

Redesigning Alfalfa for Dairy Cattle and Novel Uses

2006 Western Business Conference & Expo
October 24, 2006, Red Lion Hotel in the Park, Spokane, WA

Neal P. Martin, David Mertens and Hans Jung
USDA-ARS, Madison, WI and St. Paul, MN



This talk will explore . . .

- Trends in alfalfa production and uses
- Barriers to increasing alfalfa in dairy diets
- Redesigning alfalfa for dairy cows
- Redesigning alfalfa for biofuel and new products

Trends . . .

2005 U.S. Alfalfa Production

- **Hay**

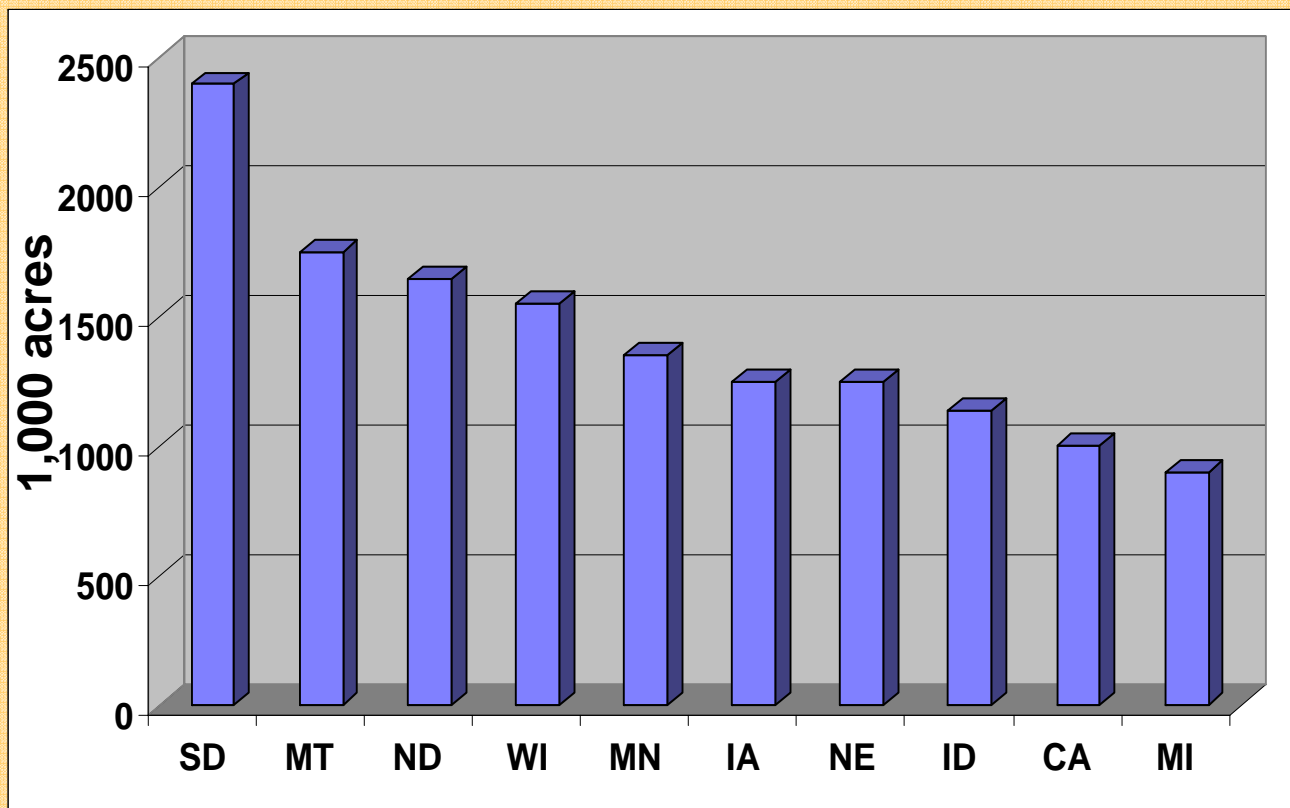
- 75.8 million tons
- 22.4 million acre
- \$ 7.3 billion
- 3rd following corn and soybeans

- **Forage**

- 86.3 million tons
- 24.4 million acres
- ~ \$91.3 billion
- 3rd following corn and soybeans

Trends . . .

Leading Alfalfa Hay States, 2005

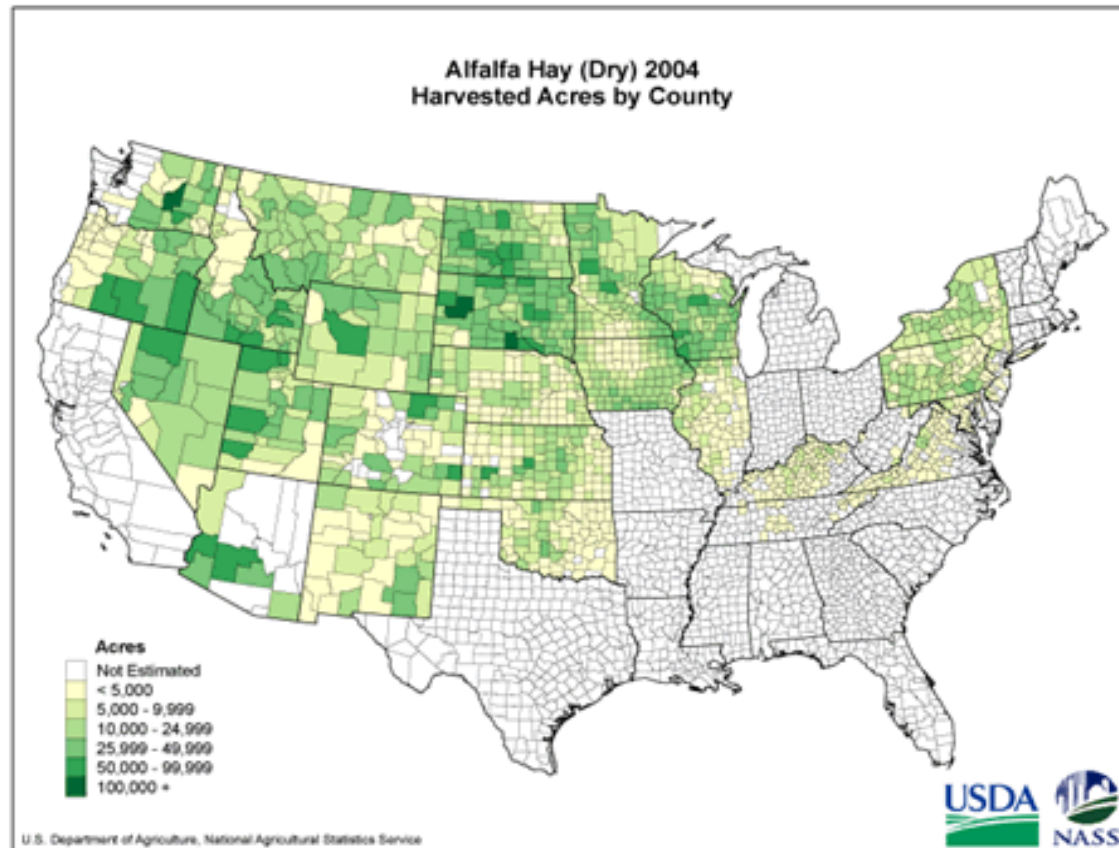


Top 10 States

- 64 % of U. S.
- 59 % of Acres
- 7 states NC
- 3 states West
- 4 Lead Dairy

Trends . . .

Alfalfa Hay Production



Trends . . .

- Hay acreage remains unchanged



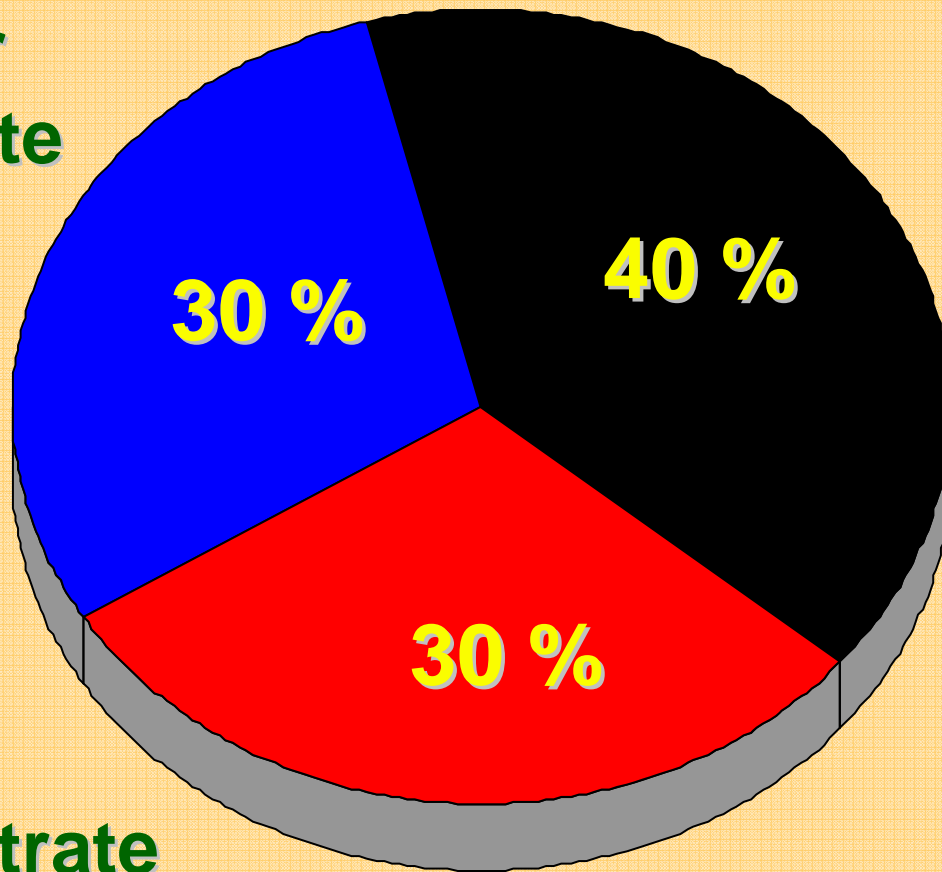
- Dairy cattle feeding – declining amounts



For many years the **Rule of Thumb** for feeding alfalfa to dairy cattle was. . .

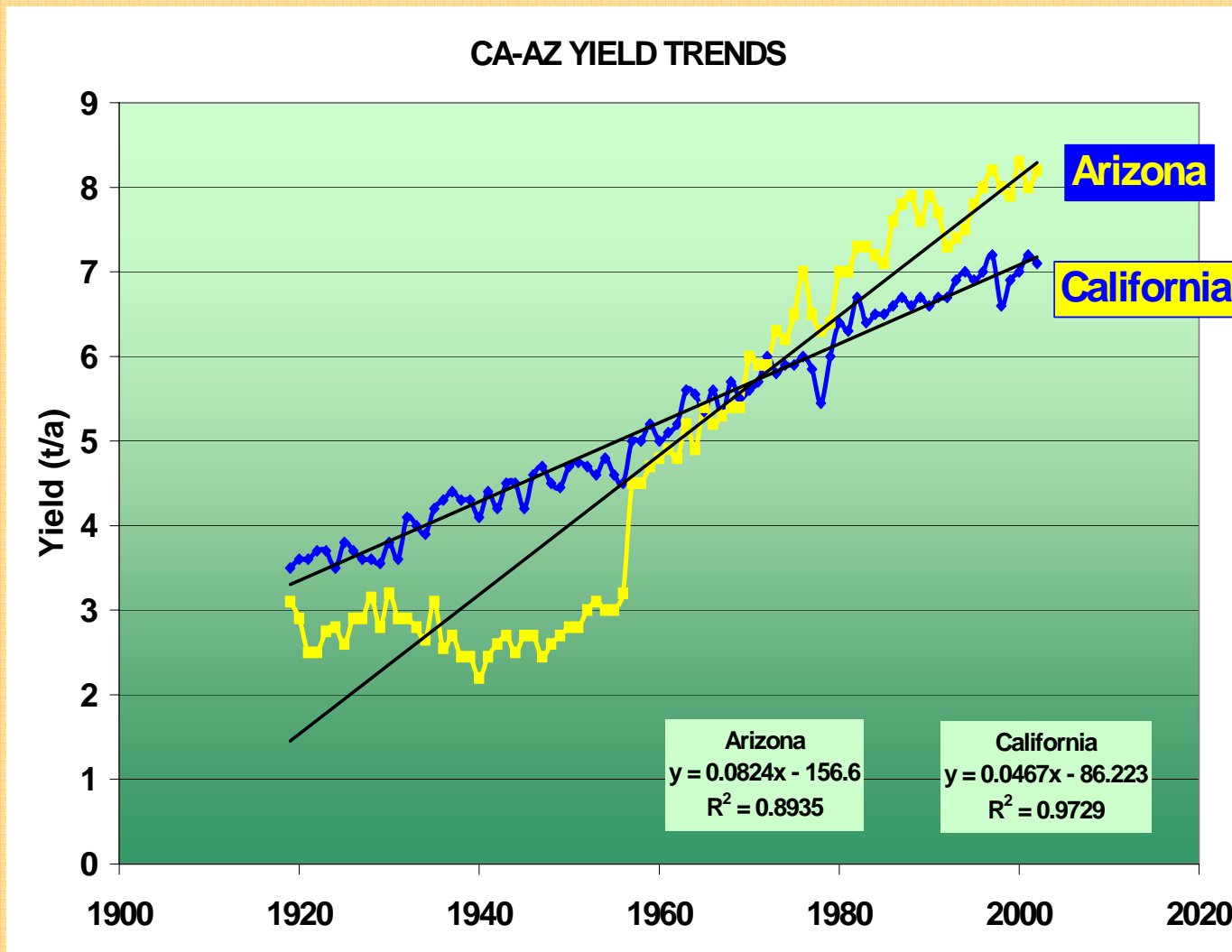
Forage or Concentrate

Forage

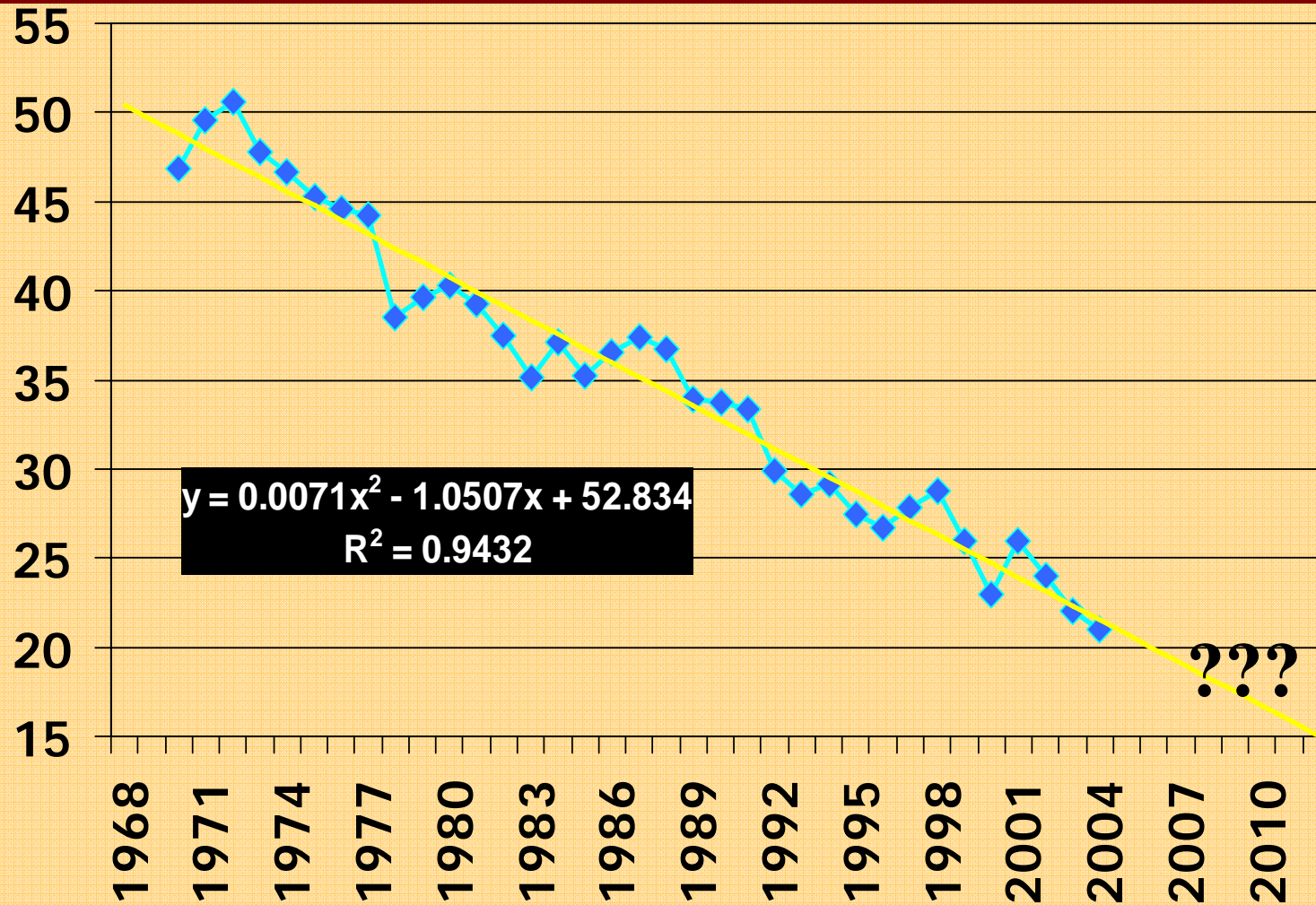


Concentrate

Alfalfa Yield Trends . . .



