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You Can't Judge a Bunker Silo by Its Cover
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For more than 30 years, recommendations for managing bunker silos at filling have included:

- Filling as quickly as possible;
- Filling in thin layers (6 inches);
- Packing with a heavy tractor which concentrates the load on a small footprint;
- Packing each layer for an extended period of time;
- Covering as soon after filling as possible;
- Covering with plastic which is weighted to maintain close contact with the silage and arranged to shed water.

Throughout this period, producers have sought less time-consuming and less difficult alternatives to these recommendations. The process of covering with plastic and adding weights (boards, tires, soil, etc.) requires much hand labor for covering and uncovering and can be a wet and dirty job. For these reasons, stories abound how producers have used alternative covers with "pretty good" success. Some of these alternatives include: candy, lime, molasses, small grain sod, manure solids, small grain straw, corn fodder, soil, sawdust, no cover, etc.

Visual observations of the silo top frequently reveals a 2- to 8-inch layer of spoiled (black) feed. Producers consider this thin layer as a small loss which can be sacrificed so as to avoid the labor of covering the bunker silo with plastic. This visible loss is only a portion of the loss which is occurring in the silo. When the blackened material is removed prior to feeding, it is referred to as visible waste.

Some covers allow precipitation to percolate into and through the silage. That which percolates through the silage is called effluent. This effluent carries soluble organics, including organic acids, from the silage. These removed organics represent a dry matter loss. The reduction in organic acids causes the pH to rise. The higher pH makes the silage more easily decomposed by microbial activity. Percolating water can also transport oxygen into the silage. The oxygen supports microbes which cause silage deterioration. Microbial deterioration is called gaseous dry matter loss. The effluent and gaseous dry matter losses are not visible to producers, so they are not aware of the magnitude of the loss.

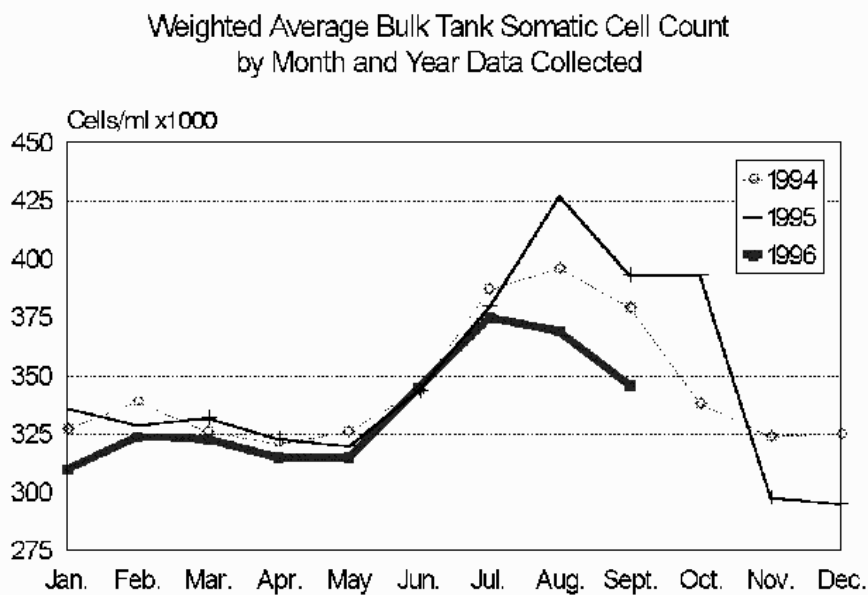
Minson and Lancaster [1965] conducted research in New Zealand in 1963 and 1964 to determine the impact of silo cover on dry matter loss. They harvested grass at about

Emerging Mastitis Pathogens: Are these “bugs” in your future?
Pamela Ruegg, DVM, MPVM
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Introduction

Since the early 1970s control of mastitis on dairy farms has been based upon reduction of contagious mastitis pathogens through the use of the “5 point plan.” Worldwide, by adopting the 5 basic principles of mastitis control: post milking teat disinfection, universal dry cow antibiotic therapy, appropriate treatment of clinical cases, culling chronically infected cows and regular milking machine maintenance, farmers have achieved tremendous success in reducing the incidence of mastitis in dairy cows. The greatest impact of adopting the 5-pt. plan has been on infections caused by contagious bacteria such as *Staphylococcus aureus* and *Streptococcus agalactia*. It has been estimated that these agents are now responsible for less than one third of all mastitis cases compared with >75% of all cases 20 years ago.¹ Herds that successfully adopt these control strategies generally experience a reduction in subclinical mastitis that is evidenced by a decrease in bulk tank and individual cow somatic cell counts. The continued decrease in average US bulk somatic cell count (BTSCC) levels indicates that this trend is continuing (Figure 1). In recognition of this trend, there have been recent recommendations to consider decreasing the national upper limit for BTSCC from 750,000/ml to 400,000/ml.²

