REINVENTING ALFALFA FOR DAIRY CATTLE AND NOVEL USES

N.P. Martin and D.R. Mertens [1]

ABSTRACT

Alfalfa use by dairy cattle has decreased in recent years because of excessive non-protein nitrogen and low fiber digestibility. Ideal attributes for plant modification of alfalfa may include those that: increase milk potential per acre and/or per ton; enhance digestible NDF; improve protein content and amino acid balance; and 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 and testing these attributes will accelerate with the use of biotechnology. Livestock and hay enterprises will benefit from alfalfa that is 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. Value-added traits of alfalfa are needed to provide farmers new high value products. Phytase from transgenic alfalfa has been tested in poultry and swine rations and found to improve animal performance.

Key Words: alfalfa, protein, fiber digestibility, transgenic, and Phytase

INTRODUCTION

In 2004, U.S. farmers harvested 24.7 million acres of alfalfa. Alfalfa harvested as hay and haylage produced 83.9 million tons valued approximately at $8.4 billion, ranking behind only corn, soybeans and wheat. Alfalfa hay supports dairy, beef, sheep, and horse production in the U.S. as well as a growing export market. Harvested alfalfa acreage declined in 2004 to an all-time low of (23.3 million acres).

Alfalfa production has increased in the west to support a rapidly expanding dairy industry. Lactating dairy animals 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 feed supplements. We believe scientific capacity is available to genetically engineer alfalfa to solve current limitations of increased inclusion levels in diets of high producing dairy cows.

Novel traits to improve alfalfa are available in **Table 1**. Redesigning alfalfa to reverse declining amounts in diets and from acreages will improve alfalfa for other livestock uses. We will discuss

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new novel concepts being investigated with alfalfa, which have potential to increase acreage, add product value and offer new products.

Major factors of increased utilization are yield enhancement, forage quality

Table 1. Summary of alfalfa biotechnology research as reported by the North American Alfalfa Improvement Conference (Brummer et al., 2004).

USA	
Arizona State Univ.	Over-expression of salt tolerance genes.
Forage Genetics Int'l, West Salem, WI	Commercialization of Roundup Ready gene and down regulation of lignin genes to increase digestibility.
Iowa State Univ.	Identifying alfalfa genes controlling yield and winter-hardiness.
New Mexico State Univ.	Identifying genes controlling salt and drought stress.
Purdue Univ.	Cloning genes for vegetative storage proteins.
Samuel Roberts Noble Foundation, Ardmore, OK	Developing molecular markers, studying down regulation of lignin genes, insertion of genes for condensed tannins, identifying and introgression of drought and aluminum tolerance genes.
USDA-ARS, Beltsville, MD	Developing molecular markers and using markers for identifying genes for yield and winter survival.
USDA-ARS Madison, WI	Characterize genes controlling post-harvest proteolysis.
USDA-ARS, St. Paul, MN	Insertion of genes to allow remediation of atrazine and genes to control pectin in cell walls.
USDA-ARS, Prosser, WA	Using molecular techniques for quick identification of disease pathogens.
Univ. of California, LA	Expression of plant genes controlling nodulation and nitrogen fixation.
Washington State Univ. and USDA-ARS	Molecular markers to characterize diversity among alfalfa accessions.
Canada	
Agriculture and Agri-Food Canada, Saskatoon	Alter expression of condensed tannin genes to reduce bloat, greenhouse gases, and protein bypass.
Agriculture and Agri-Food Canada, Ste-Foy	Identification of genes for improved persistence, yield and cold tolerance.
Medicago Inc., Ste-Foy	Using alfalfa as factory to produce pharmaceutical products.
Univ. of Guelph	Genetic engineering to modify winter-hardiness.
Mexico	
National Univ. of Mexico	Using anti-sense and over-expression approaches to study nodulation and nitrogen fixation.
Europe	
AgroBioInstitute, Bulgaria	In vitro selection to increase tolerance to abiotic stresses.
INRA, Toulouse, France	Genomic approaches for nitrogen fixation, disease resistance, and abiotic stress tolerance.
BAP, Toulouse, France	Induction of plant defenses during disease infection via genetic and cellular approaches.
INRA, Lusignan, France	Developing molecular markers for candidate genes for aerial morphogenesis and genetic mapping.
Univ. of Perugia, Italy	Study of aluminum tolerance genes and examining methodology to remove antibiotic resistance during selection of transgenes.

improvements, environmental enhancements, and new products.