CA Hay Production Per Dairy Cow (lbs alfalfa/cow/day)



Source: Dan Putnam, 2005 Consortium for Alfalfa Improvement



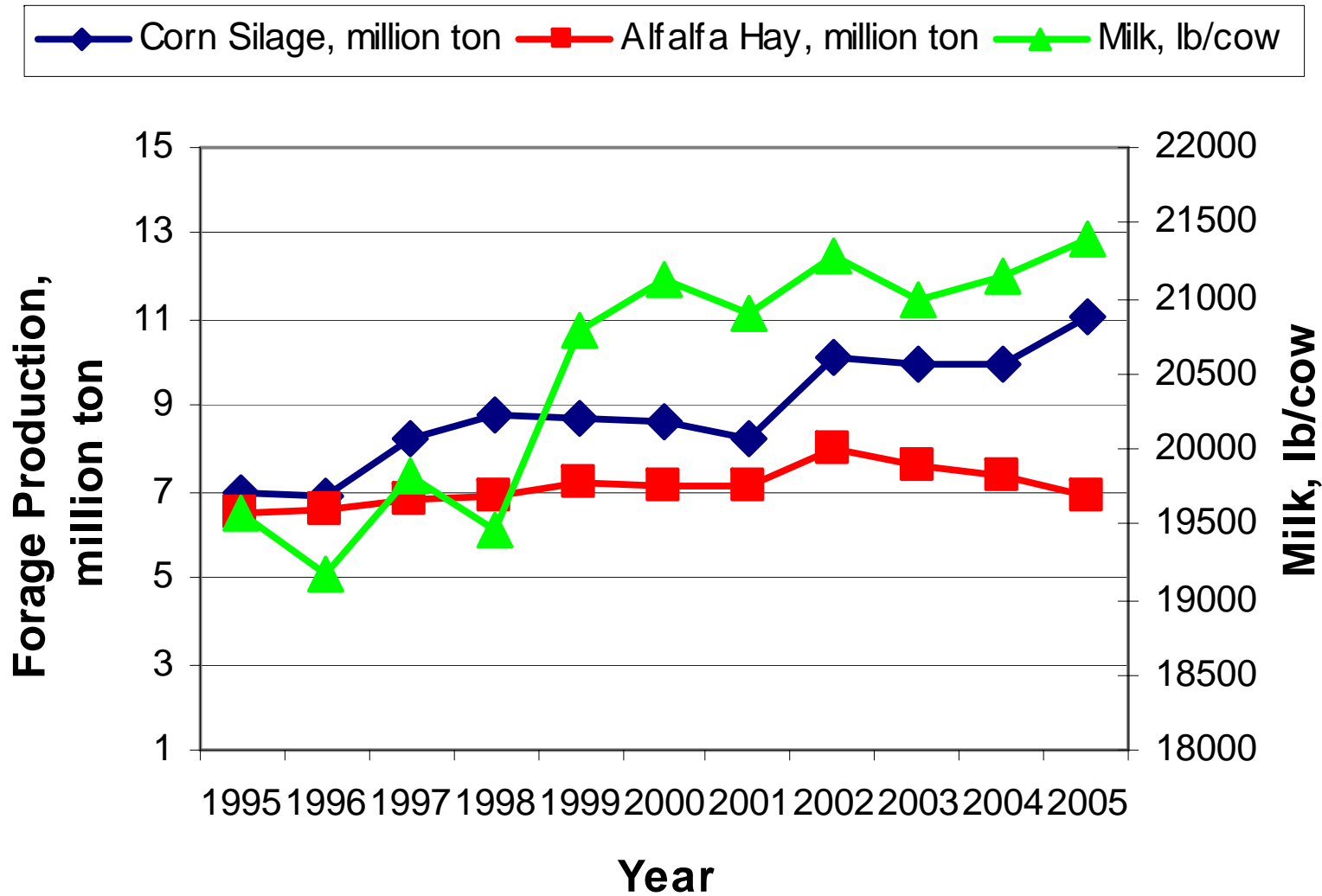
Why this declining trend?



Competition with
corn silage

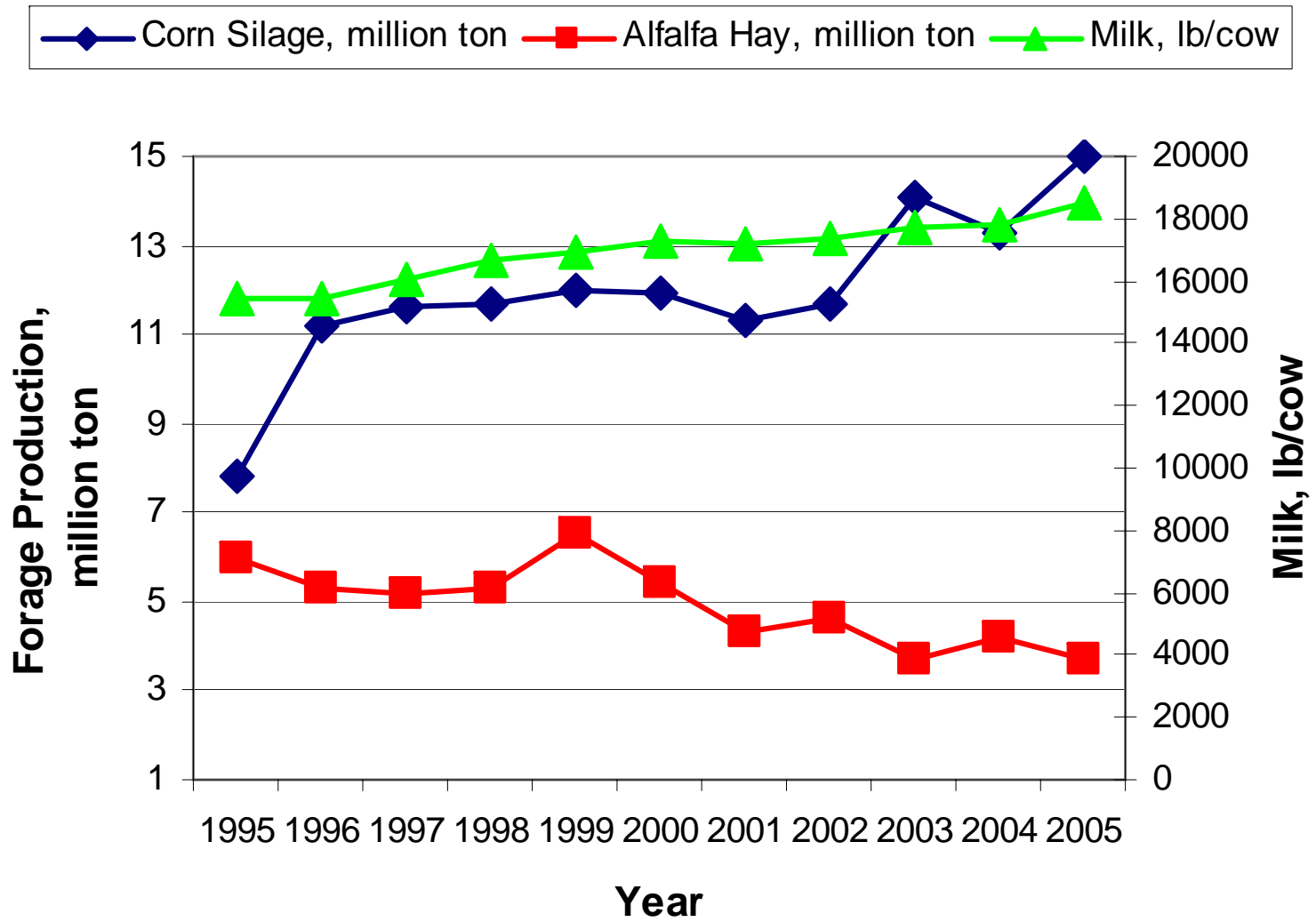


California



SOURCE: Jim Linn, 2006 NAAIC

Wisconsin



SOURCE: Jim Linn, 2006 NAAIC

Why this declining trend?

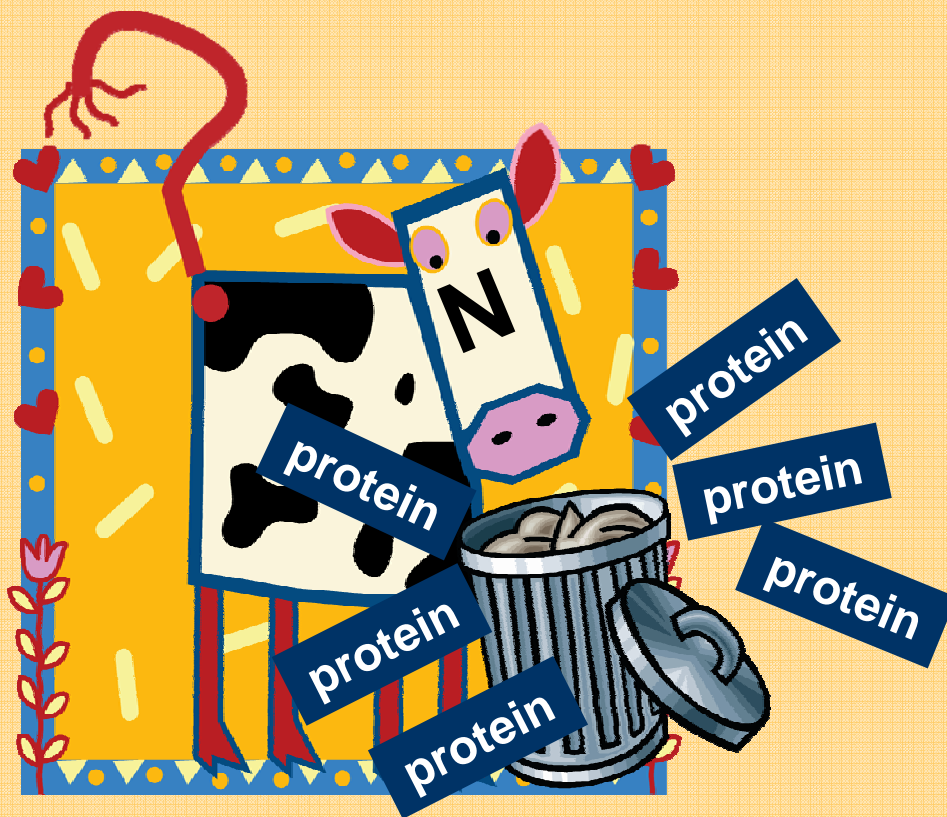
Competition from byproducts



- Canola Meal
- Soybean Meal
- Cottonseed
- Distillers Grains
- Bakery By-Products
- Almond Hulls
- Citrus Pulp
- Tomato Pumice
- Etc. Etc. Etc. Etc. Etc.

Why this declining trend?

Many of these byproducts are high in protein



Protein Sources

	CP, %	RDP, % CP
Alfalfa	20+	70
Dist grains - ethanol	30	60
SBM – biodiesel	50	65
Corn gluten feed	22	70
Corn gluten meal	67	45
Wheat midds	19	75
Blood	87	30
Corn silage	8	65
Corn grain	10	50
Dairy Cow Ration	<17	65

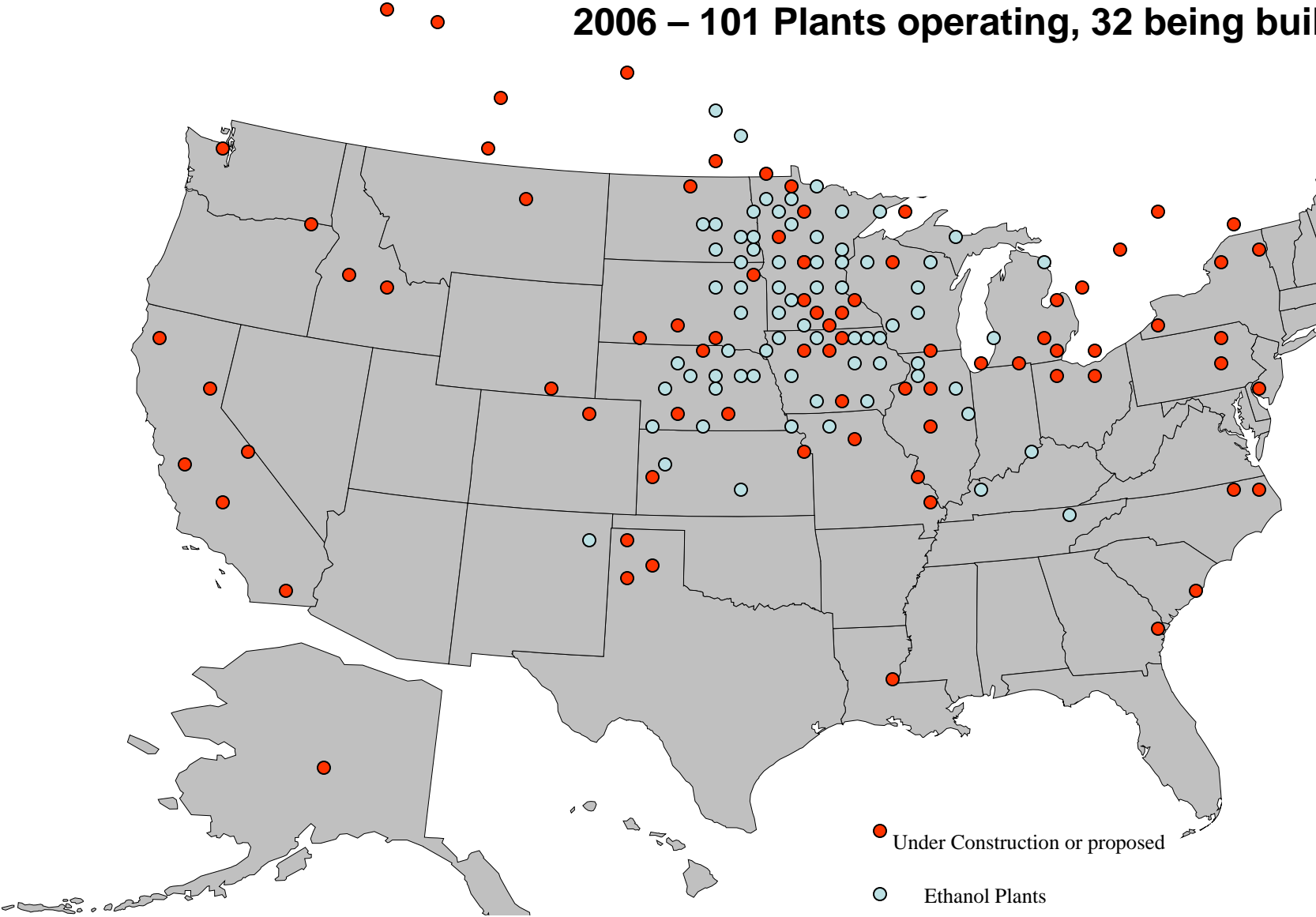


SOURCE: Jim Linn, 2006 NAAIC



Ethanol Plants in North America - June 16, 2004

2006 – 101 Plants operating, 32 being built



SOURCE: Jim Linn, 2006 NAAIC

Forage Fiber Sources – Dairy Rations

Straw – Use is increasing

- Low nutrient value
- Effective fiber

Hay Price, Particle Size and \$

- ▲ Ground hay
 - Quality (125 – 175 RFV) may not have extra value
- ▲ Long hay - Unchopped
 - Quality has value (\$)

