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Alfalfa: Forage Crop of the Future

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FORAGE TRENDS

In 2007, U.S. farmers harvested 23.6 million acres of alfalfa. Alfalfa harvested as hay and haylage produced 82.8 million tons valued at approximately $9.4 billion, ranking behind only corn and soybeans. Alfalfa hay supports dairy, beef, sheep, and horse production in the U.S. as well as a growing export market.

Alfalfa production has increased in the west to support an expanding dairy industry. Lactating dairy cows fed physically effective alfalfa fiber are healthy and very productive. Western dairy operators have demanded higher quality alfalfa, often at the expense of yield. However, quantities of alfalfa hay fed to dairy cattle have declined in recent years, often being replaced by corn silage and by-product feeds. We believe scientific capacity is available to genetically engineer alfalfa to solve current limitations in its production, its utilization by high producing dairy cows, and its use for renewable energy.

Determining what plant modifications are needed to increase alfalfa's role in any one dairy enterprise requires a holistic approach. Alfalfa traits that should be modified depend upon: crop growing environment; how it is harvested, stored and fed; its nutritive and economic value relative to the availability, quality and consistency of other feed ingredients; the type of diet in which it is fed, the animal and its nutrient requirements, its value in crop rotations and environmental impacts. For example, the ideal characteristics for grazing alfalfa may be quite different for alfalfa grown for hay production and sale.

CURRENT ROLE OF ALFALFA IN DAIRY DIETS

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. Corn silage and alfalfa are the main forages providing energy, protein, digestible and effective fiber, minerals, and vitamins to dairy cattle. Alfalfa, often called the “Queen of Forages,” is the most important forage legume for dairy cows. It is relatively low in fiber and high in protein compared to other forages and typically results in high intakes and levels of milk production. In addition to its excellent nutritional properties, crop rotations utilizing alfalfa have a positive environmental impact in terms of stabilizing soils, decreasing nutrient inputs, and increasing wildlife habitat. The major disadvantages of alfalfa are 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 also expose the forage to multiple harvesting environmental risks, 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 by dairy cows. Alfalfa’s low fiber and high protein content makes it an excellent complement for grains and other forages in dairy rations. Although there are genetic differences in nutritional value among alfalfa varieties, currently the nutritional quality of alfalfa is established primarily by harvest management. Although there are differences among seasons and cuttings, the composition and dry matter digestibility (DMD) of alfalfa is related primarily to plant maturity at harvest.

Table 1. 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 percent-units more protein than required. 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 non-fibrous carbohydrates

(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, 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 fiber 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. The fiber component of forage represents a major source of energy; however, less than 50% of this fraction is readily digested and utilized by the animal. If a 10% increase in fiber digestion was obtained, the dairy industry could realize a benefit of $380 million in milk and meat sales while reducing manure solids by 2.5 million tons and grain input into dairy rations by 3.3 million tons.

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.

REDESIGNING ALFALA FOR LIVESTOCK DIETS

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 biotechnology tools may be needed 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 alfalfa's environmental benefits (N fixation, excellent nutrient sink, stand longevity, etc.). Developing alfalfa that could retain nutrition quality with fewer 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 increases have 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. Developing germplasm that has greater pest resistance and winter hardiness and selection for increased quality under a frequent cutting regime has accompanied most recent gains in yield. 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 animal would readily utilize the digestible cell walls of leaves 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 haymaking 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. The U.S. Dairy Forage Research Center has discovered technologies such as alfalfa maceration that improve alfalfa production by preserving biomass yield and improving quality at the same time. A macerator mat harvester could keep harvest losses well below the current 6 to 19% loss.

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 pre-harvest intervals; crop injury risks; requirements for soil incorporation; a narrow weed control spectrum; and crop rotation restrictions. With the development of Roundup Ready® alfalfa, growers can spray alfalfa fields with glyphosate herbicide to control more than 200 species of weeds without injuring the alfalfa crop or negatively affecting the quality of the forage. Use of this product is currently under a court ordered injunction that prohibited new seeding after March of 2007.

Fiber Digestibility. Fiber digestibility is an important component of forage having an impact on intake and digestibility by the dairy cow. Lignin is a phenolic compound found in most plant secondary cell walls that 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 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) leading to improved animal performance.

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 rapidly degraded by rumen microbes producing acetate and propionate, but do not result in acidosis like rapidly fermented starch. The U.S. Dairy Forage Research Center has been collaborating with 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%. 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 digestibility. 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.