Figure 1: US Average Bulk Tank SCC



Source: 8 U.S. Milk Market Orders, USDA-AMS

The success of the 5-pt. plan against clinical and subclinical mastitis caused by *Staph aureus* and *Strep agalactia* has not been demonstrated for clinical mastitis caused by other agents. In a study of 9 Ohio herds that had controlled contagious pathogens (as evidenced by low BTSCC) the incidence rate of clinical mastitis varied from 15.6% to 63.7% of cows.³ This study reported a mean cost per clinical case of \$107

Future Employment of Cows in Wisconsin

Robert Bremel, Ph.D.

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Summary of the findings

Our efforts have resulted in a highly efficient means of producing transgenic cattle. A replication defective retroviral vector is used to transfer genes, as RNA, into an oocyte before fertilization. Reverse transcription of the retroviral RNA integrates a single DNA copy of the gene into the maternal genome as a finite event. No replication of the vector can occur. When the oocyte is fertilized, the novel gene is present in all cells of the resulting embryo as though it had been contributed by the maternal germline.

Significance

Efforts to produce transgenic livestock have been underway for over a decade. The primary motivation is the desire to express proteins in milk as a cost effective means to produce high value biopharmaceuticals. Several companies have this as a primary business goal. Previously, the method of choice for gene introduction to create transgenics has been pronuclear microinjection of embryos. Since the birth of Dolly in 1997, cloning has been explored as an alternative method of transgenic production. This paper describes an entirely novel approach focussing on the female gamete. This technology produces approximately a 100-fold increase in efficiency of transgenic production.

Transgenic efficiency

Enhancing the efficiency of production of transgenic animals has become the "holy grail" of the biopharmaceutical industry.

The "first generation" approach to production of transgenic livestock uses pronuclear microinjection, in which many copies of DNA are injected into the male pronucleus of the fertilized embryo. Efficiency in production of transgenic domestic livestock using this approach had not surpassed 1%. Cloning seeks to overcome this problem of low efficiency by replicating a rare founder animal, or by introducing genes to a stem cell line used to create many identical copies of the same founder. To date, the efficiency of cloning does not greatly surpass that of pronuclear microinjection.

In contrast, the "second generation" method described by Gala Design founder Bremel's group results in nearly 100% efficiency and in germline transmission.

When efficiency is low, the cost of maintaining recipient herds of surrogate mothers for injected embryos is a major factor in the cost of production of transgenic livestock.

Simply introducing a new transgene does not guarantee production of an animal of optimal characteristics. An animal carrying a gene may not always express the gene product at an ideal level. When transgenic founders are produced as one-in-a-hundred events, there is little opportunity to select animals with ideal production characteristics to be founders of new

herds. When transgenic founders can be produced in large numbers, the possibility of selecting the ideal lines for further breeding are much improved.

Evolutionary implications

Some 10% of the normal genome is made up of transposable elements, or retrotransposons. Evolutionary patterns in the distribution of retrotransposons have been known many years. Dr. Barbara McLintock described "jumping genes", later defined as retrotransposons. A recent Nature paper (Agrawal et al, Nature 294: 744-751, 1998) described movement of a transposable element as the key evolutionary event in the emergence of the immune system. Until now it has not been possible to reproduce the effect of retrotransposons movement in a way that resulted in stable germline transmission. The replication defective retroviral elements used as gene delivery vectors in the PNAS publication are synthetic equivalents of retrotransposons. We hypothesize that the mechanism described to introduce a gene could also mimic the naturally occurring mechanism. Retrotransposons relocating during development of the oocyte could result in quantum genetic changes, could be incorporated in the germline, and could offer selective advantage.

Other applications

While Gala Design's primary motivation for production of transgenic livestock has been to increase efficiency of production of biopharmaceuticals, this technique also offers potential for adding genetic traits to agricultural livestock.

The method described in the PNAS publication would be applicable in other species where the oocyte is accessible during the key metaphase developmental stage. This includes primates. Gala Design has no intention of using the technology except in livestock for biopharmaceutical production, xenotransplantation, and for improving the productivity of livestock. We strongly believe that ethical guidance is necessary to determine whether there are socially acceptable applications of the technology in humans.

Comparisons to other methods

Relative to pronuclear microinjection

Following pronuclear microinjection, tandem arrays of gene copies are integrated as a result of DNA repair processes. Such multiple insertion arrays often prove unstable upon replication of the DNA.