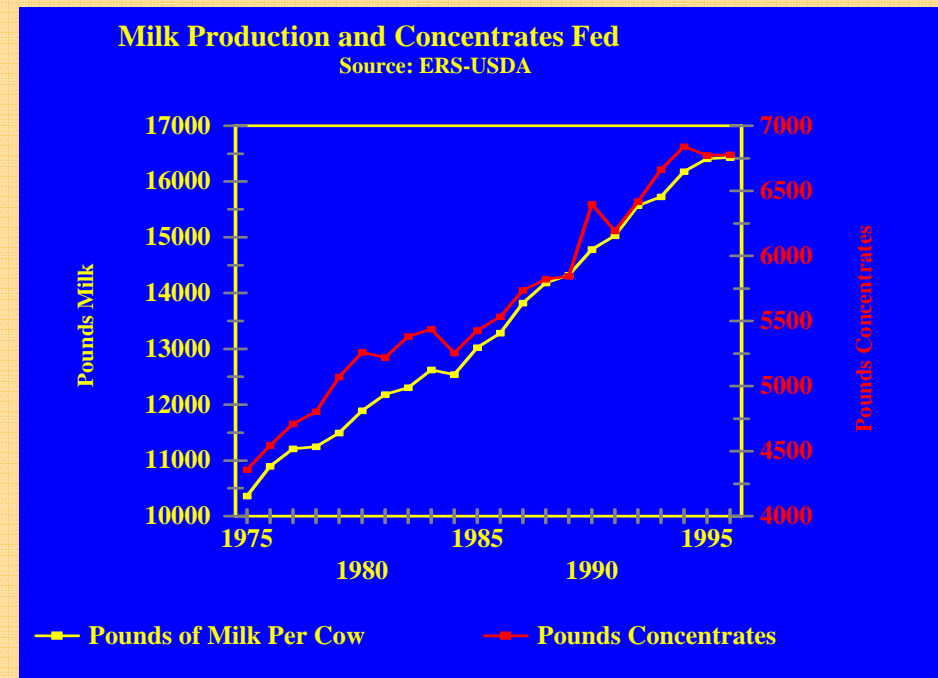


Key Issues with alfalfa quality/value

- It must be measured
- Breaking the yield/quality tradeoff
- Problems with rapid lignification of alfalfa stems under hot conditions
- Enhancing/complementing other feeds
- Solving Waste Problems

Less alfalfa being fed in dairy rations

- Lower yield of alfalfa than other crops
- Increased use of corn silage
- Minimized forage in ration
 - Cheap grain
 - Greater quality consistency of grain
 - Inability to accurately estimate energy of forage



Dairy Nutritionist Survey

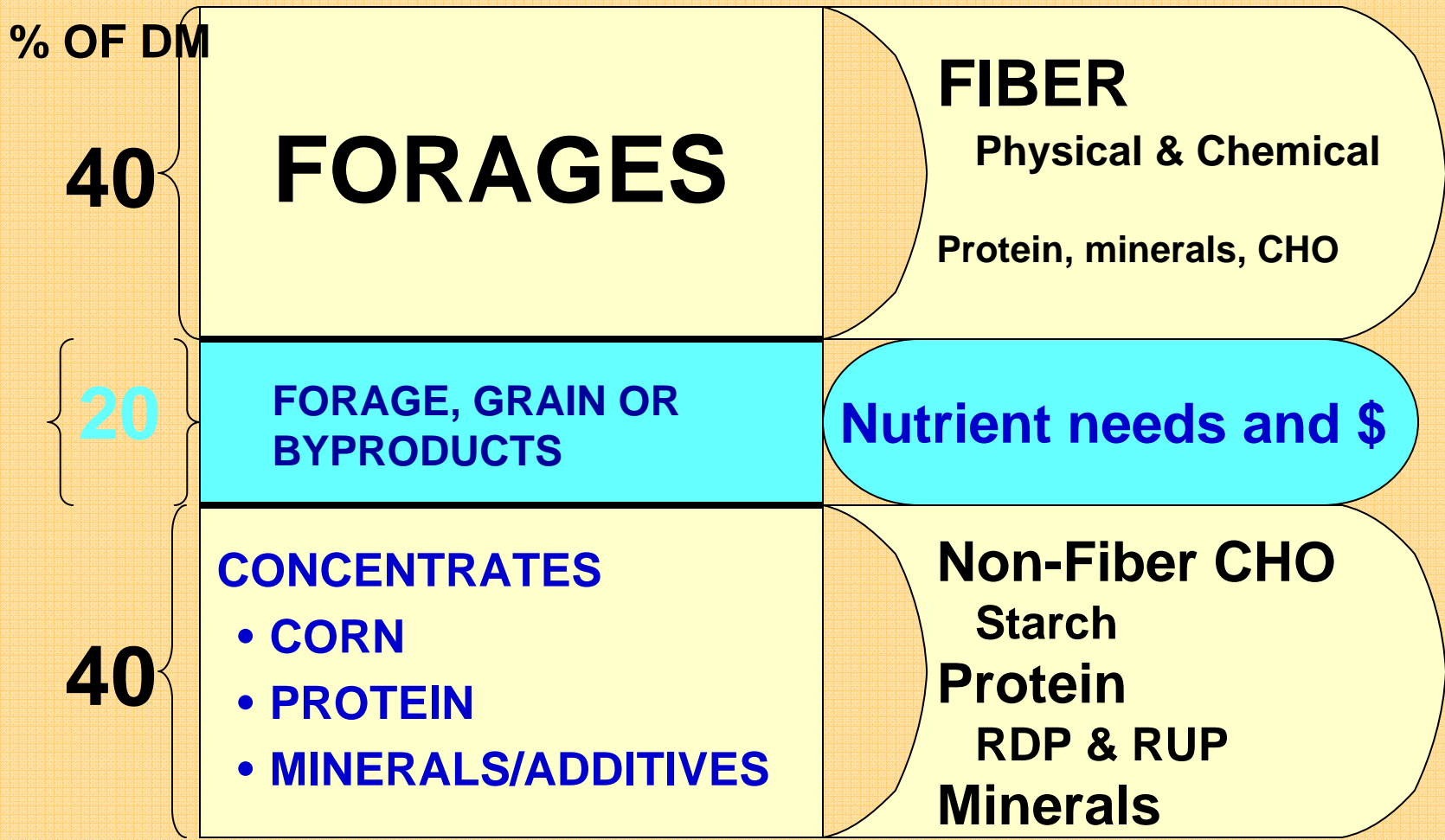
MAJOR CHALLENGES

1. Forage quality - consistency
2. N and P excretion
3. Transition cows
4. Ethanol – starch and Distillers Grains
5. Ration formulation – modeling
6. Fiber digestion
7. Milk price and feed cost

WATER AVAILABILITY



Dairy Ration Overview



We don't want to see reduced perennial forage crops in rotation because . . .

- Perennial forage crops are good for environment
- Good for cow health



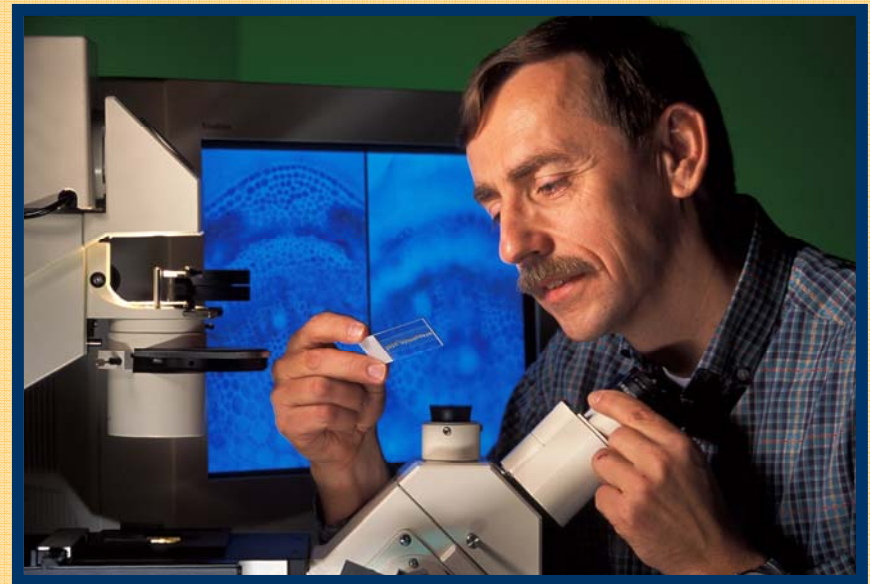
Challenges . . .

. . . of the dairy forage industry



Research strategies and opportunities . . .

. . . of the U.S. Dairy Forage
Research Center





Barriers to increasing alfalfa in dairy diets

Redesigning alfalfa for dairy cows

- **Improve protein utilization**
 - **Increase fiber digestion**
 - **Increase yield**

Forage Quality...

Description	CP	EE	Ash	Starch	Pectin	aNDF	ADF	ADL
ALFALFA HAY								
Exceptional	25.4	2.7	10.4	3.1	14.2	30.0	24.0	4.53
Very high	24.0	2.6	9.9	2.9	13.2	34.1	27.0	5.38
High quality	22.5	2.5	9.5	2.7	12.3	38.2	30.0	6.23
Good quality	21.0	2.4	9.1	2.5	11.4	42.2	33.0	7.08
Fair quality	19.5	2.2	8.7	2.3	10.5	46.3	36.0	7.93
CORN SILAGE								
V. high grain	8.3	3.2	4.1	31.1	1.7	36.0	21.0	1.57
High grain	8.6	3.1	4.6	27.2	1.6	40.5	24.0	1.91
Normal	8.8	3.0	5.1	23.2	1.5	45.0	27.0	2.25
Low grain	9.0	2.8	5.7	19.2	1.4	49.5	30.0	2.59
V. low grain	9.3	2.7	6.2	15.3	1.3	54.0	33.0	2.93



Source: Mertens, 2003.



Apparent Dry Matter Digestibility of AH and CS

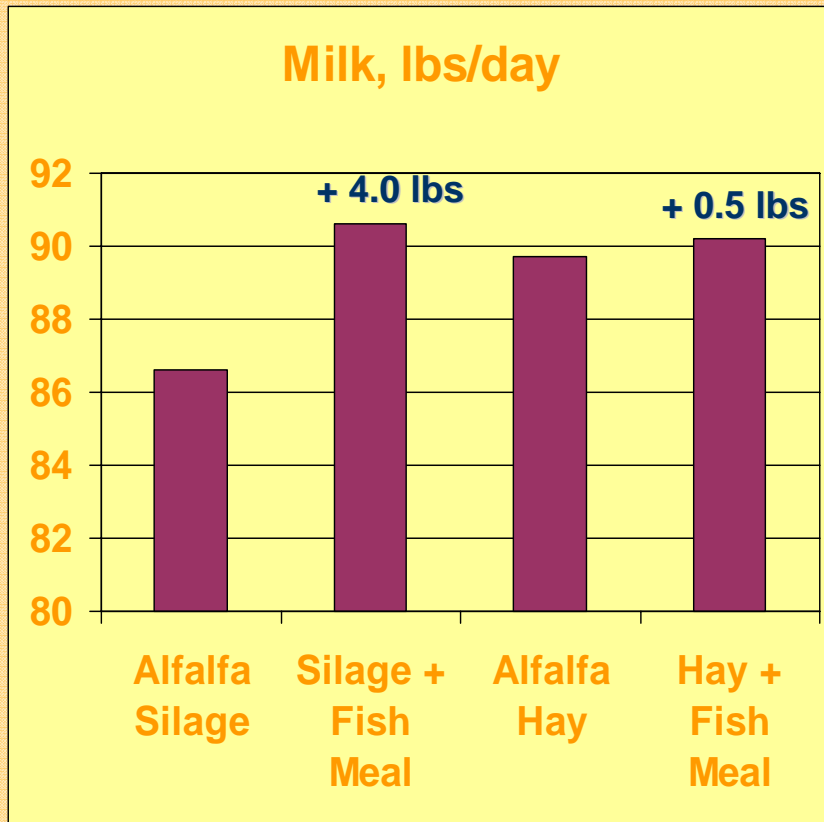
Item	AH 24%ADF	AH 27%ADF	CS proc 24%ADF	CS proc 27%ADF
% aNDF	30.0	34.1	40.5	45.0
% dNDF	15.6	16.0	24.9	27.3
% NDS	70.0	65.9	59.5	55.0
% dNDS	68.6	64.6	58.3	53.9
% True DMD	84.2	80.6	83.2	81.2

Source: Adapted from Mertens, 2003.