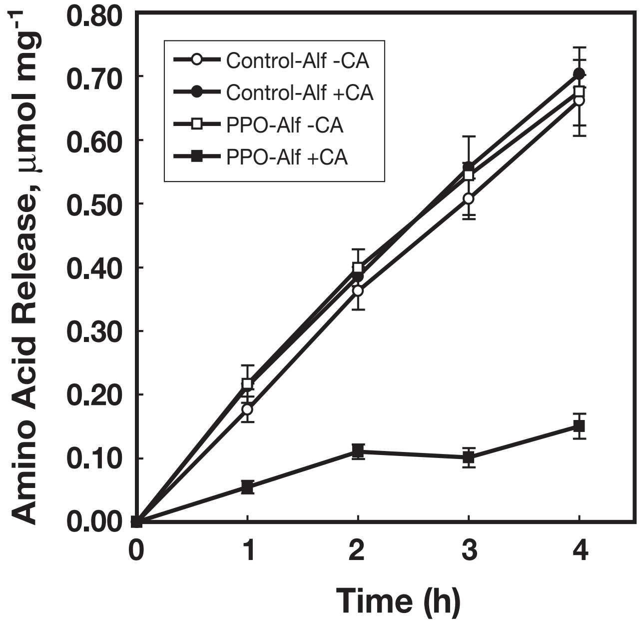


Figure 1. PPO inhibits postharvest proteolysis in an o-diphenol-dependent manner. Amino acid release during a 4-hour incubation at 37°C was used to measure proteolysis in extracts of control or PPO1-expressing alfalfa as indicated in the presence (+CA) or absence (-CA) of 3 mM caffeic acid. (Sullivan and Hatfield, 2004).

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 magnify the problem. This loss is due to plant proteases that degrade 44 to 87% of forage protein into ammonia, amino acids, and small peptides during silage fermentation. These nitrogen fractions are rapidly degraded and lost by the animal in urine, resulting in losses of up to $28 per acre for alfalfa. Decreasing protein degradation during ensiling and in the rumen would decrease the need for supplemental protein and reduce 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. 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 and 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 is related to the presence of a soluble polyphenol oxidase (PPO) and *o*-diphenols in red clover.

Recently, the U.S. Dairy Forage Research Center has successfully tested the hypothesis that PPO and *o*-diphenols inhibit proteolysis in plant extracts. Researchers have further demonstrated the role of PPO in proteolytic inhibition using a transgenic alfalfa system. 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, **Figure 1**. 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.

Using PPO and *o*-diphenols to slow the rate of alfalfa protein degradation in the rumen is more difficult. 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. However, tannins may more easily 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. An optimum level of tannins supported an increase of 11 pounds per day of milk from cows fed normal tannin containing birdsfoot trefoil over alfalfa silage, **Figure 2**. Unfortunately, alfalfa does not produce tannins except in the seed coats.

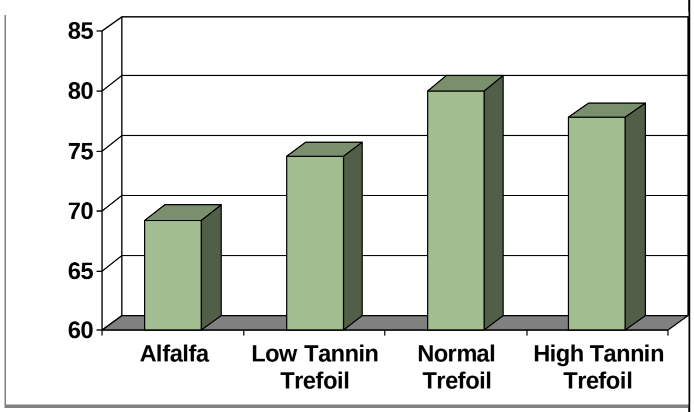


Figure 2. Comparison of alfalfa and birdsfoot trefoil silages on milk yield (pounds per day). SOURCE: Hymes Fecht et al., 2005.

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. Because many of the “raw materials” needed to produce the building blocks of tannin polymers are already being produced by alfalfa; it’s just a matter of diverting some of these into a new pathway.

