source in the diet and the concentrate portion of the ration is high in starch, then emphasis in selecting corn for silage should be placed on improving fiber digestibility and effectiveness. Thus, there may not be a single ideal corn silage, but a continuum of improved corn silage attributes that fit specific dairy enterprises.

Plant Modifications

Plant modifications have been made through traditional breeding and/or biotechnology to accomplish some of these goals toward an ideal corn silage. High-quality corn silage hybrids have been obtained through genetic selection for improved stover digestibility (stalk, leaves, husk and cob) and grain yield. Fiber digestibility and grain yield have been research areas of interest for many years because the two are not highly related (Argillier et al., 1995; Hunt et al., 1992). Recently, University of Wisconsin workers have focused on kernel texture and starch digestibility (http://www. silagebreeding.agronomy.wisc.edu/Corn/Quality%20assessment/quality_assessment.htm). Kernel density and amount of vitreousness starch may be significantly correlated with starch digestibility (http://www. silagebreeding.agronomy.wisc.edu/Corn/Nutritional%20Studies/Starch/Description.htm). Soft kernel texture may result in improved starch availability, but kernel processing could offset hard kernel texture.

There are many different types of commercial hybrids developed through conventional breeding to consider for use as silage for cattle such as waxy, Opaque 2 (high lysine), brown midrib (bmr), high oil, multileaf (leafy) and high NDF. Each of these modifications will be discussed below.

Waxy: This starch variant was found in China in 1908 but it wasn't until 1936 in which Iowa State researchers started developing hybrids. Waxy endosperm hybrids contain 100% amlyopectin starch (branched polysaccharide), rather than the normal dent corn ratio of 75% amylopectin and 25% amylose. Amylopectin has been shown to be highly digestible in the rumen (Mohd and Wootton, 1984) and waxy corn when fed as silage and corn to lactating cows had 6% more apparently digestible starch (Akay and Jackson, 2001). Diets with increased ruminally available starch increase microbial protein synthesis (Sniffen and Robinson, 1987) and thereby increase amino acid flow to the duodenum of dairy cows (Poore et al., 1993a), if the pH of the rumen is adequate. This results in higher milk yield and protein percentage (Poore et al., 1993b). Feeding waxy corn silage to lactating dairy cows (Moreira et al., 2000; Akay and Jackson, 2001) resulted in more milk, FCM and fat than those fed the control diet. However, in a pooled three location lactating dairy cow study, where waxy corn silage was fed (31 - 49% of diet dry matter) for 28 days, no differences in milk yield and composition were noted (Monsanto, unpublished). Also, Schroeder et al. (1998) observed no effect on milk yield or total milk solids and serum urea nitrogen when lactating cows were fed waxy corn versus dent corn. Overall, studies have not consistently indicated an economic benefit.