In contrast, reverse transcription of RNA into a proviral DNA results in a single gene insertion, and is far less prone to excision during subsequent cell replication.

Relative to Cloning

Cloning, or the replication of an animal from a single somatic cell, has been used to try to overcome the obstacle posed by the inefficiency of gene transfer. Cells from rare founder animals can be used to generate new "vegetatively reproduced" individuals. Alternatively, new genes can be introduced into stem cell lines established in vitro. These transgenic stem cells are then used to produce cloned animals. However, cloning is still plagued by low efficiencies, failures of placentation and, by definition, a narrowing of the underlying gene pool.

Gala Design's methods can conserve valuable genetic diversity, while efficiently incorporating new, desirable genes.

Mosaicism

Genes inserted by pronuclear microinjection are sometimes not uniformly incorporated, resulting in a condition known as mosaicism. Mosaic animals possess the transgene in some, but not all, cells. Some mosaic animals do not transmit the transgene because only somatic cells have the new DNA. Mosaicism can be difficult to identify quickly in livestock species. By contrast, Gala's technology for the introduction of single genes into the oocyte prior to fertilization means that all embryonic cells will include the transgene.

The Animals

Five transgenic founder cattle have resulted from the work described in this publication. Now two years old, these animals are all healthy and normal in all respects. The two bulls (Gremlin and Samson) are housed at an artificial insemination center. The two single-born heifers (Cressy and Buttons) have been artificially induced to lactate and are in Gala Design's research milking herd. They have recently been shown to produce the marker protein -- hepatitis B virus surface antigen protein -- in their milk in significant quantities. Calf #4, Delilah, the female twin who may have acquired her transgenes by sharing a placental blood supply with her co-twin, Samson, is also in the herd but does not express marker protein in her milk. Gremlin has sired healthy twin offspring - Nip and Tuck - who are also transgenic.

Authors of "Transgenic cattle produced by reverse transcribed gene transfer". Proceedings of the National Academy of Sciences 95: 14028-14033

Anthony Chan, a citizen of Hong Kong, completed his Ph.D. in Bob Bremel's lab at the University of Wisconsin. Upon graduation, he worked for a time with Gala Design but has since returned to academic research, at the Oregon Primate Research Lab.

Robert Bremel has been a faculty member of the Department of Dairy Science at the University of Wisconsin-Madison for 25 years. Over the last ten years his research group worked on development of transgenic technologies applicable to livestock. In 1996, along with several colleagues, Dr. Bremel founded Gala Design.

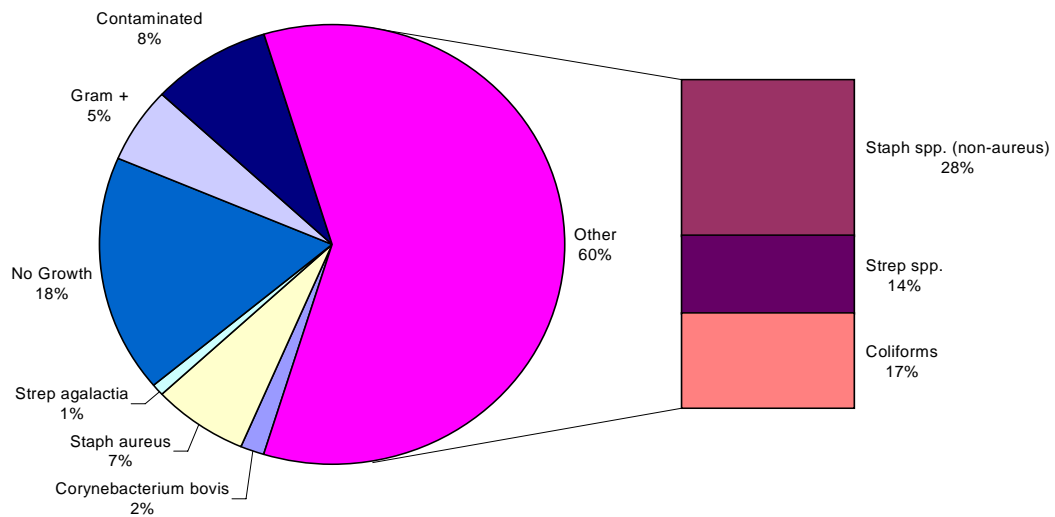
Jane Homan is a veterinary virologist, formerly at the University of Wisconsin, a co-founder and Operations Manager of Gala Design.