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 (Hartnell et al., 2005).

Determining what plant modifications would be needed to supply dietary ingredients for any one dairy enterprise requires a holistic approach. 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.

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.

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. Crop rotations utilizing alfalfa have a positive environmental impact in terms of stabilizing soils, decreasing nutrient inputs, and increasing wild life habitat. 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 harvest management. Although there are differences among seasons and cuttings, in general the composition and dry matter digestibility (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).

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

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, 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.

Immature alfalfa is high in protein, but the protein is rapidly fermented in the rumen to ammonia and not used efficiently (Broderick and Satter, 1998). Because alfalfa protein is used inefficiently, dairy rations containing predominantly alfalfa forage are formulated to contain 1 to 3 percent-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, **Figure 1.**

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

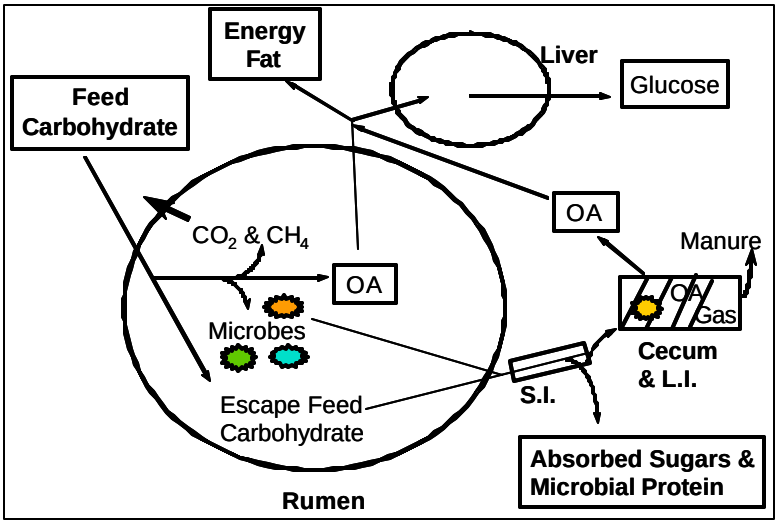

Figure 1. Protein metabolism in the rumen of dairy cow. SOURCE: Adapted from M.B. Hall, 2003.

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 biotechnology tools may be needed in a timely manner to move a desirable trait into the elite germplasm. 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. 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. There are technologies being developed such as hay maceration (U.S. Dairy Forage Research Center) that will improve hay production to preserve biomass and improve quality at the same time. A macerator mat harvester 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; have relatively long preharvest intervals; risk crop injury; have requirements for soil incorporation; have a narrow weed control spectrum; and have 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. This product is approved for limited commercialization in 2005.

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 produce 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 rapidly degraded by rumen microbes producing acetate and propionate, but do not result in acidosis like rapidly fermented starch (Hatfield and Weimer, 1995). The U.S. 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 U.S. 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), **Figure 2**. 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