Brown Midrib: The brown midrib trait was first reported in dent corn at St. Paul, MN in 1924. It first appears at the 4-6 leaf stage as a reddish-orange coloration down the underside of the leaf mid-vein or midrib. The color is associated with lignified rind and vascular bundles. Coloring eventually disappears on the leaves, but remains in the stalk (Lauer and Coors, 1997). Since its initial discovery four brown midrib mutants have been identified which include *bm*1, *bm*2, *bm*3, and *bm*4. Brown midrib corn is an example of a natural mutation that caused a “knock-out” of one of the lignin biosynthetic enzymes. The bmr mutant, *bm*3, consistently decreases the lignin content of the corn plant by approximately 40% because the activity of the enzyme, O-methyltransferase, is reduced, which increases in vitro NDFD (Barriere and Argillier, 1993). Of the available corn hybrid types, the bmr trait is the only one that more frequently shows an improvement in milk production (Oba and Allen, 1999; Bal et al., 2000; Moreilra et al., 2000; Ballard et al., 2001). However, there are studies where no effect on milk production was observed (Tine et al., 2001; Monsanto, unpublished). Where no difference in total tract NDFD was observed, there was greater dry matter intake for bmr hybrid and the improved milk response could be attributed to the increase in dry matter intake. Oba and Allen (2000) evaluated corn silage from a brown midrib (*bm3*) hybrid and its near isogenic counterpart at two concentrations of dietary NDF (29 and 38%). Ruminal or total tract NDF digestibility were not affected by corn silage hybrids. The bmr corn silage resulted in decreased starch digestibility in the rumen and total tract, increased postruminal starch digestibility, increased microbial N flow to the duodenum and enhanced microbial efficiency as compared to the control corn silage. The authors concluded that enhanced in vitro NDFD does not necessarily result in increased rumen or total tract NDFD but improved milk yield may result from increasing rate of passage and DMI and improving efficiency of microbial N production. Tine et al. (2001) fed bmr corn silage to dry cows at maintenance and found hybrid differences in terms of TDN and metabolizable energy concentrations; however, when the same silage hybrids were fed to lactating dairy cows in a mixed diet (60% silage, 40% concentrate DM) at 4x maintenance, no differences were observed. Some concerns with bmr corn include yield reduction, potential of increased lodging (downed stalks), brown color has lead to harvesting too early so there is more seepage and increased risk of an undesirable fermentation, and diets not properly formulated to account for increased digestion resulting in ruminal acidosis. However, brown midrib corn silage can be successfully incorporated into dairy diets when managed properly.

Multi-leaf (Leafy): The leaf gene was first identified in the 70's. Since then, corn hybrids have been developed and commercialized with increased leaf content (two to four extra leaves above the ear) as compared to other hybrids of the same maturity. Having extra leaves above the ear often results in increased forage digestibility because of the increased leaf to stem ratio. Growing environment, maturity, and height of the cutter bar at harvest, can alter stover composition considerably and thus affect fiber digestibility and performance in animals. Thomas et al. (2001) and Clark et al. (2002) reported significant increases in milk production when multi-leaf hybrids were fed as corn silage. However, several researchers have reported small or no differences in milk production when lactating cows were fed leafy silages (Kuehn et al., 1999; Bal et al., 2000; Moreira et al., 2000; Ballard et al., 2001; Nennich et al., 2003). Kuehn et al. (1999) fed lactating dairy cows a diet containing 40% CS (multi-leaf, high-yielding grain, and a blend of hybrids) on a dry matter basis for the first 22 weeks of lactation. Silage yields were 5.5, 6.0, and 5.8 tonnes of DM/acre for the grain, leafy and blend silage, respectively. In vitro digestible dry matter (66.8, 69.2, and 66.7% for grain, leafy and blend silages, respectively) and digestible NDF (34.6, 38.0, and 34.4% of DM for grain, leafy and blend silages, respectively) were measured. No significant corn silage effect on cow performance was reported. Nennich et al. (2003) fed leafy hybrid as compared to conventional corn hybrids to lactating cows for 120 days (40% of diet dry matter as corn silage) and observed no differences in DMI, NDF intake, milk yield or composition.

High Oil: As a result of the enhanced oil content being primarily in the grain, the increased percentage of oil in the whole plant is less due to the dilution by stover. Assuming no associative effects, unprocessed high oil corn silage had 8.2% more TDN than unprocessed conventional corn silage due primarily to the increased oil content (Weiss and Wyatt, 2000). Processing conventional corn silage increased TDN (8.4%) to values similar to high oil corn silage, but processing high oil corn silage did not influence TDN. Increased TDN in the processed conventional corn silage was due to improved starch digestibility. In a lactating cow trial where cows were fed diets containing 63% corn silage for 12 weeks, no effect was seen on DMI (Weiss and Wyatt, 2000). Cows fed high oil corn silage did produce 1 kg more ($P < 0.08$) more milk and 1.3 kg more ($P < 0.05$) FCM than cows fed the conventional control. Atwell et al. (1988) reported similar numerical differences in milk and FCM yields when feeding cows diets containing 50% of the diet as high oil corn as compared to conventional controls. LaCount et al. (1995) reported no differences in milk or FCM yields when high oil corn silage comprised 25% of the dietary dry matter and fed to dairy cows for an entire lactation. Moreira et al. (2000) reported no differences in milk or FCM yields when high oil corn silage comprised 37.5% of the dietary dry matter when fed to dairy cows for 28 days. Holistically, there are times when it is advantageous to increase the energy density of dairy rations. In these cases, high oil grains or silages may be beneficial; however, adding fat decreases the proportion of readily available substrate in the diet for microbial growth and polyunsaturated fats when combined with low ruminal pH could lead to the production of trans-fatty acids and milk fat depression. When feeding high oil grains or forages, the nutritionist must make sure that dairy rations are formulated to take advantage of the energy in the corn oil.