GROWING ALFALFA FOR RENEWABLE ENERGY

The United States is experiencing an economic shock due to recent hikes in the price of oil and natural gas. For farmers, the prices of nitrogen fertilizer and fuel have risen to unprecedented levels, putting profits at risk. The President, Congress, industry, and the public are all calling for independence from imported oil. Ethanol produced from cellulosic biomass may be a sustainable and achievable alternative to help fuel America’s transportation system. At the same time, biomass crops must help support profitable agricultural systems, vital rural towns, and public demand for environmental protection.

Alfalfa has considerable potential as a feedstock for production of ethanol and other industrial materials because of its high biomass production, perennial nature, ability to provide its own nitrogen fertilizer, and valuable co-products. Unlike other major field crops like corn and soybeans, which are commonly refined for production of fuel and industrial materials, refining of alfalfa remains underdeveloped. Instead, alfalfa is primarily processed and used on-farm as livestock feed. Although alfalfa remains tied with wheat as the third most important field crop after corn and soybeans, declining dairy cow numbers and shifts in feeding practices have caused a reduction in alfalfa acreage over the last 25 years. The end result has been an increase in continuous row cropping of corn and soybeans with little rotation to perennial forages. As a result, the risks of soil erosion, contamination of surface and ground water by nitrate and pesticides, and loss of valuable soil organic matter have increased. Growing more alfalfa for biofuel production would contribute to making the United States energy independent, improving our natural soil resource, reducing greenhouse gas emissions, and protecting water quality.

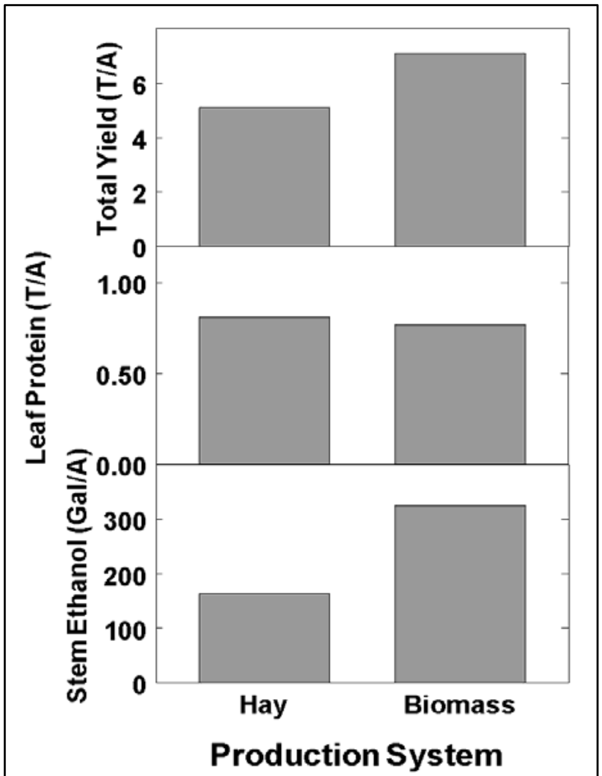
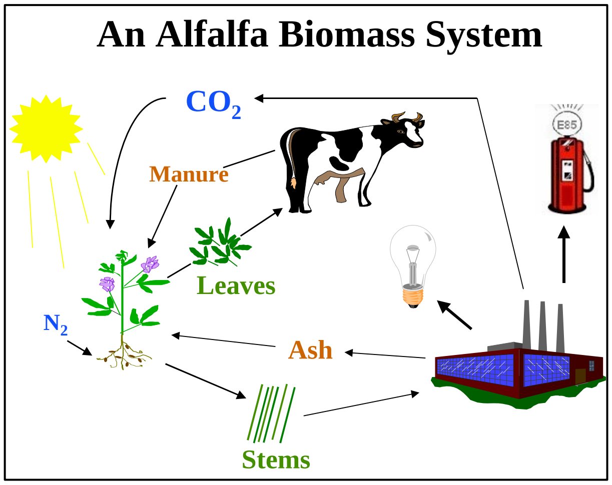


Figure 3. Dry matter, leaf protein, and theoretical ethanol yield from stems of hay-type alfalfa grown under conventional hay management compared to biomass-type alfalfa grown under a biomass management system. SOURCE: Lamb et al., 2007.