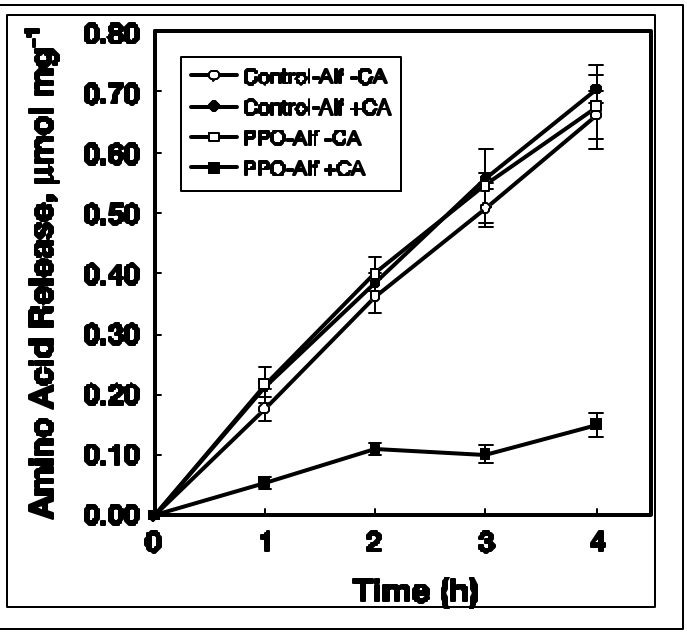

Figure 2. PPO inhibits postharvest proteolysis in an o-diphenol-dependent manner. Amino acid release during a 4-hour incubation at 37C was used to measure proteolysis in extracts of control or PPO1-expressing alfalfa as indicated in the presence (+CA) or absence (-CA) of 3 mM caffeic acid. (Sullivan and Hatfield, 2004).

providing some protection in the rumen creating greater bypass protein. It is clear that tannins provide protection of plant proteins from ruminal degradation. An optimum level of tannins supported an increase of 11 pounds per day of milk from cows fed normal tannin containing birsdfoot trefoil over alfalfa silage (Hymes-Fecht, 2005), **Figure 3**. 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.

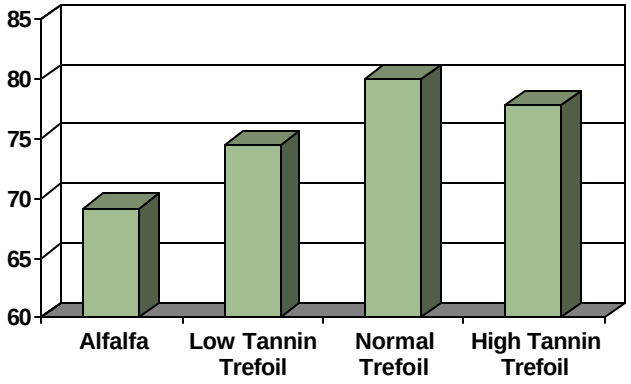

Figure 3. Comparison of alfalfa and birdsfoot trefoil silages on milk yield (pounds per day). SOURCE: Hymes Fecht et al., 2005.

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. 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 biotechno-logical 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.

High Phytase Transgenic Alfalfa. Buildup of phosphorus in the environment and the resulting degradation of water resources are of mounting concern. Much of this buildup is traceable to human activities. Important among these is livestock production. Monogastric animals, such as poultry and swine, which can solubilize only a small fraction of the phosphorus in their grain-based rations while excreting the remainder, have come under increased scrutiny. Supplementation of inorganic phosphorus into rations to meet animal nutritional requirements exacerbates the problem.

Much of the phosphorus in grain is in the form of insoluble phytates. Researchers have shown that supplementing poultry and swine rations with the enzyme phytase can lead to solubilization of the phosphorus, thus eliminating the need for phosphorus supplementation and concurrently reducing the level of phosphorus in animal excrement to approximately one-half of that normally experienced.

The enzyme phytase derived from *Aspergillus niger* has, to date, generally been produced in fermentation vats using genetically engineered microorganisms. It has been estimated that the cost of phytase supplementation with this material would be about three times the cost of conventional supplementation with dicalcium phosphate.

As an approach to reducing the cost of phytase production, a multi-disciplinary USDA-ARS-UW team at Madison, Wisconsin has produced transgenic alfalfa with the capability of expressing phytase (Austin-Phillips and Bingham, 1997). This phytase can be recovered with juice extracted from herbage. Leaf meal, which typically has protein and fiber contents greater than 25 % and less than 20 %, respectively, has also been used successfully.