Opaque 2 (high lysine): High lysine grain is of limited value to ruminants until a more ruminally undegradable lysine is developed.

NDFD: Digestibility of NDF is an important component of forage quality. [Note: digestible NDF (dNDF) has the units % of DM whereas NDF digestibility (NDFD) is the digestion coefficient of NDF and has the units % of NDF.] Increased NDFD may result in reduced physical fill in the rumen over time and allow greater voluntary feed intake (Dado and Allen, 1995) as well as increasing the energy density of diets and microbial N production (Oba and Allen, 2000). Oba and Allen (1999) evaluated the importance of NDFD and reported a one unit increase in forage NDFD in vitro or in situ was associated with a 0.17 kg increase in DMI and a 0.25 kg increase in 4%FCM yield. Ivan et al. (2005) reported increased feed intake (% of BW) and 4% FCM production for those cows where corn silage with higher NDF content and digestibility was substituted (either DM or NDF basis) for silage with lower NDF content and digestibility. Increase in fiber digestibility can be accomplished by using bmr hybrids, selecting highly digestible fiber hybrids such as leafy hybrids or by raising the cutter bar and leaving more of the lower stalk in the field. University of Wisconsin corn breeders are developing a high digestible NDF germplasm that is equivalent in terms of NDFD to bmr but without the agronomic detriments such as yield reduction and lodging.

Biotech – Agronomic traits: Using the tools of biotechnology insect-protection and herbicide-tolerant traits have been incorporated into corn. The insect protection traits have resulted in protection against the European corn borer, Corn rootworm and other pests. The insecticidal proteins used in this products have been found to be safe with no adverse effects when fed to dairy cattle (Hartnell et al., 2001; Barrière et al., 2001; Folmer et al., 2002; Donkin et al., 2003; Grant et al., 2003; Clark and Ipharraguerre, 2004). Benefits from the use of these traits include higher yields; increased tonnage due to less insect damage and less lodging; decreased mycotoxin (primarily fumonisin); reduced pesticidal use, exposure to humans, and the environment; and reduced grower costs. Corn containing herbicide-tolerant traits have also been shown to be safe with no adverse effects when fed to dairy cattle (Donkin et al., 2003; Grant et al., 2003; Ipharraguerre et al., 2003). Benefits from the use of herbicide-tolerant traits include the use of a more environmentally friendly production system for corn through the utilization of the no-till system; increased tonnage as a result of less competition for nutrients from weeds; reduced herbicide costs; a more simplified weed control program; and reduced grower costs.

Agronomic traits under development or future possibilities for development in corn for silage include: drought tolerance for improved biomass production when water is limited or there is insufficient water at key times such as pollination and kernel fill; cold tolerance for improved germination in colder soil temperatures allowing for earlier planting; insect-stalk snap resistant for less field loss; increased grain to stover ratio; slower grain dry-down (this isn't as important now with the use of kernel processors on the harvester equipment); and mycotoxin resistance (primarily aflatoxin). The aforementioned traits are primarily driven by the desire to improve grain production, which also includes higher grain yields per acre.