Linda Ballou was a member of the laboratory team at the University of Wisconsin and is also a co-founder of Gala Design, where she is laboratory manager.

Jane Burns is a Professor at the University of San Diego Medical School and a founder of Pangenix. Her work led to the development of the pseudotyped vectors.

(equivalent to \$142 current dollars assuming a 3% rate of inflation). A recent study looked at 65 Ontario dairy herds with above average milk production, low BTSCC and an average herd size of 52 cows (range in herd size of 24 to 216).⁴ In this study, clinical mastitis occurred in almost 20% of cows. The microorganisms isolated in this study (Figure 2) indicate that other pathogens have emerged to fill the niche vacated by *Staph aureus* and *Strep agalactia*.

Figure 2: Frequency of Bacteriological Isolation from Clinical Mastitis



Organisms such as coagulase negative staphylococci (CNS), environmental streptococci, *Mycoplasma* spp, and *Serratia* spp, will be increasingly implicated in mastitis in Wisconsin dairy herds. The objective of this paper is to discuss diagnosis, treatment and control of these emerging mastitis pathogens.

Coagulase negative Staphylococcus (CNS)

Details. Coagulase-negative Staph (CNS) refer to staphylococcus bacteria that are not *Staph aureus*. The nomenclature “coagulase-negative” refers to a laboratory test that differentiates this species of bacteria from the “coagulase-positive” *Staph aureus*. CNS have been isolated from 7-30% of quarters in various herd surveys. They are one of the most frequent organisms isolated from milk samples in herds that have controlled major pathogens.⁵ CNS are a part of the normal skin flora and can colonize the teat canal. Anything that decreases the patency of the teat sphincter can allow infections to occur. Both clinical and subclinical mastitis can be caused by infections with CNS. Cows in their first lactation have been consistently shown to have a higher rate of infection with CNS as compared to older animals. This is usually attributed to the effectiveness of dry cow therapy in controlling these

organisms. Infections with CNS are highest immediately after calving, decline in mid-lactation and increase again until the cow receives dry cow therapy.

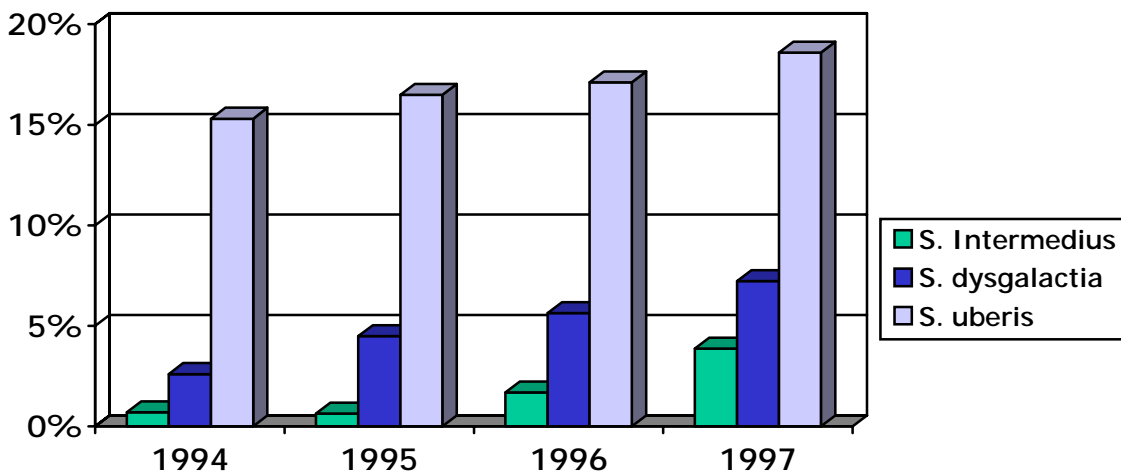
Effect on SCC and Clinical Mastitis. Somatic cell counts of quarters infected with CNS are generally 2-3 times higher than uninfected quarters, but the magnitude of SCC response is usually less than the magnitude experienced with other pathogens. While the SCC of infected cows can vary, a typical SCC for a cow infected with this pathogen would range between 250,000 – 400,000. While it is not a frequent cause of clinical mastitis, surveys in herds that have controlled major pathogens generally attribute 3-10% of clinical cases to CNS.

Treatment and Control. Treatment of cows that experience subclinical infections with CNS during lactation is not recommended. The relationship of CNS infections to milk production losses is unclear and spontaneous cure rates of up to 73% have been reported. Post-milking teat dip is the most effective method of controlling this pathogen. The benefit of pre-dipping to control this organism is unclear.⁶ When teat dips are not used (i.e. during the dry period, or during very cold weather) infections with CNS increase. Routine dry cow therapy is effective in decreasing intramammary infection rates. Milking routines, environmental conditions and equipment performance that damage teat ends may result in increased infections.