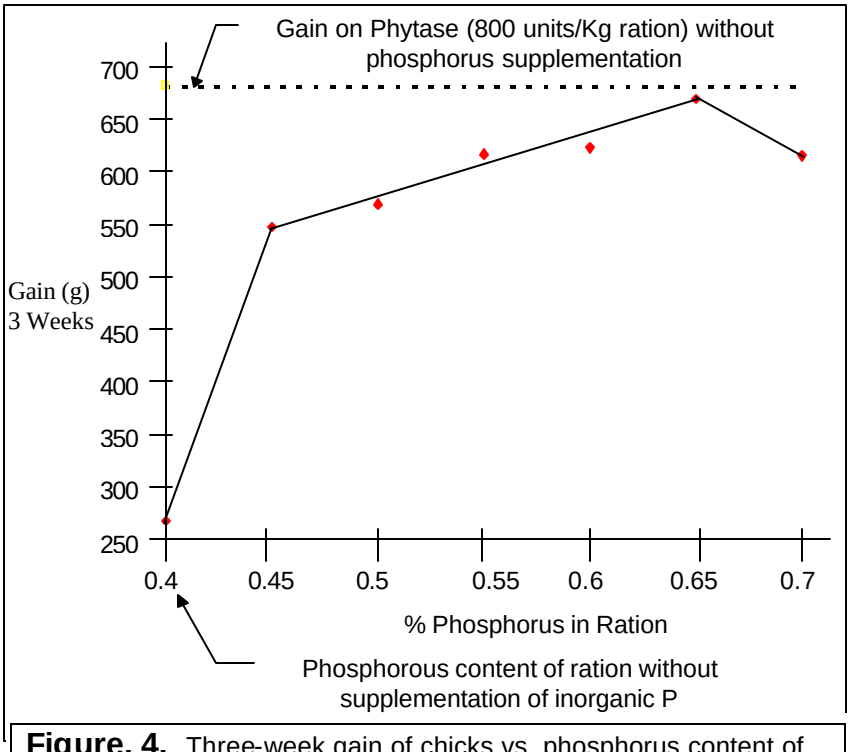

Figure. 4. Three-week gain of chicks vs. phosphorus content of ration compared to phytase with no phosphorus supplementation. SOURCE: Koegel et al., 1999.

Koegel et al. (1999) reported feeding growing chicks with alfalfa-produced phytase, which at appropriate levels can totally replace the inorganic P supplementation, **Figure 4.**

Replacing inorganic P with phytase resulted in a reduction of P concentration in poultry feces to less than one-half. They further reported that alfalfa phytase in the form of fresh juice, dried juice, or leaf meal was effective with swine and poultry (data not shown). The quantity of phytase, which can be produced in transgenic alfalfa, is on the

order of 200 X 10^6 units/acre/year, equivalent to an amount able to treat 500 tons of poultry ration. At current cost of inorganic P supplementation, the value of phytase would be $750 - $1,500 per acre-year. The value of xanthophylls and protein content of alfalfa as well as the environmental benefits would be in addition to this.

Fuel or Adhesives in Wood Products. While solid fuel yields the highest net energy and has the lowest processing cost, its use is generally limited to electric power generation. An alternative to solid fuel is the saccharification and fermentation of the ligno-cellulosic, fiber fraction to ethanol (Koegel et al., 1999). While conversion of fiber to ethanol results in less total energy and is a more complicated process, its versatility and potential use as a transportation fuel make it an interesting alternative.

USDA 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 (Weimer et al., 2003). Fermentation residues (consisting of incompletely fermented fiber, adherent bacterial cells, and a glycocalyx material that enhanced bacterial adherence) were obtained by growing the anaerobic cellulolytic bacteria *Ruminococcus albus* 7 or *Clostridium thermocellum* ATCC 27405 on a fibrous fraction derived from alfalfa (Weimer et al., 2005). 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 for PF that contained much smaller amounts of fillers or extenders. Glycocalyx has potential to replace phenol-formaldehyde resin currently used in forming plywood panels.

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, 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

Alfalfa is a key forage to build effective diets for dairy, horse and beef owners. Enhancing the nutrient utilization of alfalfa in dairy diets offers potential new products and expanded acreage. 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 and hay enterprises.

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