Biotech – Forage enhanced traits: Biotech tools are available to up-regulate, down-regulate or knock-out certain key enzymes in a metabolic pathway, insert new pathways, etc. through genetic manipulation. However, every metabolic alteration has consequences that need to be understood. If carbon is diverted towards the production of more starch, then there is less carbon for oil and protein production. An understanding of the key metabolic pathways in corn and the genetic components that control and influence them will be crucial in developing improved corn silages. Using the tools of biotechnology it may be possible to reduce or alter lignin for enhanced fiber digestibility; alter carbohydrates for improved microbial efficiency in the rumen and reduce its impact on fiber digestibility and ruminal pH; increase protein content, quality and amino acid balance; enhance digestible biomass or milk production potential per acre and per ton; incorporate rate limiting digestive enzymes in the corn plant; and produce fermentation adjuvants in the plant that aid fermentation in the silo as well as in the rumen. The key is to identify those targets that will have the biggest economic impact to the dairy enterprise without sacrificing any of the key agronomic traits.

Alfalfa

Current Role of Alfalfa in Dairy Diets

Alfalfa (*Medicago sativa*), often called the "Queen of Forages" is the most important forage legume for dairy cows. Production of alfalfa forage in 2002 was over 90 million tons (hay and haylage tons, 68.5 and 23.4, respectively) on 26.1 million acres. Crop rotations utilizing alfalfa have a positive environmental impact in terms of stabilizing soils, decreasing nutrient inputs and increasing wild life habitat. Also, well managed alfalfa stands effectively absorb mineralized N from manure and remove residual nitrate from the subsoil, irrigation water, and shallow ground water, and fix less N from the air (Russlle, 2004). The major disadvantage of alfalfa is low yields when compared to corn silage and the need for multiple harvests. Multiple harvests not only increase the labor and equipment costs for alfalfa, but expose the forage to multiple harvesting environments, such as rain damage, that increase the variability in nutritional quality. Intensive cutting schedules may also be the root cause of poor stand survival and reduced yields.

High quality alfalfa is palatable and often maximizes intake and production of dairy cows. Alfalfa is low in fiber and high in protein compared to other forages, which makes it an excellent complement for grains and other forages in dairy rations. Although there are genetic differences in nutritional value among alfalfas, currently the nutritional quality of alfalfa is established primarily by harvesting management. Although there are differences among seasons and cuttings, in general the composition and DMD of alfalfa is related to plant maturity. Alfalfa hay composition in **Table 2** was derived from relationships among chemical components obtained from several data sets (Mertens, 1973; Onstad and Fick, 1983; Fick and Onstad, 1988) and analyses obtained from ten commercial forage testing laboratories that were used to develop standards for reporting hay market prices (Mertens and Getz, 2004). The crude protein, ash, crude fat, fiber, and lignin values in

Table 2 agree with those of similar quality found in the dairy NRC (2001). The forage quality descriptions in **Table 2** are relative and may not reflect the economic or nutritional value of the alfalfa in a given situation. For example, an exceptional quality hay as described in **Table 2** may provide too much protein and not enough aNDF in a particular dairy ration and its value may not be exceptional. As with corn silage, a holistic approach for the specific dairy enterprise should be taken when selecting the traits for the ideal alfalfa.

Table 2. Typical composition (% of dry matter) of alfalfa hays varying in fiber content (adapted from Mertens, 2002).

Forage description	CP[a]	EE[b]	Ash	NFC[c]	Star[d]	Pec[e]	aNDF[f]	ADF[g]	ADL[h]
Exceptional quality	25.4	2.7	10.4	31.5	3.1	14.2	30.0	24.0	4.53
Very high quality	24.0	2.6	9.9	29.4	2.9	13.2	34.1	27.0	5.38
High quality	22.5	2.5	9.5	27.4	2.7	12.3	38.2	30.0	6.23
Good quality	21.0	2.4	9.1	25.3	2.5	11.4	42.2	33.0	7.08
Fair quality	19.5	2.2	8.7	23.2	2.3	10.5	46.3	36.0	7.93