The USDA-Agricultural Research Service is actively researching many potential biomass crops. The Plant Science Research Unit (PSRU) in St. Paul, MN has been actively involved in alfalfa biomass energy research since 1993 in collaboration with the University of Minnesota. The PSRU has developed a biomass-type alfalfa that is taller and does not lodge at later maturity stages. These traits allow less frequent harvesting than conventional forage-type alfalfa, reducing harvest costs, and protects nesting birds in early summer. When biomass-type alfalfa is grown under a biomass management system with less dense seeding and only two harvests per year, compared with standard hay-type alfalfa production practices, total yield of alfalfa increases 42%, leaf protein yield is equal, and potential ethanol yield from stems doubles, **Figure 3.**

Alfalfa produces high net energy yield – that is, the energy required for production is far lower than the total energy contained in the crop. This is due, in part, to biological nitrogen fixation. Unlike corn and other grass crops, alfalfa can obtain nitrogen from the air. This saves the farmer money, but also represents a tremendous energy savings. Furthermore, alfalfa leaves enough plant-available nitrogen in the soil to meet the needs of the next crop of corn. In many cases, there is even enough nitrogen to satisfy one-half the need of the second crop of corn. Clearly, inclusion of alfalfa in rotation with corn increases the efficiency of energy production from corn.



To maximize energy yield, cellulosic biomass production and processing likely will need to be locally based. Recently, PSRU scientists analyzed the spatial distribution of net energy yield for soybeans, corn, and alfalfa in a prospective fuelshed, and the results demonstrate how net energy yield varies with soil type and decreases with distance from processing facilities. This approach has shown how biofuel facility planners can minimize costs and maximize energy production by contracting with nearby, high-producing farms. In addition to the many environmental benefits of growing a perennial legume, the efficiency of energy production by alfalfa is 2 to 3 times better than corn grain or soybeans. Below are examples for specified biomass yields in fields located 15 miles from a processing facility.

Crop (yield)	Energy input	Delivered energy	Ratio of output:input
	Million BTU/acre		
Soybean (40 bu/a)	2.3	18.3	7.1
Corn grain (180 bu/a)	6.0	59.0	8.8
Corn stover (3.6 tons/a)	2.6	51.1	19.7
Alfalfa (6 tons/a)	3.0	78.2	***25.0***

In contrast to new crops and native perennials, production practices and machinery are well developed for alfalfa. There is agronomic expertise available in most states through the Extension service and private consultants. Additionally, several value-added products can be produced from alfalfa leaves before conversion of the rest of the crop to fuel or energy. Examples are feed and food grade proteins, and nutriceuticals such as lutein. Scientists in the PSRU have produced bio-degradable plastic in alfalfa leaves.

Fuel or Adhesives in Wood Products. U.S. Dairy Forage Research Center scientists have identified a potential high value by-product from bacteria fermenting alfalfa fiber for ethanol. This material is the glycocalyx, a sticky resin formed by the bacteria that adhere to the fiber Fermentation residues (consisting of incompletely fermented fiber and adherent bacterial cells with their glycocalyx material) were obtained by growing the anaerobic cellulolytic bacteria *Ruminococcus albus* 7 or *Clostridium thermocellum* ATCC 27405 on a fibrous fraction derived from alfalfa. Dried residue served as an effective co-adhesive for phenol-formaldehyde (PF) bonding of aspen veneer sheets to one another. Testing of resulting plywood panels revealed that the adhesive, formulated to contain 30% of its total dry weight as fermentation residue, displayed shear strength and wood failure comparable with those of industry standards (plywood normally contains 55 % by weight of PF). Microbial glycocalyx has potential to replace phenol-formaldehyde resin currently used in forming plywood panels.

Further research on alfalfa germplasm improvement, development of valuable co-products, and providing guidelines for the most environmentally beneficial deployment of alfalfa in cropping systems will strengthen the economic and environmental benefits to be gained from using alfalfa to provide the future energy needs of the United States.

CONCLUSIONS

Alfalfa is a key forage to build effective diets for livestock producers. Enhancing the nutrient utilization of alfalfa in dairy diets offers potential performance enhancements for beef, horse, and sheep producers. Research into improving biomass yield and conversion of biomass to liquid fuel via fermentation or gasification can generate new products and expanded acreage. Past progress relying on traditional breeding has enhanced alfalfa's quality, but yield improvements have been limited and alfalfa does not possess certain special attributes, such as tannins, that could dramatically enhance the crop. With the modern biotechnology tools, rapid advancement of alfalfa with improved agronomic and nutritional traits, along with the addition of novel traits, should lead to more efficient and environmentally friendly dairy and hay enterprises.

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