Environmental Streptococcus species

Details. Environmental streptococci refer to species of streptococcus other than *Strep agalactia* that are isolated from bovine mastitis. These organisms are also referred to as “non-ag” streptococci. The most common mastitis causing environmental streps are *S. uberis* and *S. dysgalactia*. A retrospective survey from 1984-1994 of 67 herds in 14 states reported that environmental streps were isolated from 11.5% of cows and 3.9% of quarters.⁷ Other studies generally have generally reported infection rates of 12-15%. Recently, there have been reports of sporadic high raw bacterial counts that have been traced to small numbers of cows infected with *S. uberis* and *S. dysgalactia*.^{8,9} In 1993, one western processor began speciating the bacteria present in milk sample with >60,000 raw counts. They have noted a steady increase in the number of strep species that have been isolated (Figure 3).

Figure 3: Isolation of Strep spp. From High Raw Counts in WA



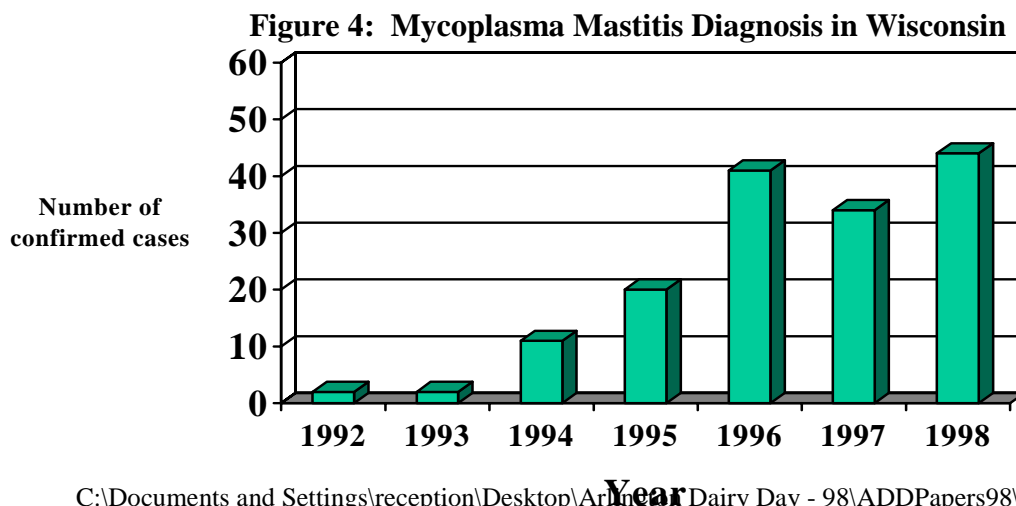
Environmental streps are ubiquitous in the environment and have been isolated from bedding sources such as straw. Exposure to these pathogens can occur at anytime and the risk of infection is much higher during the dry period as compared to during lactation. Infection rates for environmental streps are highest before calving, during early lactation and near dry off.¹⁰ Most studies have shown an increased incidence of infections with environmental streps during winter months.

Effect on SCC and Clinical Mastitis. Environmental streps can cause both subclinical and clinical mastitis. A study of *Strep uberis* intramammary infections demonstrated that the ratio of subclinical to clinical infections increased from 10 subclinical per 1 clinical case in early lactation to 24 subclinical per 1 clinical case in late lactation.¹⁰ In other words, the risk of an infected quarter becoming clinical decreases with stage of lactation.

Treatment and Control. Traditional methods of controlling mastitis (routine dry cow therapy, pre and post dipping) are helpful in controlling these organisms. Additionally, because these organisms thrive in organic bedding, herds that experience problems with environmental streps may need to consider non-organic bedding sources such as sand. While the spontaneous cure rate for IMI caused by these pathogens approaches 50%, clinical cases of mastitis caused by environmental streps should be treated with approved intramammary antibiotic products. Researchers have documented a shorter duration of infection and fewer relapses in infected cows treated appropriately with approved intramammary antibiotics.¹¹

Mycoplasma species

Details. Mycoplasma refers to a group of bacteria of which over 20 different species have been isolated from cattle. The most important mastitis causing species of this organism is *Mycoplasma bovis*. *M. bovis* lives naturally in the upper respiratory tract of cattle and is an important component of bovine respiratory diseases such as shipping fever and calf pneumonia. Mastitis caused by *M. bovis* has been reported throughout the world. Until recently, mastitis caused by *M. bovis* was considered to be a regional problem of western dairy herds. Prior to 1992, only 2 confirmed cases of mycoplasma mastitis had been reported in Wisconsin.¹² Since 1992 the incidence of mastitis caused by *M. bovis* appears to be increasing (Fig 4 -data from personal communication with Dr. Chet Thomas, SVM, UW, Madison, 1998 data YTD Jan-Sept).