[a] Crude protein
[b] Ether extract or crude fat
[c] Nonfiber carbohydrates calculated by difference (NFC = 100 – CP – EE – Ash – aNDF)
[d] Starch
[e] Pectin, estimated from NFC
[f] Amylase-treated neutral detergent fiber determined with sodium sulfite and amylase
[g] Acid detergent fiber
[h] Acid detergent lignin using 72% sulfuric acid

Immature alfalfa is high in protein, but the protein is rapidly fermented in the rumen to ammonia and not used efficiently. Because alfalfa protein is used inefficiently, dairy rations containing predominantly alfalfa forage are formulated to contain 1 to 3 %-units more protein. When used as the sole forage source, the high protein and low fiber concentrations in immature alfalfa can make it difficult to formulate rations that meet the protein, energy and fiber requirements of dairy cows. As alfalfa matures, the proportions of crude protein and NFC decrease. The main NFC in alfalfa is pectin of which 10 to 20% is not extracted by acid detergent causing the difference between aNDF and ADF to underestimate hemicellulose in alfalfa. Because pectin ferments rapidly and completely without a decrease in ruminal pH (Hatfield and Weimer, 1995), it may be desirable to maintain or increase its proportion in alfalfa because alfalfa is relatively deficient in rapidly fermentable carbohydrates when compared to corn silage.

As in other forages, the proportions of fiber and lignin increase with maturity in alfalfa. Alfalfa fiber contains a high proportion of lignin relative to grasses resulting in low digestibility relative to grasses. Whereas, 60 to 80% of grass fiber is potentially digestible, the potential extent of digestion of alfalfa fiber is only 40 to 60% due to its high lignin content. However, alfalfa has a great advantage over grasses because the rate of digestion of its potentially digestible fiber is 2 to 3 times that of grasses. It also appears that the indigestible fiber in alfalfa disintegrates into particles that rapidly pass out of the rumen. The higher intake and digestibility often observed with alfalfa based diets compared to those containing grass is not due to greater digestibility of alfalfa fiber, but due to alfalfa's low fiber content and the rapid rates of digestion and passage of that fiber.

Attributes of Ideal Alfalfa

An ideal alfalfa would contain a better balance of protein and rapidly fermentable carbohydrate. At an optimum aNDF concentration of about 40% (DM basis), it would be desirable to have about 18% crude protein, less ash and about 30% NFC. It would also be beneficial to have a better balance of amino acids in the protein and with a slower rate of degradation in the silo or rumen to minimize its losses as ammonia. Increasing the fat to 4% might also be energetically advantageous to dairy cows. The rate of digestion and passage of alfalfa fiber is excellent when compared to other forages and nothing should be done to diminish these attributes. However, it might be desirable to improve the potential extent of fiber digestion by modifying lignin content or characteristics. For grazing or green chop purposes, removal or suppression of the bloat causing properties would be beneficial. Above all, the yield of alfalfa should be enhanced with a reduction in the number of cuttings needed to produce dairy-quality alfalfa forage.

Plant Modifications

Over the past fifty years great advances have been made in the development of varieties with improved winter hardiness and pest resistance (insects, nematodes, and pathogens), providing even greater potential utilization in modern farming systems. To develop alfalfa varieties with physical and biochemical properties that fit the needs of the high producing dairy cow (i.e., greater cell wall digestibility, less protein degradation during ensiling, increased by-pass protein, increased yield without quality loss, insect and pathogen resistance, herbicide tolerance, reduced bloat, and winter hardiness) requires input from several disciplines. Strategies that embrace traditional genetic selection methods as well as precision breeding and other tools biotechnology may be needed in a timely manner to move a desirable trait into the elite germplasm in a timely manner. The goal is to have alfalfa varieties that can meet the needs of the dairy enterprise and at the same time maximize their use in farming systems that improve the ecological environment (N fixation, excellent nutrient sink, stand longevity, etc.). Developing alfalfa that could retain nutrition quality with few cuttings, increased yields, and better water use efficiency would be a major improvement in the profitability of alfalfa production.