Alfalfa for Dairy Rations

- **Currently using harvesting management to improve alfalfa quality**
 - **Immature alfalfa has many appealing nutritional properties**
 - **Low in fiber**
 - High digestibility
 - High intake potential
 - **Rapid rate of digestion**
 - **High in crude protein**

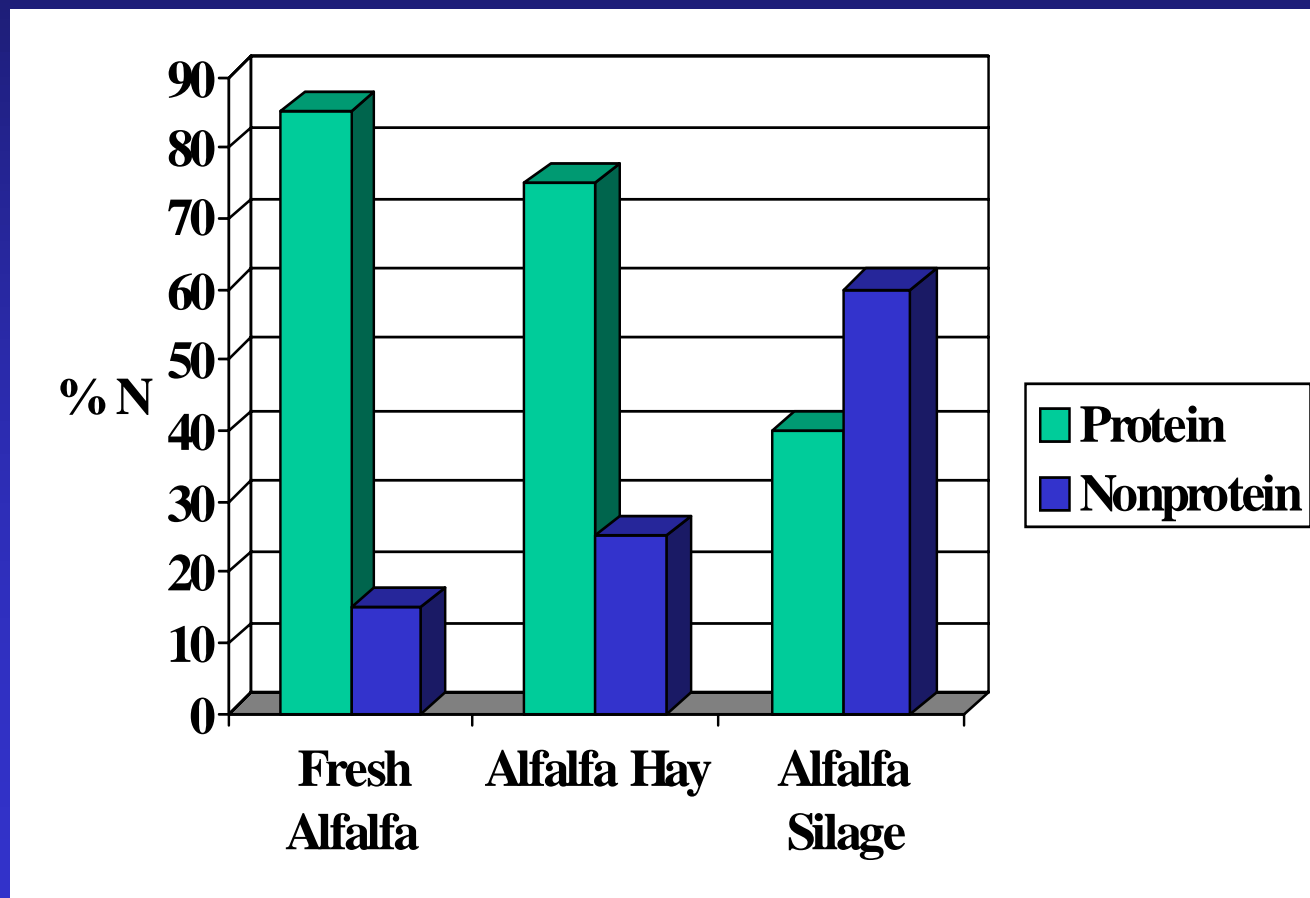
Milk Yield from Alfalfa Silage and Hay Diets

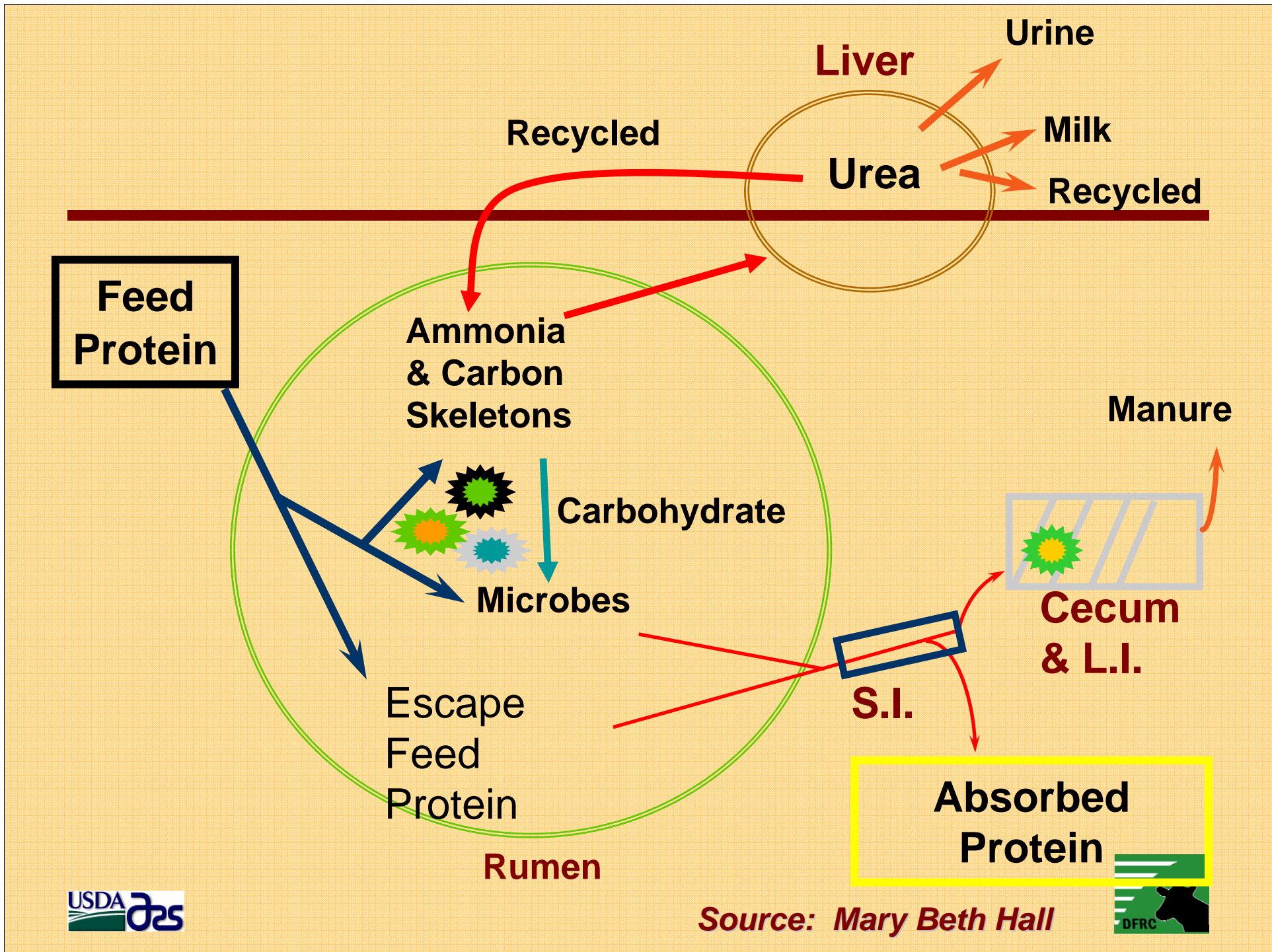


- Fish meal is beneficial in alfalfa silage diets, but not alfalfa hay diets.
- Bottom line: alfalfa silage nitrogen is not efficiently used by the cow

Feed Storage Problems

- However in alfalfa, our primary forage:





Research Challenge/ Opportunity . . .

- **Protein utilization:**
 - high-quality forage reduces N use efficiency . . .
 - leading to higher manurial N loading back to fields . . .
 - creating an increased risk of N leaving farm via runoff, leaching, or ammonia emissions.

Protein utilization: PPO

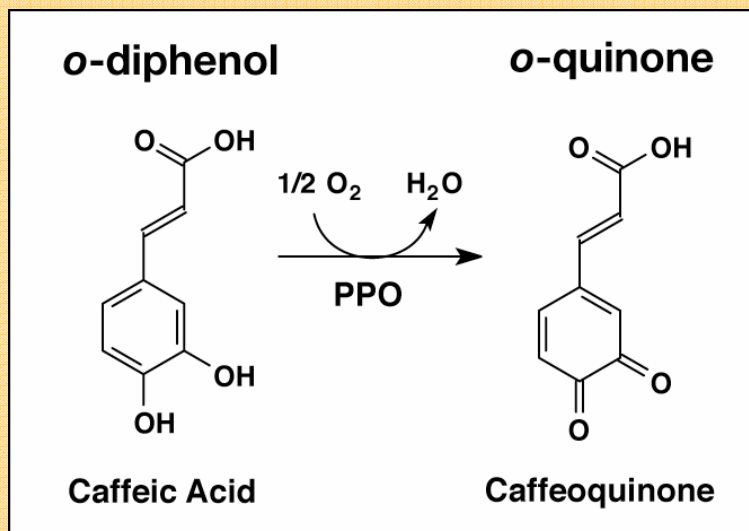
Polyphenol oxidase (PPO) and *o*-diphenols

**--A process for preserving protein
in ensiled forages**



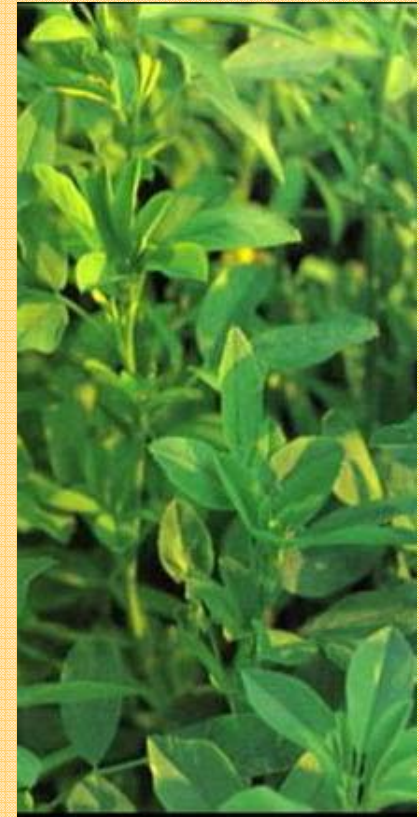
Polyphenol oxidase (PPO) and *o*-diphenols in red clover

- PPO oxidizes *o*-diphenols to *o*-quinones
- Responsible for post harvest browning
- PPO and *o*-diphenols are abundant in red clover

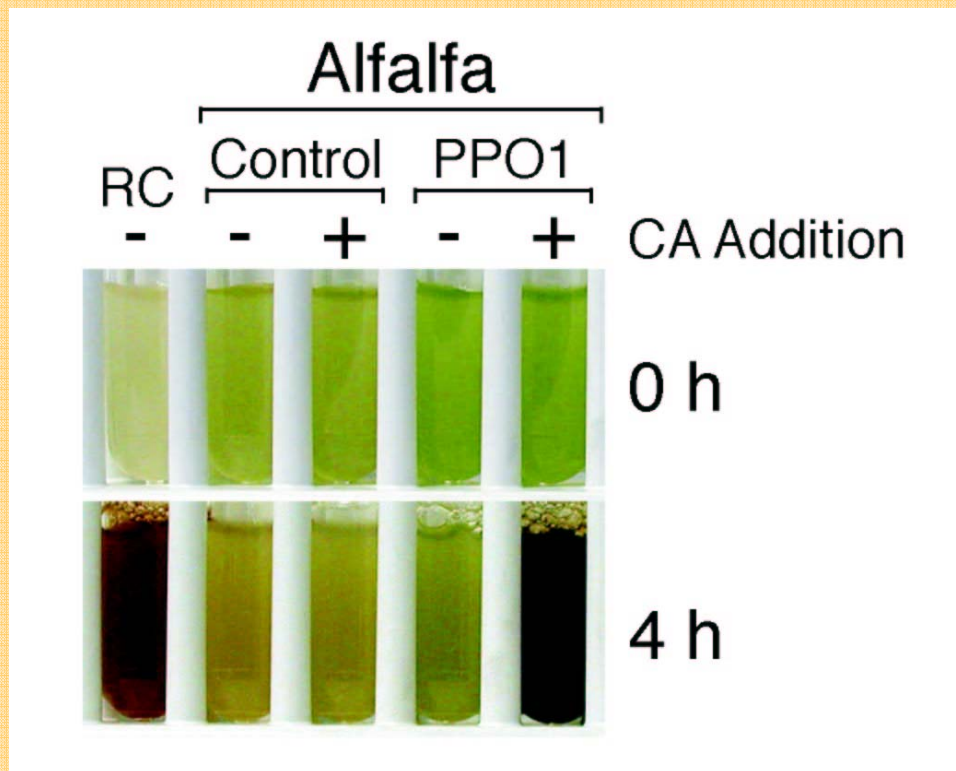


PPO and *o*-diphenols prevent post-harvest proteolysis

- Evidence for PPO/*o*-diphenol role
 - Alfalfa lacks PPO/*o*-diphenols
 - Proteolytic inhibition O₂-dependent
 - » Inhibition involves a heat labile factor
- Experimental demonstration
 - Loss-of-function in red clover
 - Gain-of-function in alfalfa



Expression of red clover PPO1 in transgenic alfalfa



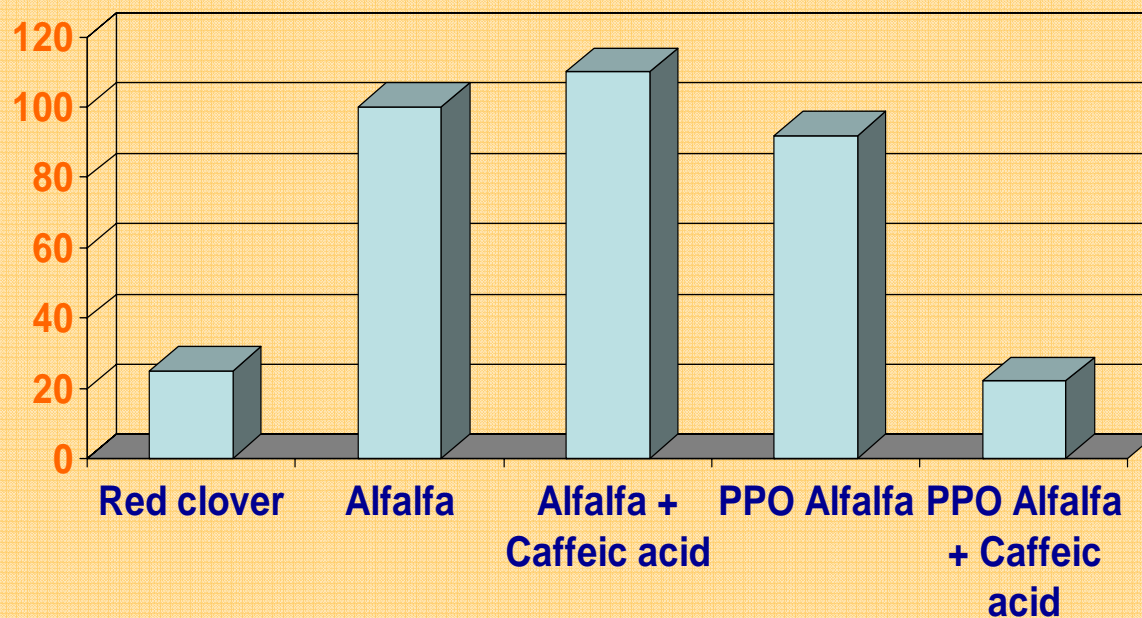
In alfalfa, browning is dependent on:

- A PPO transgene
- Exogenous o-diphenol, e.g. caffeic acid

SOURCE: Sullivan, Michael L. and Ron D. Hatfield. 2003 DFRC Research Report

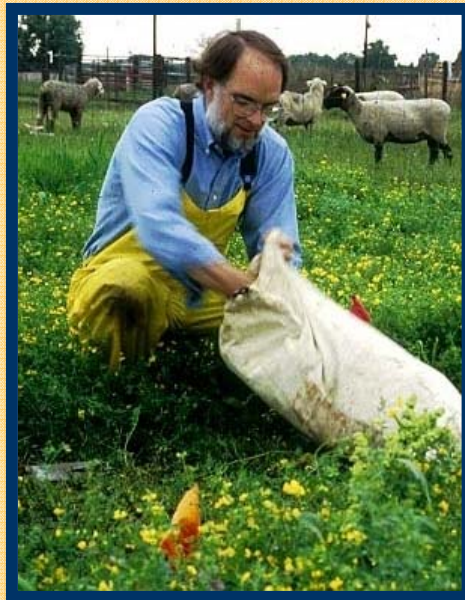
Red Clover vs. Alfalfa Silage

Protein breakdown (% of alfalfa)

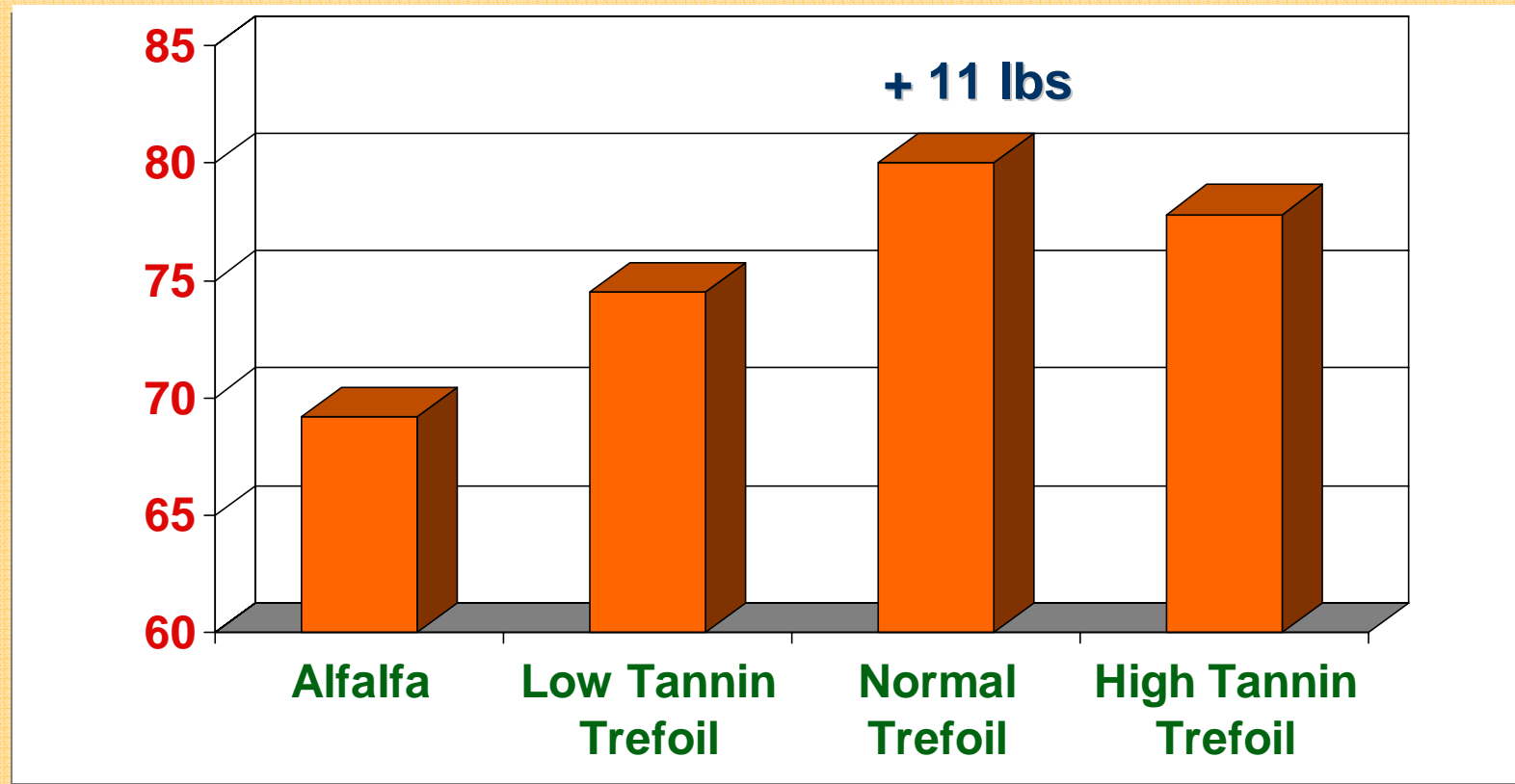


Protein Utilization: Tannins

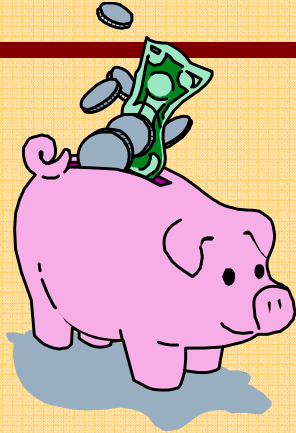
- Tannins have been shown to improve protein utilization and animal performance.



Milk Yield (lbs/day)-Alfalfa and Birdsfoot Trefoil Silages



Added value of forage with tannin (per ton dry matter)



Alfalfa silage **\$ 23**

Alfalfa hay **\$ 11**

Strategies: reducing post-harvest proteolysis in alfalfa silage

- **Some compounds bind with alfalfa protein to decrease rate of post-harvest proteolysis. Transgenic alfalfa will be produced that contain these compounds.**
 - **Tannins – altered expression of genes for alfalfa tannin biosynthesis**
 - **Polyphenol oxidase (PPO) – gene isolated from red clover (USDA)**

Research Challenge/ Opportunity . . .

. . . fiber digestion

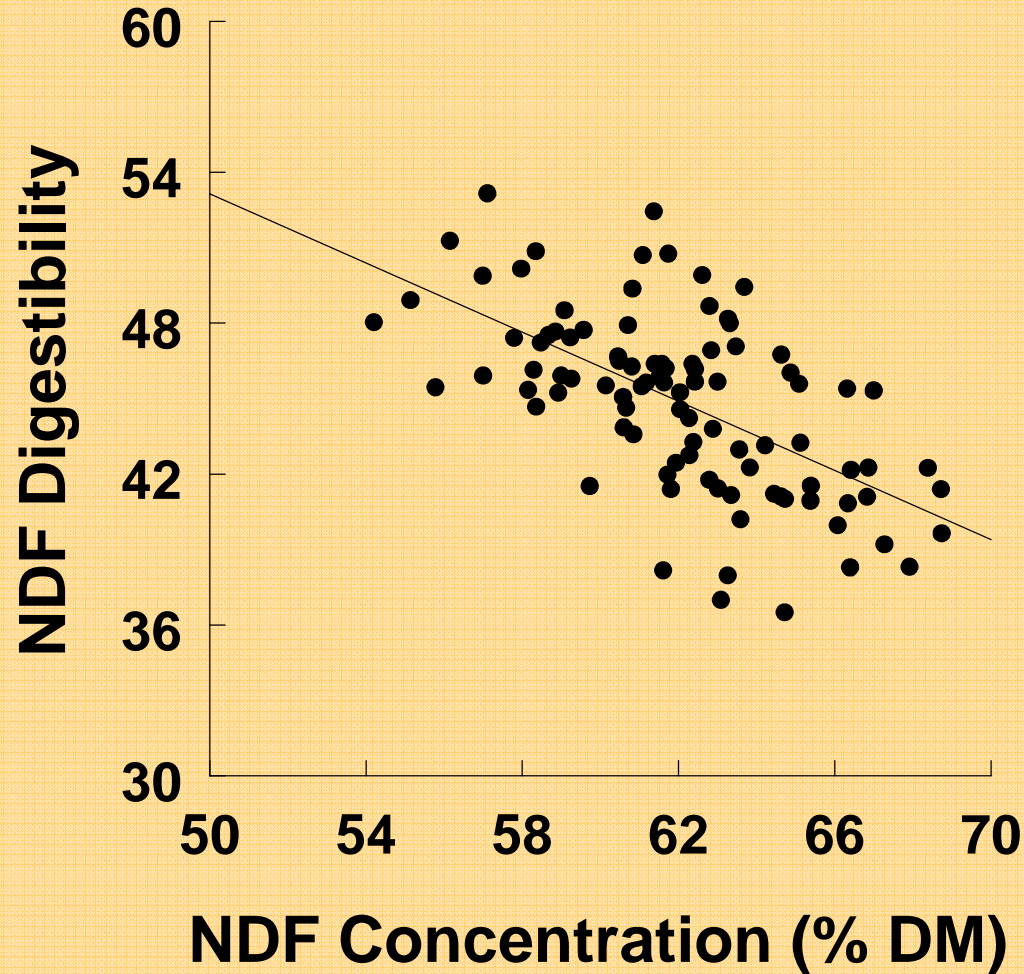


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% True DMD	84.2	80.6	83.2	81.2

Source: Adapted from Mertens, 2003.

NDF Digestibility of Alfalfa Stems



Source: Jung and Lamb, 2002. Unpub USDA-ARS. St. Paul, MN



Down regulation

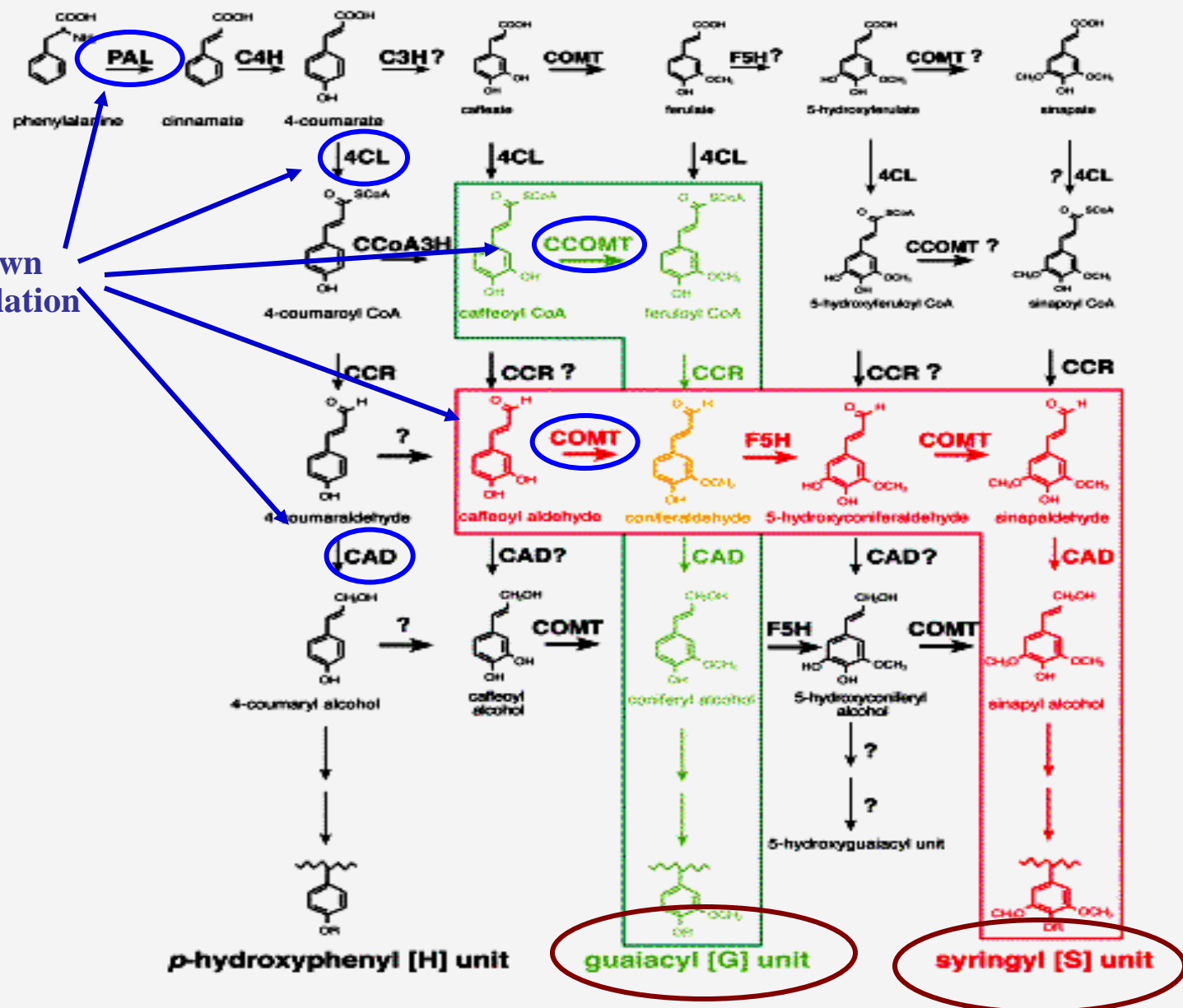
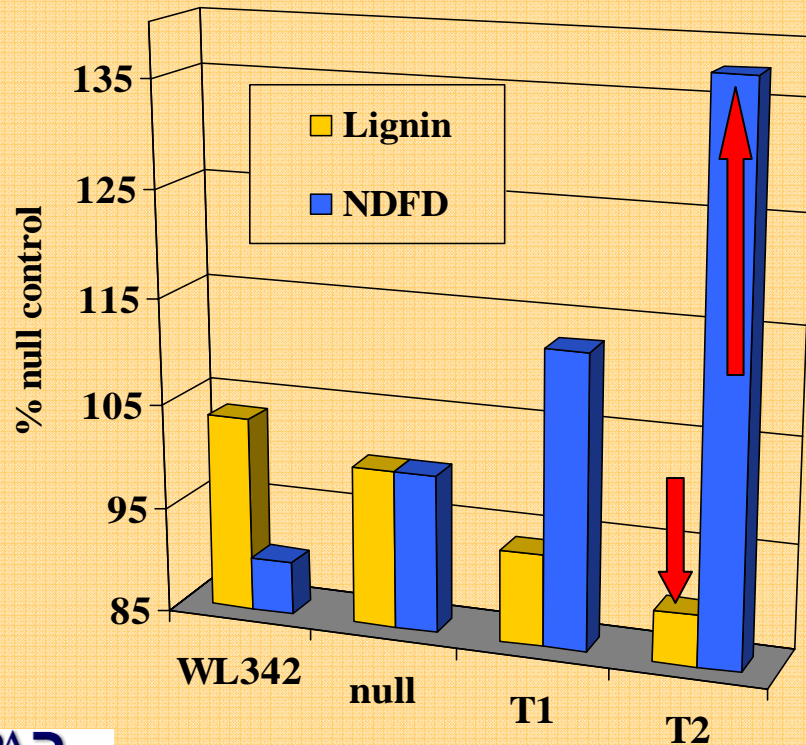


Figure 2. Biosynthesis of monolignols in alfalfa

Low Lignin Alfalfa... Higher Fiber Digestibility

Fiber digestibility of alfalfa stems in transgenic lines at Nampa, ID.



Transgenic plants have been generated that show decreased lignin content and increased fiber digestibility.



Source: McCaslin et. al., 2002



Alfalfa Improvement Opportunities

- **Modify fiber composition**
 - Replace with soluble CHO (pectin, etc.)
- **Improve fiber digestibility**
 - Lower lignin
 - Modify lignin
 - Replace lignin with cellulose
 - Reduce physical limitations
 - Increase rate of digestion

Redesign Alfalfa for Dairy Cattle

Consortium for Alfalfa Improvement

- Noble Foundation
- Forage Genetics International
- Plant Science Research Unit,
USDA-ARS
- US Dairy Forage Research Center,
USDA-ARS



Consortium
for
Alfalfa Improvement



Before we switch gears . . .