The most important risk factor for mycoplasma mastitis is purchase of an infected carrier but outbreaks of this organism have occurred without a history of purchasing cows. An informal survey of WI herds that had experienced mycoplasma mastitis, reported that an outbreak of respiratory disease often preceded the mastitis outbreak (personal communication – Chet Thomas, UW, Madison).

Effect on SCC and Clinical Mastitis. There is considerable variation in the appearance of milk from glands infected with mycoplasma. The classic description of mastitis caused by mycoplasma is severe clinical signs of mastitis from multiple quarters in cows experiencing a dramatic decrease in milk production. However, mycoplasma can cause subclinical infections, severe clinical mastitis or be shed from asymptomatic quarters. Mycoplasma mastitis should be suspected when mastitis that does not respond to conventional mastitis treatment occurs in multiple quarters.

Treatment and Control. Segregation and preferential culling of infected cows are the recommended strategies for control of mycoplasma mastitis. Cows that are segregated should be milked last and great care taken to minimize transmission between cows. Weekly bulk tank cultures should be performed for one year after isolation of mycoplasma from a mastitis case. It is important to note that special media and culture techniques are required to successfully culture this organism.

Serratia spp.

Details. *Serratia spp.* are common inhabitants of soil and water. Two species (*Serratia marcescens* and *Serratia liquifaciens*) have been implicated in a number of outbreaks of mastitis in dairy cows. Originally, mastitis caused by this organism was associated with contaminated chlorhexidine teat dips but more recently a number of outbreaks associated with unknown or environmental sources have been reported.¹³ Susceptibility to infections caused by *Serratia spp.* is highest during the dry period and several outbreaks have been related to adverse weather conditions.

Effect on SCC and Clinical Mastitis. The majority of intramammary infections caused by *Serratia spp.* are subclinical in nature. These infections tend to become chronic and persist for a considerable amount of time (>60 days). SCC's of cows infected with *Serratia* are increased. Up to 50% of cows infected with *Serratia* may exhibit mild clinical signs of mastitis (abnormal milk without appearing ill).

Treatment and Control. Spontaneous cure rates exceed the reported cure rates of infected cows treated with intramammary antibiotics, so antibiotic therapy during lactation is not recommended. Control measures for herds experiencing outbreaks of mastitis caused by *Serratia* should include strict attention to milking hygiene, pre and post dipping with appropriate iodine based dips and routine dry cow therapy.

Conclusion

Tremendous progress has been made in the control of contagious mastitis pathogens. The adoption of accepted methods of mastitis control have greatly decreased the amount of mastitis caused by *Staph aureus* and *Strep agalactia* in many herds. While BTSCC levels have dropped in response to these measures, the rate of clinical mastitis (and milk discard) remains unacceptably high on many dairy farms. Mastitis causing pathogens such as CNS, environmental Streps, Mycoplasma spp. and others have emerged to fill the niche vacated by control of the major pathogens. BTSCC

levels do not necessarily reflect clinical mastitis rates caused by these emerging pathogens. It is imperative to have systems in place to identify outbreaks of these organisms. To continue to make progress in mastitis control, surveillance methods must shift from strict reliance upon decreasing BTSCC to routine recording of clinical mastitis and regular bulk tank culturing.

¹ Hillerton, J.E., A. J. Bramley, R. T. Staker and C.H. McKinnon. 1995. Patterns of intramammary infection and clinical mastitis over a 5 year period in a closely monitored herd applying mastitis control measures. *J Dairy Res*, 62:39-50.

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³ Hoblet, K.H., G. D. Schnitkey, J.S. Arbaugh, J. S. Hogan, et al. 1991. Costs associated with selected preventive practices and with episodes of clinical mastitis in nine herds with low somatic cell counts. *J Am Vet Med. Assoc.* 199:190-196.

⁴ Sargeant, J. M., H. M. Scott, K. E. Leslie, M. J. Ireland, and A. Bashirl. 1998. Clinical mastitis in dairy cattle in Ontario: frequency of occurrence and bacteriological isolates. *Can Vet J*, 39:33-38.