Yield: Although quality has improved, alfalfa yield has not kept pace with corn. This is becoming more of an issue as land, labor, and energy costs continue to rise placing a greater burden on obtaining sufficient value from the harvested crop. Most recent gains in yield have been made by developing germplasm that has greater pest resistance and winter hardiness and selection for increased quality under a frequent cutting regime. There would seem to be sufficient genetic diversity to select for much larger plants that would provide significantly higher yields per acre (JoAnn Lamb, personal communication), but forage quality cannot be sacrificed. There are other opportunities for improving total biomass production that involve specific tissues of the alfalfa plant such as leaves and stems.

Reducing leaf loss has potential for enhancing biomass and quality. One of the problems with large plants in a typical seeding pattern is the loss of leaves that are shaded in the lower portions of the crop canopy. A solution to the problem could involve genetic selection for increased leaf retention or possibly using a molecular approach to disable genes that are responsible for leaf drop. This would require identification of specific cell wall hydrolases involved in the disruption of cells in the attachment area of alfalfa leaves to the stem. For other plants it has been shown that cellulases and pectinases are critical for leaf drop. If plants could maintain leaves after they have passed senescence this would increase total biomass. The digestible cell walls of leaves would be readily utilized by the animal even though the senesced leaves would not contain much protein or soluble carbohydrate. Increasing the mechanical strength of leaf attachment may also improve harvest recoveries of leaves.

Harvesting techniques can greatly impact biomass recovery. Harvest losses with conventional hay-making equipment are typically in the range of 6 to 19 percent. Utilizing haylage versus hay probably has the greatest single impact both in terms of preserving total biomass and quality. Even though more alfalfa haylage is being produced in the Midwest as a rain damage alleviator, production and marketing of haylage outside the dairy enterprise is difficult. There are technologies being developed such as hay maceration (US Dairy Forage Research Center) that will improve hay production to preserve biomass and improve quality at the same time. A macerator mat harvestor could keep harvest losses well below the 6 to 19% loss (Koegel et al., 1992).

Weeds in alfalfa are a major challenge. They can inhibit successful stand establishment, reduce yields, lower forage quality, reduce stand life and be toxic to livestock. Current weed control products have a narrow window of application, relatively long preharvest intervals, risk crop injury, have requirements for soil incorporation, narrow weed control spectrum, and there are crop rotation restrictions. With the development of Roundup Ready® alfalfa, like other Roundup Ready crops, growers can spray alfalfa fields with Roundup herbicides to control more than 200 species of weeds without injuring the alfalfa crop or negatively affecting the quality of the forage.

Fiber Digestibility: Fiber digestibility is an important component of forage having an impact on intake and digestibility by the dairy cow. Hatfield et al. (1999) provides background, on the molecular basis for improving forage digestibilities, Barrièrer et al. (2004) on the genetic and molecular basis of grass cell wall biosynthesis and degradability, and Ralph et al. (2004) on lignins. Lignin is a phenolic compound found in most plant secondary cell walls, is indigestible, and cross-links with other cell wall components resulting in decreased cellulose digestibility. Lignin content increases, and cell wall digestibility decreases as alfalfa plants mature. Almost every enzyme involved in the synthesis of lignin monomers has been investigated in one species or another (http://www.psb.ugent.be/research/molgen/lignin_details.htm) with variable impacts upon the concentration of the final lignin polymer deposited within the cell wall matrix. Some have had dramatic effects upon the total lignin (>50% reduction), but producing a phenotype that is fragile and with poor agronomic qualities. Dixon's group (Guo et al., 2001)) at the Noble Foundation have altered the lignin pathway in alfalfa by decreasing the expression of two genes which are involved in the biosynthesis of coniferyl and sinapyl alcohol, the main building blocks of lignin. The changes in lignin were on the order of 20% reduction (Guo et al., 2001) that translated into increases in digestibility of 2-5%. This improvement can be compared to conventional breeding where over 15 years selection has resulted in a 2-3% increase in cell wall digestibility.