**Any
questions ?**

Value-Added Products From Alfalfa

- Potential of biofuel from alfalfa
- Potential products from dry fractionation
- Value-added processing of alfalfa
- Transgenic high phytase alfalfa

Potential of biofuel from alfalfa

Can alfalfa be a
major biomass
crop?



Alfalfa Traits That Will Impact Bioenergy Production

Hans-Joachim G. Jung
USDA-ARS, Plant Science Research Unit,
St. Paul, MN

2006 North American Alfalfa Improvement Conference, St. Paul, MN



Bioenergy Goals and Requirements

- USDOE and USDA have set a national goal of replacing 30% of petroleum consumption with biomass-derived energy by 2030.
- This goal would require more than 1 billion tons of biomass.
- Projected that 77% of total biomass could come from agricultural lands (377 million tons from perennial biomass crops).

Alfalfa Would Be a Good Biomass Crop

- Perennial (less erosion)
- Nitrogen fixation (lower fertilizer requirement and benefits next crop)
- Established infrastructure (seed, management, harvest equipment)
- Valuable co-product (leaf meal as supplemental protein feed for livestock)

Alfalfa saves energy

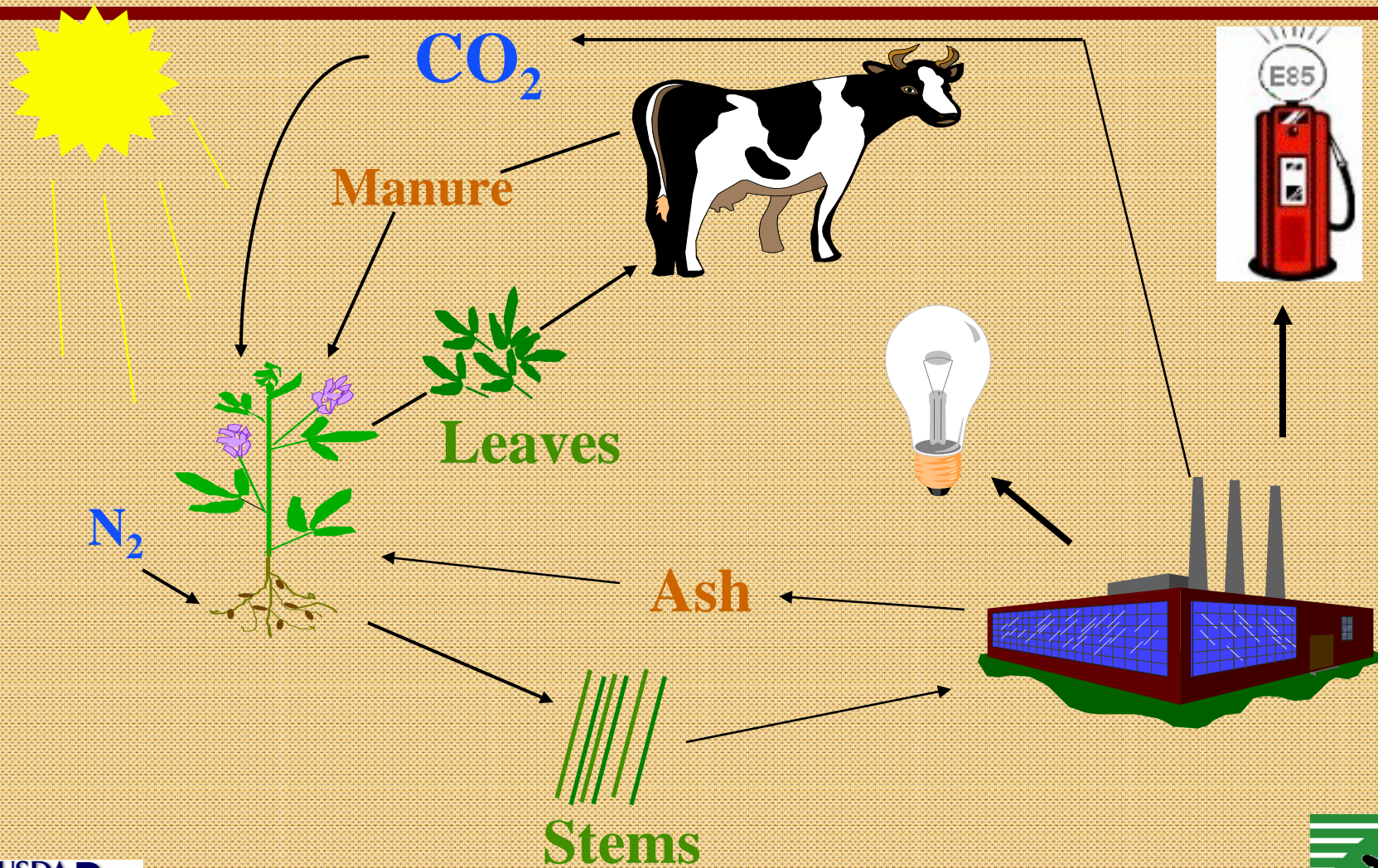
- Lower energy input costs than corn
- Much greater net energy yield than corn or soybean

Crop (yield)	Energy Input	Energy Output	Ratio Out : In
	----- MMBTU/a -----		
Corn (180 bu/a)	6.0	59.0	8.8
Soybean (40 bu/a)	2.3	18.3	7.1
Alfalfa (6 t/a)	3.0	78.2	25.0

Major Issues for Biomass Crops

- Energy from coal and oil is still relatively inexpensive.
- Projected prices to be paid for biomass are low (\$30 to 50 per ton).
- Starch is less expensive to convert to ethanol than are biomass cell wall polysaccharides.
- Biomass crops must produce high yields, at low cost, of constituents that can be economically converted to energy.

An Alfalfa Biomass System



Energy from Biomass

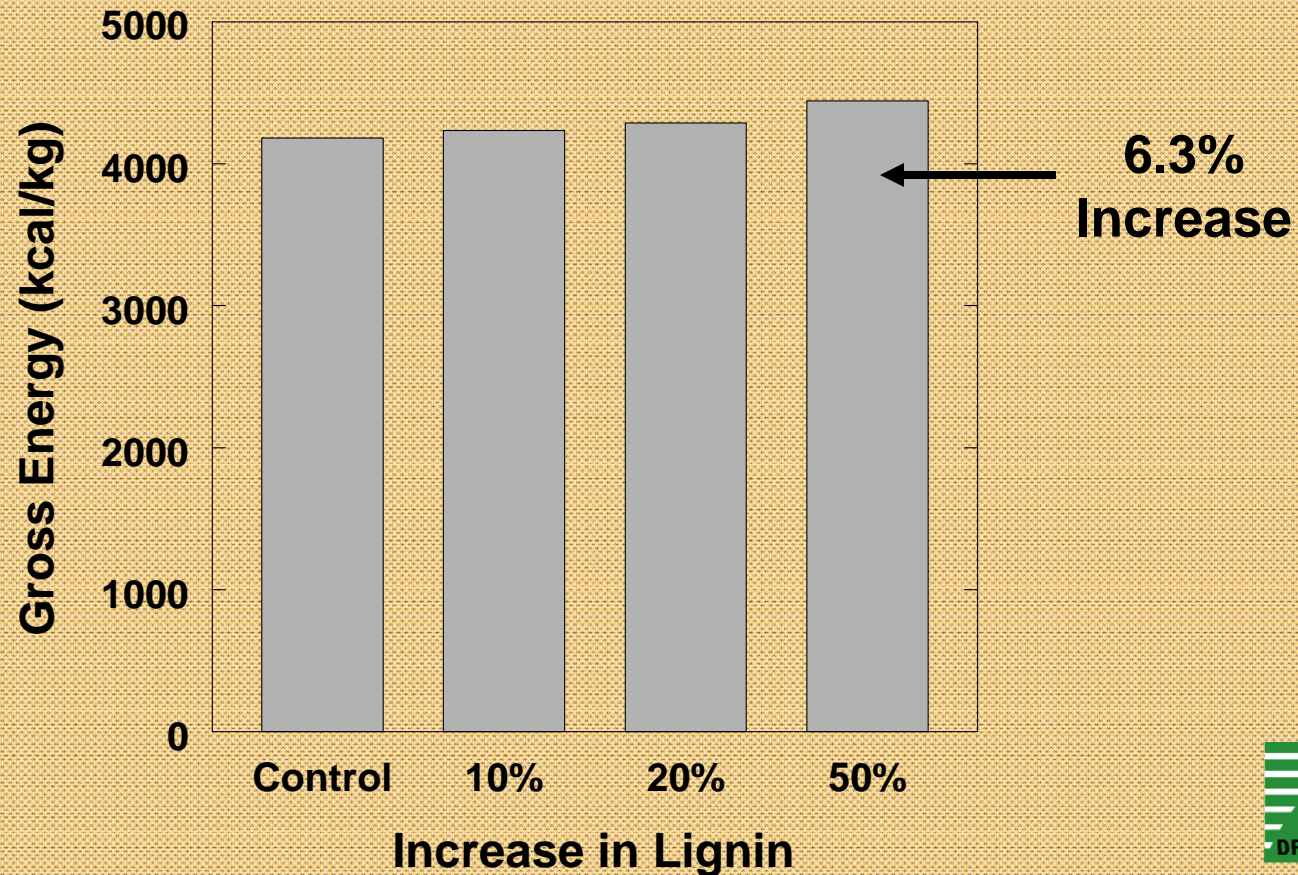
- **Combustion of biomass to produce electricity or industrial processing heat (bioenergy).**
- **Conversion of carbohydrates and lipids in biomass to liquid transportation fuels (biofuel).**

Bioenergy

- Gross energy of coal is ~17,000 kcal/kg.
- Gross energy of all herbaceous biomass is 4300 to 4500 kcal/kg.
- Organic constituents differ in gross energy.
 - carbohydrates 4000 kcal/kg
 - protein 5700 kcal/kg
 - lignin 7000 kcal/kg
 - lipid 9000 kcal/kg

Impact of Changing Alfalfa Composition

(At full flower stage of maturity stems - 88 protein, 7 lipid, 175 lignin, 598 carbohydrate in g/kg DM)



Suggested Improvement Criteria for Bioenergy

- Yield

- Yield

- and More Yield ! ! !

The Process: Lignocellulosic Biomass to Fermentable Sugars



Grind

Pretreatment to Remove Inhibitors

Enzymatic Breakdown of Polysaccharides

Electricity & Processing Heat

Residual Solids

Fermentation

Product Recovery

Sugars

Sugars

Biofuel

- Ethanol is produced by yeast fermenting carbohydrates (glucose).
- Corn grain contains 70+% starch (glucose) plus 10% cell wall polysaccharides (mixture of sugars).
- Biomass typically contains little starch or other non-cell wall carbohydrates.
- Biomass cell wall polysaccharides are more difficult to convert to free sugars than starch.

Advancing Renewable Energy

Advancing Renewable Energy was designed to address one of the greatest challenges facing the world: increasing the availability of clean and affordable energy to reduce global dependence on fossil fuels.

October 10–12, 2006
America's Center
St. Louis, Missouri



ABOUT the Conference

DOE and USDA are hosting this first-time event to further President Bush's Advanced Energy Initiative (AEI)—an aggressive plan to reduce U.S. dependence on foreign oil and ensure a secure energy supply.

The goal of the conference is to help build the necessary partnerships and strategies for hastening the commercialization of domestic, commercially viable renewable energy industries and distribution systems.

Featured speakers at the event will include **DOE Secretary Samuel Bodman** and **USDA Secretary Mike Johanns**.

[Click Here to Register NOW](#) 



TOPICS

- Building Supply and Distribution Chains
- Encouraging Demand
- Developing Infrastructure
- Creating Effective Market Models and Partnerships

Conference Registration Fee
The fee to attend the conference is \$495. See the Registration page for details and regulations.

[About the AEI](#) [Visit DOE](#) [Visit USDA](#)



Advancing Renewable Energy

- In 2008, U.S. will produce 7 bil gal of ethanol
- 16 % of U.S. corn yield will produce 5 bil gal ethanol
- Using corn hulls to produce ethanol increases ethanol production by 15 %
- Current biodiesel production is 200 mil gal
- Demand for biodiesel in 2010 – 7.4 bil gal
- Small ethanol plants dispersed widely across rural America is a safer system relative to terrorism and accidents that have major impacts than concentrated infrastructure, like large oil refineries or nuclear plants
- We need to scale up cellulosic ethanol production soon

Recent Changes

	1996	2000	2005	2006
	Billions of gallons			
Ethanol	1.0	1.6	3.9	5.0
Gasoline	121	130	140	141
Ethanol Share	0.8%	1.2%	2.8%	3.5%
Ethanol	'96 - 06	4.0	'00-06	3.4
Gasoline	'96 - 06	20.0	'00-06	11.0
Share Met By Ethanol:		20%		31%

SOURCE: Keith Collins, Chief Economist, USDA. 2006

MINNESOTA BIOENERGY AND BIOPRODUCT YIELDS (per acre)

<u>Crop</u>	<u>Ethanol</u> gallons	<u>Biodiesel</u> gallons	<u>Electricity</u> kWh	<u>Feed</u> Tons
Corn	410	0	0	1.3
Soybeans	0	60	0	1.0
Alfalfa	130	0	358	1.6

How Should Alfalfa be Produced in a Biomass System?

- Total biomass yield can be increased by less dense seeding.
- Harvesting at more advanced maturity can increase yield.
- Non-lodging germplasm have greater biomass yield.
- Higher yield, less frequent harvesting, and lower seeding density would reduce production costs.

Alfalfa Breeding: St. Paul, MN



- Developed a biomass-type alfalfa to increase stem yield without loss of leaf production.



Grass Seed Straw Utilization Outlook in the Pacific Northwest

*Forage Seed & Cereal Research Unit
National Forage Seed Production
Center*

Corvallis, OR

January 21, 2005

ARS research shows farmers ...