⁵ Harmon, R. J., and B. E. Langlois. Mastitis due to coagulase-negative *Staphylococcus* species. 1995. Pp 56-64, in *Proc Natl Mastitis Council*. Vol 34, National Mastitis Council, Madison WI.

⁶ Ruegg, P.L., and I. R. Dohoo. 1997. A benefit to cost analysis of the effect of premilking teat hygiene on somatic cell count and intramammary infections in a commercial dairy herd. *Can Vet J*, 38:632-636.

⁷ Hogan, J. S., and K. L. Smith. 1997. Occurrence of clinical and subclinical environmental streptococcal mastitis. Pp. 36-41 in *Proc. Symp. in Udder Health Mgmt for environmental Streptococci*. Ont Vet College, Guelph, Ontario, Canada.

⁸ Mickelson, A., L. Hansen, and N. Morris. The impact of environmental mastitis on milk quality in the Pacific Northwest. 1998. Pp 26-34 in *Proc. Natl. Mastitis Council. Reg. Meeting.*, Bellevue, WA. Natl. Mastitis Council, Madison, WI.

⁹ Britten A. 1998. Is Strep mastitis causing high bacteria counts in your bulk tank? Pp 35-39 in *Proc. Natl. Mastitis Council. Reg. Meeting.*, Bellevue, WA. Natl. Mastitis Council, Madison, WI.

¹⁰ Oliver, S. P., B.M. Jayarao, E.E. Gillespie, M.J. Lewis, and H.H. Dowlen. 1998. Epidemiology of *Streptococcus uberis* intramammary infections in a dairy herd. Pp 14-25 in *Proc. Natl. Mastitis Council. Reg. Meeting.*, Bellevue, WA. Natl. Mastitis Council, Madison, WI.

¹¹ Morin D.E., R. D. Shanks, G.C. McCoy. 1998. Comparison of antibiotic administration in conjunction with supportive measures versus supportive measures alone for treatment of dairy cows with clinical mastitis. *J Am Vet Med Assoc.* 213:676-684.

¹² Thomas, C.B. 1998. Bovine Mycoplasmas: a practitioners orientation to host and agent interactions. Pp 255-264 in *Proceedings of the WI VMA, WVMA*, Madison WI.

¹³ Ruegg, P. L., W. M. Guterbock, C.A. Holmberg, J. M. Gay, et al. 1992. Microbiologic investigation of an epizootic of mastitis caused by *Serratia marcescens* in a dairy herd. *J Am Vet Med Assoc.* 200:184-189.

80% moisture and placed it into bunker silos to a depth of 3.5 feet. The following cover systems were applied.

None	Exposed to the atmosphere.
Roof	No cover touching the silage. Structural roof shed rain from bunker.
Sawdust	5-inch layer of sawdust on top of silage.
Soil	5-inch layer of soil on top of silage.
Limestone	3-inch layer of ground limestone on top of silage.
Plastic	Plastic on top of silage weighted with 5 inches of soil.

Precipitation onto the bunker silos was about 20 inches during the 167- to 224-day storage period. Effluent was collected from the bottom of all but one of the silos. Spoiled feed was weighed as it was rejected by the herdsman. Gaseous losses were determined by subtracting effluent and visible waste losses from the total dry matter loss. The results of the study are shown in Table 1. In the case of the plastic covered bunker silo, visible waste was very low. Effluent loss occurred because the forage was harvested at such a high moisture content that some juice was expressed. The effluent DM loss from the roofed silo was quite similar. The other covers did not exclude precipitation as well as the plastic and roof which resulted in an increased effluent loss. The large visible waste loss (4 inches and 10% DM) in the roofed structure was probably facilitated by the diffusion of oxygen deeper into the silage as the top surface dried out. The other cover systems had similar visible waste losses except for the plastic which had very little.

Table 1. Effects of Cover Type on Dry Matter Loss from a 3.5-Foot Deep Bunker Silo [Minson and Lancaster, 1965]

Cover Type	None	Roof	Sawdust	Soil [†]	Limestone	Plastic
Visible Waste (in)	3.0	4.0	3.0	2.0	2.0	0.0
Cause of Loss	Two-year Average DM Loss (%)					
Visible Waste	5.6	10.0	4.2	6.3	5.8	0.8
Effluent	7.5	3.0	6.5	5.0	‡	2.5
Gaseous	21.1	19.6	19.3	13.8	---	8.6
Total	34.2	32.6	30.0	25.1	23.6	11.9
Moisture Content at Recovery (%)	82.0	78.6	81.6	79.4	80.4	78.6

[†]Vegetation grew last 60 days.

[‡]Leak caused effluent not to be collected.