An alternative way to improve digestibility is to selectively increase specific carbohydrates that make up alfalfa cell walls such as pectin. Alfalfa stems typically contain 10-12% pectin as a component of the cell wall matrix. Pectic polysaccharides are rapidlydegraded by rumen microbes producing acetate and propionate, but does not result in acidosis like rapidly fermented starch (Hatfield and Weimer, 1995). The US Dairy Forage Research Center has been involved with a consortium made up of alfalfa breeding companies to select for increased concentrations of pectin in alfalfa stems. Through two cycles of selection the total stem pectin concentration has been increased by 15-20% (Hatfield et al., unpublished data). Preliminary results indicate that in vitro total dry matter digestibility was increased. However, additional work must be done to determine what other changes have occurred within the plant because an increase in one component requires the decrease in some other component. It is encouraging that selection can be made for specific cell wall components.

Alfalfa cell walls also contain xylans and cellulose with vastly different digestibilities (Hatfield and Weimer, 1995). The xylans in alfalfa stems (20-25% of total) have a slow rate and low extent of digestion. Replacing at least part of this cell wall fraction with another polysaccharide could have major impacts upon total fiber digestion. Increasing the cellulose content without increasing lignin should result in a wall matrix that has a greater extent of degradation. The impact of manipulating xylan or cellulase upon the function of the alfalfa plant is unknown at this time. Precision breeding techniques allow altering a gene and determining its impact in a relatively short period of time. In this way one can determine right away if altering a particular component is going to improve plant function or be detrimental. With the exception of cellulose, the genes involved in xylan and other cell wall polysaccharide biosynthesis have not been identified, which eliminates this approach as a way to test the hypothesis of altering specific polysaccharides. It may be possible to use this approach with cellulose; however, most plants appear to have relatively large families of cellulose synthase genes making this approach difficult.

Protein: The full benefit of alfalfa protein is not realized due to its poor utilization by the animal. Ruminal microbes degrade alfalfa protein too rapidly resulting in excessive excretion of nitrogenous waste by the animal. In addition, protein breakdown during ensiling can be extensive. This loss is due to plant proteases degrading 44 to 87% of forage protein into ammonia, amino acids and small peptides during silage fermentation resulting in losses of up to $28 per acre for alfalfa. Decreasing protein degradation during the silage making process and in the rumen would decrease the need for supplemental protein and decrease the loss of nitrogen to the environment on the dairy farm.

Red clover has been found to have up to 90% less proteolysis than alfalfa during ensiling (Papadopoulos, 1983). This observation suggests that red clover should be an ideal legume for ensiling. Yet the widespread use of red clover is limited due to its poorer agronomic characteristics such as low stand persistency, yield, and its slow drying rate in the field. Lower extent of proteolysis is not due to differences in the inherent proteolytic activity in red clover versus alfalfa, but rather related to the presence of a soluble polyphenol oxidase (PPO) and *o*-diphenols in red clover (Jones et al., 1995a; Jones et al., 1995b; Jones et al., 1995c). This conclusion was initially based on several observations including: 1) red clover contains factors that can rapidly (<0.25h) inhibit proteolysis in both red clover and alfalfa, as determined by mixing experiments; 2) red clover leaves contain >250-fold higher levels of PPO activity than alfalfa leaves; 3) red clover contains abundant *o*-diphenol PPO substrates which are depleted as proteolysis is inhibited; 4) one of the factors involved in proteolytic inhibition is heat labile (consistent with involvement of a proteinaceous factor); and 5) proteolytic inhibition is O_2-dependent. Recently, the US Dairy Forage Research Center has been able to successfully test the hypothesis that PPO and *o*-diphenols inhibit proteolysis in plant extracts. Researchers have further demonstrated the role of PPO in proteolytic inhibition in plant extracts using a transgenic alfalfa system.