Straw-to-Energy Technology Plan

- Convert biomass on-farm to synthesis gas
- Farm-scale conversion technology
- Production scale 1-2 tons per hour
- Low capital investment: "for the cost of combine"
- Use synthesis gas to produce electricity or liquid fuel
- Local utility company support: green energy

Pilot-scale reactor (400 pounds per hour):

Western Research Institute, Laramie, WY

National Forage Seed Production Research Center

Eastern Regional Research Center



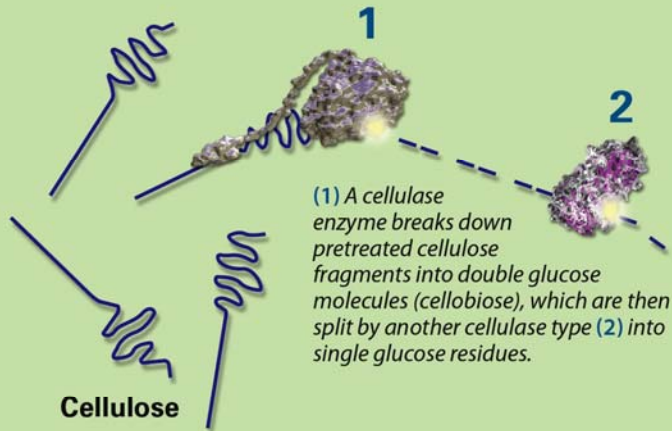
Forage Seed & Cereal Research Unit, Corvallis, OR



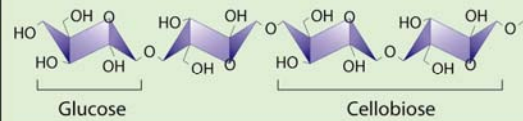
Applying Genomics for New Energy Resources

Hydrolysis

Goal: Break down cellulose into its component sugars using enzyme preparations



Cellulose molecule

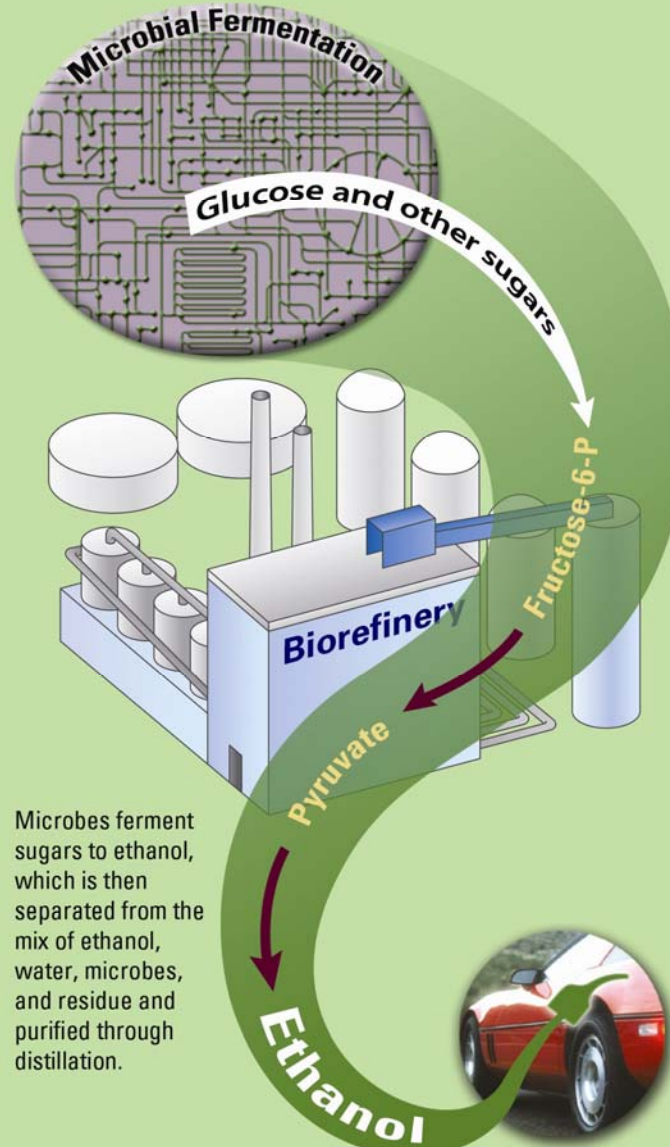


Cellulose is made up of double glucose molecules (cellobiose).

Enzymes such as cellulases synthesized by fungi and bacteria work together to degrade cellulose and other structural polysaccharides in biomass. Optimizing these complex systems will require a more detailed understanding of their regulation and activity.

Fermentation to Ethanol

Goal: Convert sugars to ethanol using microbes



Microbes ferment sugars to ethanol, which is then separated from the mix of ethanol, water, microbes, and residue and purified through distillation.

Source:
Genome Management Information System, Oak Ridge National Laboratory

Key to the Future: Cellulosics

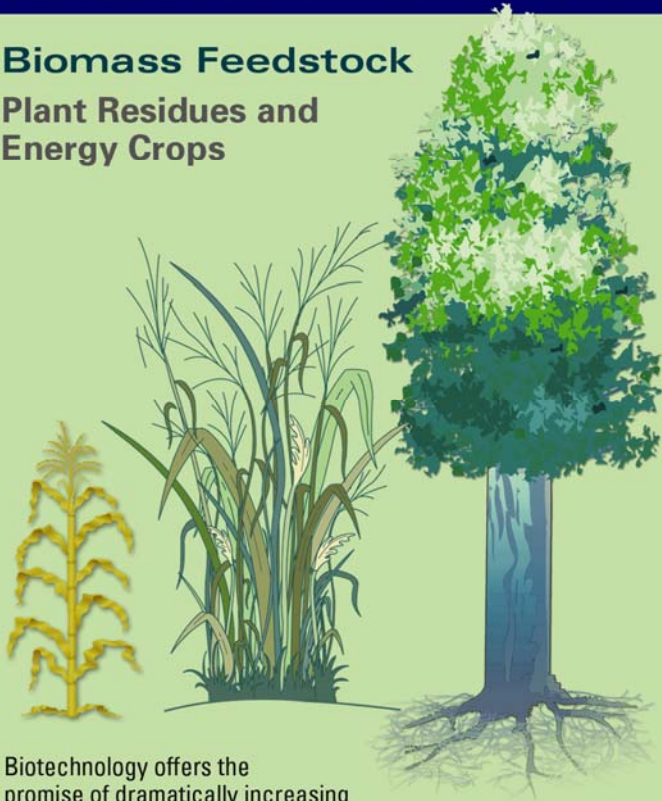
	Corn	Cellulosics
Capital Costs	\$1.25-\$1.50/gal	\$4.30-\$5.50/gal
Ethanol Yield	98 gal/ton	70-80 gal/ton
Conversion Process	Simple	Complex
Enzyme Cost	\$0.03/gal	\$0.30-\$0.50/gal
Alcohol Content	14-20%	4%
Transport Costs	Low	High

SOURCE: Keith Collins, Chief Economist, USDA. 2006

From BIOMASS to CELLULOSIC ETHANOL

Biomass Feedstock

Plant Residues and Energy Crops



Biotechnology offers the promise of dramatically increasing ethanol production using cellulose, the most abundant biological material on earth, and other polysaccharides (hemicellulose). Residue including postharvest corn plants (stover) and timber residues could be used, as well as such specialized high-biomass "energy" crops as domesticated poplar trees and switchgrass.

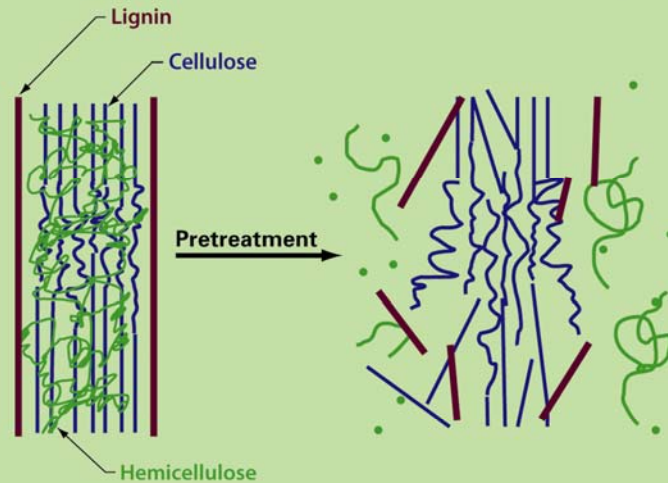
Biochemical conversion of cellulosic biomass to ethanol for transportation fuel currently involves three basic steps:

- ▶ **Pretreatments to increase the accessibility of cellulose to enzymes and solubilize hemicellulose sugars**
- ▶ **Hydrolysis with special enzyme preparations to break down cellulose to sugars**
- ▶ **Fermentation to ethanol**

Making cellulosic biomass conversion to ethanol more economical and practical will require a science base for molecular redesign of numerous enzymes, biochemical pathways, and full cellular systems.

Pretreatment

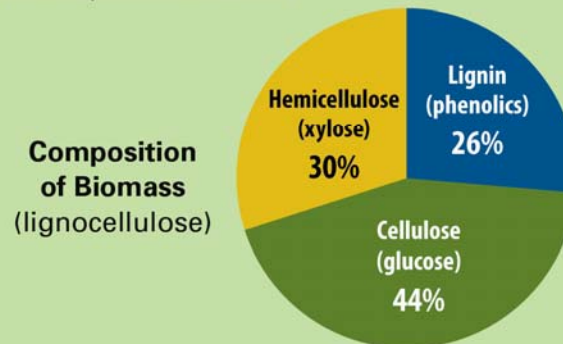
Goal: Make cellulose more accessible to enzymatic breakdown (hydrolysis) and solubilize hemicellulose sugars



Cellulose exists within a matrix of other polymers, primarily hemicellulose and lignin. Pretreatment of biomass with heat, enzymes, or acids removes these polymers from the cellulose core before hydrolysis.

Pretreatment, one of the most expensive processing steps, has great potential for improvement through R&D.

[Figure adapted from N. Mosier et al. 2005. "Features of Promising Technologies for Pretreatment of Lignocellulosic Biomass," *Bioresource Technology* 96(6), 673–86. Reprinted with permission from Elsevier.]



Source:
Genome Management Information System, Oak Ridge National Laboratory

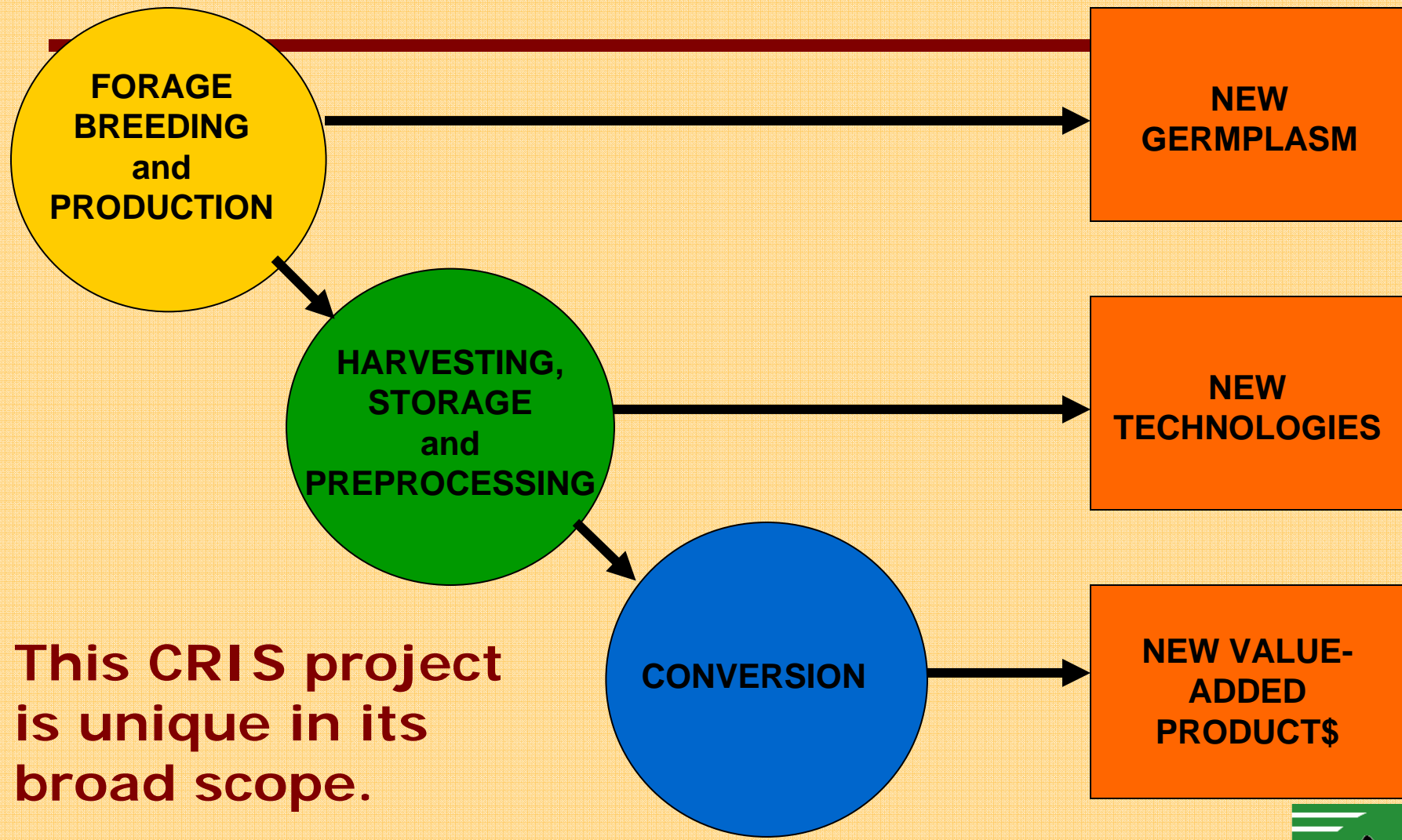
Where Should Alfalfa for Biomass be Grown?

- It has been suggested that switchgrass be grown on CRP acres.
- Corn/soybean row cropping would benefit from a more complex rotation.
- A biomass crop for row-cropped land must compete with the economic returns from corn and soybeans.
- Energy plus two co-products uniquely position alfalfa as a biomass crop for row-crop land.

Suggestions to Enhance Alfalfa for Biofuel Use

- Further develop biomass production systems that minimize costs and increase yield of stems and (at a minimum) maintain leaf yield.
- Develop alfalfa germplasm with greater yield, less lignin, and more carbohydrates under biomass production systems.
- Create new co-product opportunities using natural alfalfa constituents and biotech engineering.

USDFRC Biomass Research



This CRIS project is unique in its broad scope.



Single-Pass, Split-Stream Harvesting of Corn Grain and Stover

Grain



***All stover: Stalk, cob,
husk & leaf***

K.J. Shinnars

Addressing Challenges to Biomass Production and Processing

Objective: On-farm pretreatment of biomass materials:

- To produce a product that is more susceptible to enzymatic or chemical hydrolysis
- Add on-farm value to product



Conclusions

- **Great Economic Opportunity**
- **Attack the Challenges**
- **Pursue Public-Private Partnerships**
- **Look Beyond Our Borders**

SOURCE: Keith Collins, Chief Economist, USDA. 2006



Forage Fractionation . . .

Three methods of forage fractionation exist:

- 1. Wet fractionation:**
separation into juice and a fiber fractions
- 2. Dry fractionation:**
separation into leaves and stems
- 3. Animal fractionation:**
passage of whole plant through digestive systems of ruminant animals, leaving a high fiber residue.

Forage Fractionation . . .

Two important conditions must be met for alfalfa fractionation to be feasible and sustainable:

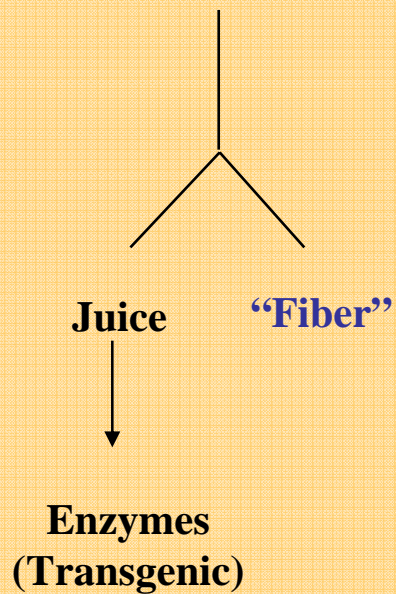
- **Total value of resulting products must be greater than the original forage plus the cost of processing;**
- **All fractions must have economic value to avoid creating a waste stream.**

Forage Fractionation . . .