Although alfalfa has at least one gene encoding PPO, expression has not been detected in any tissues except developing seed pods. Further, significant PPO activity in alfalfa leaves and stems nor significant amounts of *o*-diphenol substrates have been detected. Thus, alfalfa is an ideal model system to explore the role of PPO/*o*-diphenols in inhibition of post-harvest proteolysis. To demonstrate the role of PPO and *o*-diphenols in inhibition of proteolysis, a cloned red clover PPO gene (*PPO1*) was constitutively expressed in transgenic alfalfa (PPO1-alfalfa). Proteolysis was inhibited in leaf extracts of the PPO1-alfalfa when the *o*-diphenol caffeic acid was added (Sullivan et al., 2004). No inhibition was observed when caffeic acid was omitted. Substantial proteolysis was observed in leaf extracts of control alfalfa lacking a PPO transgene, even if caffeic acid was added to the extract, indicating that caffeic acid alone does not result in in vitro proteolytic inhibition. The extent of proteolytic inhibition seen for PPO1-alfalfa extracts with added caffeic acid was comparable to that seen for red clover extracts. These results clearly demonstrate the major role of PPO and *o*-diphenols in post-harvest proteolytic inhibition in red clover and that expression of the PPO gene in other forages can inhibit proteolysis when an appropriate o-diphenol is added.

Slowing the rate of alfalfa protein degradation in the rumen is difficult to address from the alfalfa plant. There is some evidence that PPO generated o-quinones interact with proteins in red clover providing some protection in the rumen creating greater bypass protein. It is clear that tannins provide protection of plant proteins from ruminal degradation. Tannins are phenolic compounds that generally bind with proteins, decreasing the rate and extent of protein digestion. Forage legumes (e.g. birdsfoot trefoil) that produce tannins in leaves or stems have increased stability of the protein in the rumen, thus more protein escaping degradation in the rumen. Unfortunately, alfalfa does not produce tannins except in the seed coats. With new knowledge about tannin biosynthesis (Dixon group, Noble Foundation) it may be possible to engineer alfalfa to produce tannins that provide protein protection in the rumen and may also lead to less bloat. Many of the “raw materials” needed to produce the building blocks of tannin polymers are already being produced by the plant, it’s just a matter of diverting some of these into a new pathway. Another approach is to have alfalfa produce proteins containing increased concentrations of sulfur containing amino acids whereby more disulfide bonds are present which are known to be less degradable in the rumen. Tabe et al. (1995) used a biotechnological approach to insert a gene from a sunflower plant into alfalfa that resulted in the production of a sunflower seed storage protein, rich in cysteine and methionine, in alfalfa leaves.

Other Benefits: Traditional breeding and biotechnology together could lead to other potential benefits in alfalfa such as: enhanced protein content and amino acid profile; altered carbohydrate content (more pectin) and lignin structure; bloat prevention; and increased mineral availability. The potential exists for having alfalfa produce fiber digesting enzymes such as xylanases, cellulases, hemicellulases, ferulic acid esterase, ferulase, etc. or possible fermentation adjuvants for enhancing fermentation in the silo and/or rumen. In addition, researchers at the University of Guelph are looking at producing protective antigens of M. haemolytica in alfalfa as a non-invasive means of vaccinating calves from pneumonic pasteurellosis. http://www.gov.on.ca/OMAFRA/english/research/new_directions/projects/2002/sr9104.htm. Through biotechnology, genes regulating biomass production, photosynthetic capacity, insect resistance, herbicide tolerance, virus protection, drought tolerance, cold tolerance, tolerance to saline soils, nitrogen capture and utilization, leaf attachment, etc. should be explored.

Conclusions

Corn silage and alfalfa are the key foundational forages upon which dairy rations are built. Past progress relying on traditional breeding has been slow in enhancing the quality and attributes of forages. With the recent tools of biotechnology, rapid advancement in forages with improved agronomic and nutritional traits may be possible leading to more efficient and environmentally friendly dairy enterprises.

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