FRACTIONATION METHODS

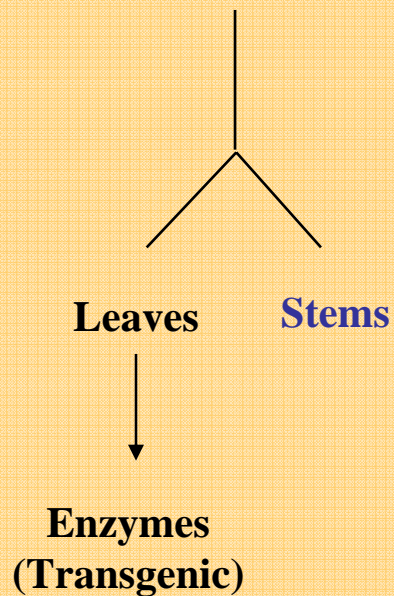
Wet

Herbage



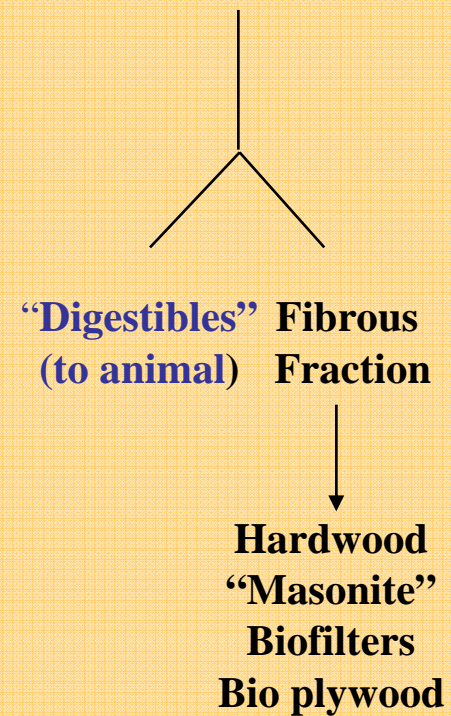
Dry

Herbage

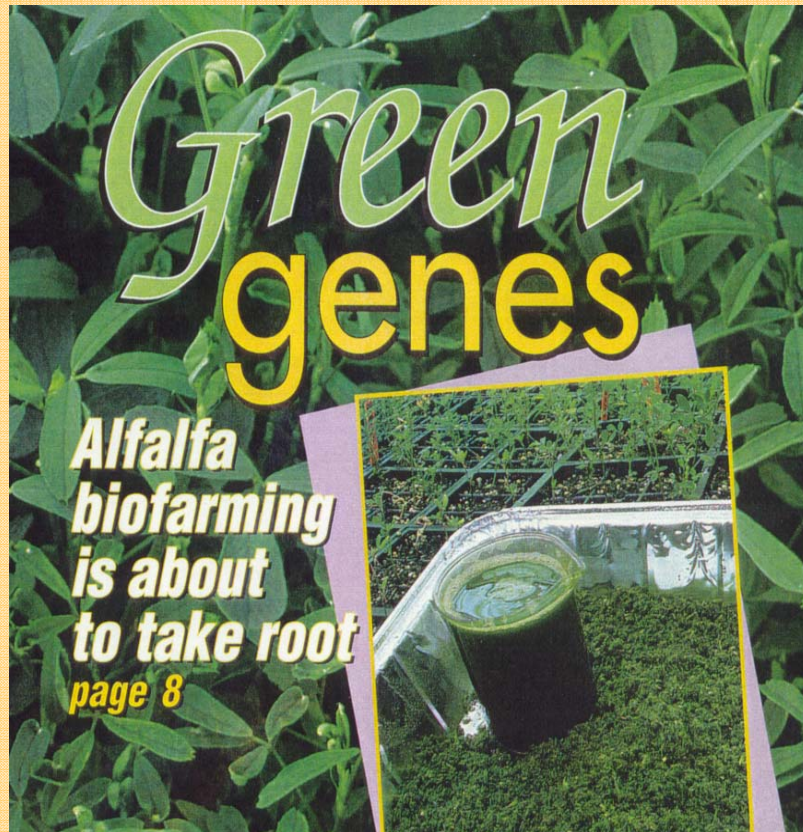


Animal

Herbage



Development of Green Genes

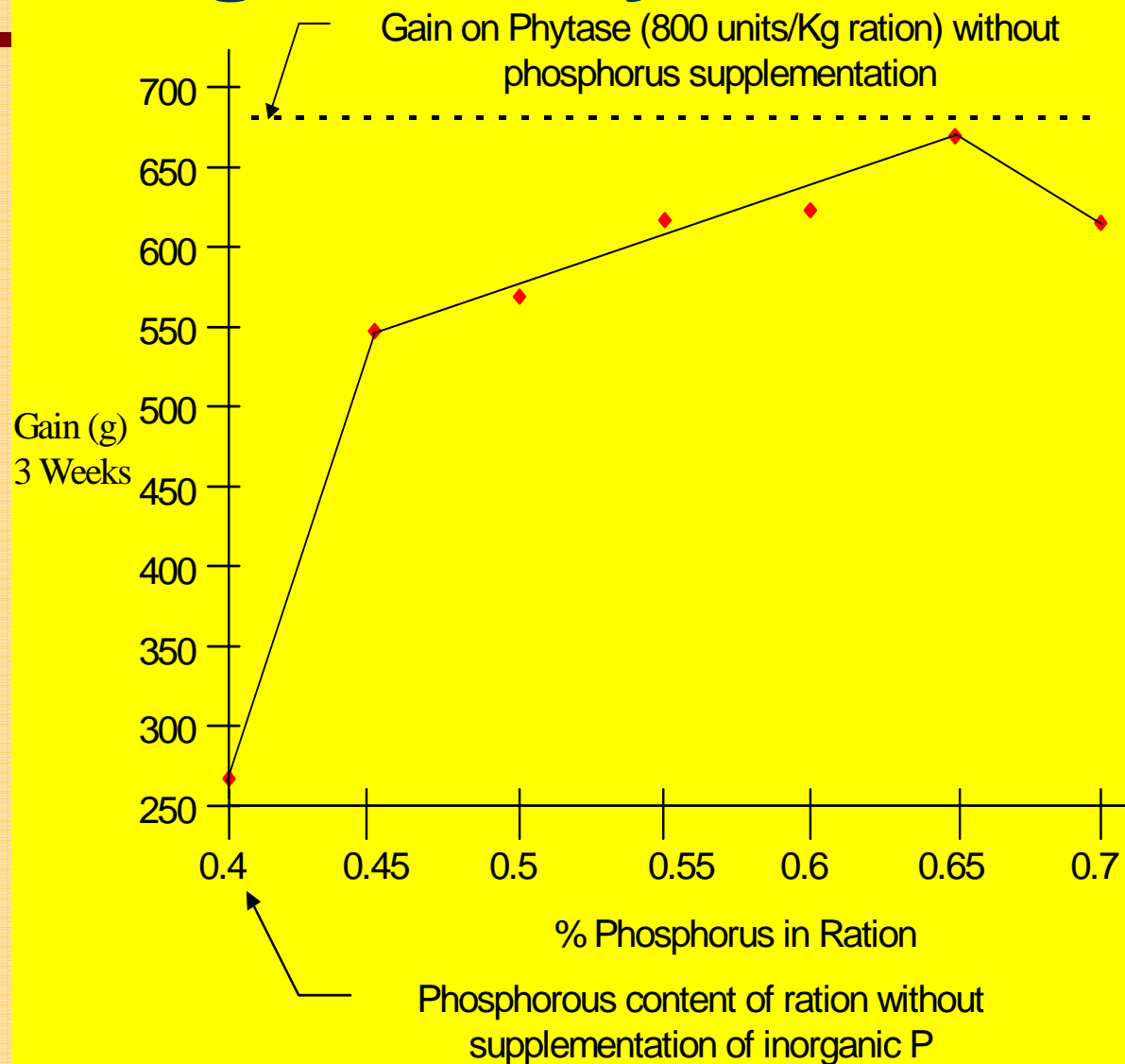


- **Fractionation of alfalfa**
 - **dry**
 - Electricity
 - Ethanol
 - Bioadhesives
 - **wet - phytase**
 - cellulase
 - biopluping
 - biobleaching
 - bioremediation

Transgenic Phytase-rich Alfalfa

- Phytase enzyme makes P in grain ration of monogastric diets more available (poultry, swine, and fish)
- Phytase enzyme levels of 1 - 2 % of soluble protein possible
- Phytase extraction with wet fractionation gives added value of xanthophyll & high protein
- Phytase is stable - alfalfa leaf meal

Weight Gain of Chicks Fed Transgenic Phytase Alfalfa



Alfalfa - Produced Phytase in Poultry Rations:

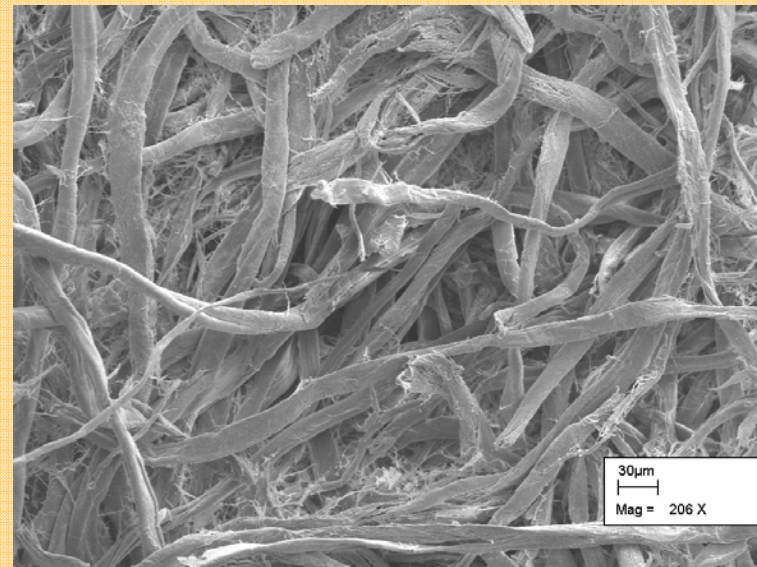
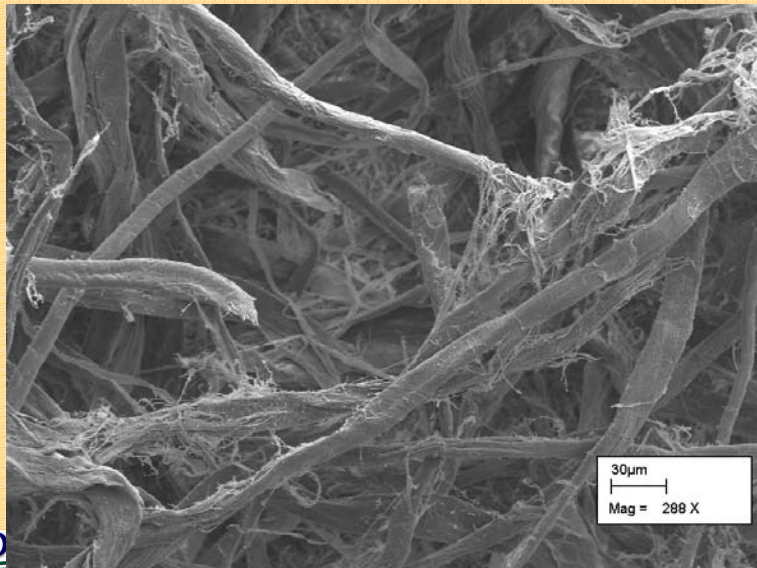
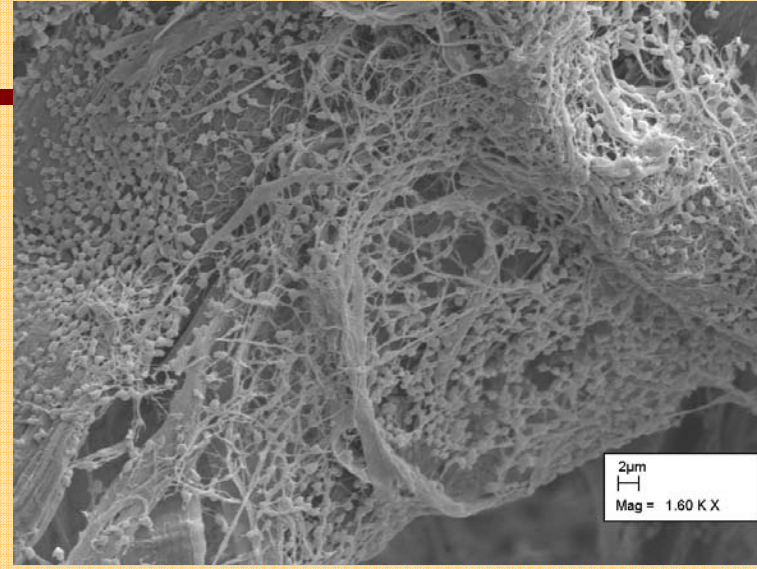
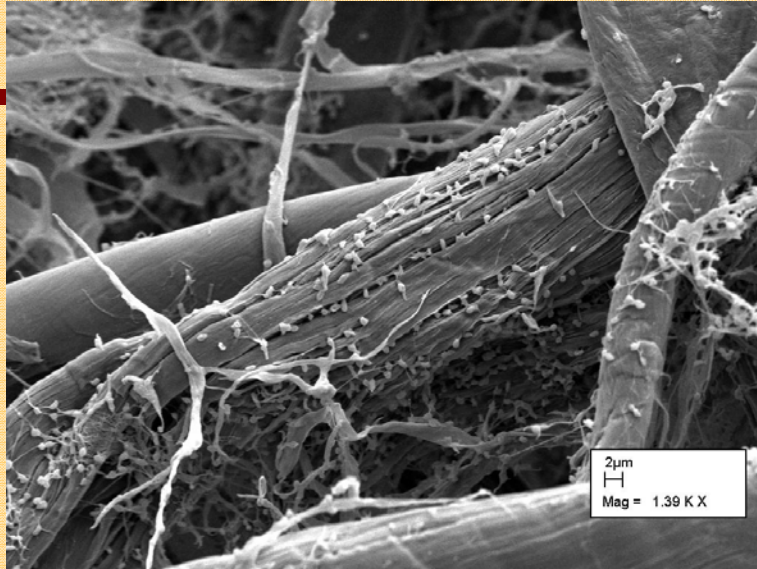
- ❖ Eliminates need for phosphorus supplementation
- ❖ Reduces the phosphorus content of feces to less than half



Direct Microbial Conversion

- **Use of microorganisms that produce their own fibrolytic enzymes and ferment the resulting hydrolytic products to useful end products**
- **Equivalent to “consolidated bioprocessing” (CBP, Lynd et al.)**

Ruminococcus albus Glycocalyx



Adhesive Testing

(A.H. Conner, C. Frihart, L. Lorenz, USDA-FS-FPL)

- Wet or freeze-dried/rehydrated residue (\pm PF resin)
- 3-ply aspen panels 7" x 7" x 1/4" each
- Press conditions: 1.12 MPa, 180 °C, 5 min
- Equilibrate at 27 °C, 26% RH for 7d
- Cut into twelve 82 x 15mm sections
- Test for shear strength and wood failure (ASTM D-5266-99) under dry and wet (VPS) conditions (6 samples each)



The Perfect Alfalfa Plant

- Yield of individual cuttings high enough to reduce number of cuts per year (2 or 3)
- Maturation that is not strongly tied to quality
- Minimal leaf loss during growth and harvest
- Total protein available to the animal, 16-18 %, of that 30-35 % ruminal undegradable
- Cell wall digestibility ~ 80 % (20-30 % rapidly fermented pectin)
- Protein loss during ensiling no greater than 10-15 %

Turning challenges into research directions

- Increased fiber digestion
- Improved protein utilization
- Understand interactions of feeding ingredients in mixed rations and forage utilization
- Management of N transformations from manure
- Reduced nutrient loads
- Reduced energy inputs or increased outputs
- More on-farm tests – improved accuracy – related to animal digestion