The gaseous loss for the plastic covered bunkers was lowest of all systems. Movement of oxygen through the remaining surface covers by diffusion and/or

percolation has contributed to significant losses caused by aerobic microbial activity. The sum of all losses results in a 2-2.9 times increase in loss compared to that of the plastic cover which did a good job of excluding oxygen and precipitation.

What does this mean to the producer in the upper Midwest who wilts hay silage valued at \$100/TDM before placing it in an 8.5-foot deep bunker silo? Let's assume adequate wilting and the 2.5% effluent loss does not occur due to juice seepage. Assume the choice is to cover with plastic and tires or not to cover at all. Assume the bottom 5 feet of silage experiences a gaseous loss of 8.6% in both cases. Assume the silage is packed to a density of 40 lb/ft³ (14 lbs DM/ft³) in a bunker silo 25 feet wide by 100 feet long. The silage placed in the bunker is 148.8 TDM valued at \$14,880. The average loss in the plastic covered silo is

$$\{[(0.8 + 8.6) \times 3.5] + (8.6 \times 5)\} / 8.5 = 8.93\%$$

The average loss in the uncovered bunker is

$$\{[(5.6 + 5 + 21.1) \times 3.5] + (8.6 \times 5)\} / 8.5 = 18.1\%$$

The dry matter loss in the plastic covered bunker is 13.3 TDM valued at \$1330 while the loss in the uncovered bunker is 26.9 TDM valued at \$2690. The \$1360 difference in lost value can defray the cost of the plastic. If the plastic costs \$100 and the labor to cover and uncover is 20 man hours, the payment for investing this labor is

$$(\$1360 - \$100) / 20 \text{ hr} = \$63/\text{hr},$$

which is not a bad wage rate for a good manager!

K. K. Bolsen [1995] studied the effect of cover type and time of application on a 3.5-foot deep bunker silo. This study also considered the effect of exposure time in storage. Table 2 shows that covering immediately with a plastic cover results in dry matter recovery exceeding 85% (15% DM loss) at all depths for storage periods up to 180 days. The top 13 inches experienced the largest dry matter loss. Depths greater than 13 inches had losses in the range of 5-8%. The average dry matter loss of 9% compares very closely with the 9.4% (corrected for seepage) found by Minson and Lancaster [1965] in their plastic covered bunkers.

When no cover was used, significant dry matter loss occurred to the 26-inch depth. Loss values of 62% in the top 13 inches and 34% in the next 13 inches were experienced after a 180-day storage period. This 34% average loss compares closely to the 32% (corrected for seepage) found by Minson and Lancaster [1965] in their uncovered bunker silos.

By delaying covering for a period of 7 days, Bolsen [1995] was able to demonstrate significant dry matter losses in the top 13 inches during the delay period. After the cover was added, further dry matter loss was similar to that of the immediately covered bunker. Thus covering is effective on a "better late than never basis" but is most effective when applied immediately. This data and analysis strongly support the recommendation of

covering the bunker silo with a material which excludes oxygen and rain water.

Table 2. Effects of Covering and Time on Dry Matter Recovery from a 3.5-foot Deep Bunker Silo [Bolsen, 1995].

Depth (in)	Time Post Filling (days)	Dry Matter Recovery (%)		
		Cover Immediately	No Cover	Cover after 7 Days
0-13	7	91.4	85.9	85.9
	21	91.7	69.4	80.9
	90	87.5	46.9	80.5
	180	86.5	37.7	78.1
13-26	7	95.6	92.6	92.6
	21	96.6	90.8	90.7
	90	93.6	67.9	89.3
	180	92.1	65.8	91.9
26-39	7	96.2	93.1	93.1
	21	96.9	93.2	92.8
	90	95.5	88.3	92.7
	180	94.6	92.6	95.6

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UPDATE ON UW-MADISON CORN SILAGE FEEDING TRIALS^a

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INTRODUCTION

There continues to be a trend for a higher proportion of corn silage in dairy cattle diets in the Upper Midwest. Some of the factors in favor of corn silage that are partially responsible for this trend include:

- Tons DM and energy per acre
- Problems with winter-kill of alfalfa
- High degradability of alfalfa protein
- Improved corn silage hybrids
- More consistent forage quality
- Rapid filling of horizontal silos
- Manure nutrient management.

Along with the trend for feeding more corn silage has come more research aimed at improving the utilization of corn silage by lactating dairy cows. We have conducted a series of feeding trials with dairy cows to evaluate the effects of stage of maturity, crop processing and chop length, and hybrid quality on lactation performance. Results of these trials will be presented and discussed in this paper.

MATURITY EFFECTS

We conducted two feeding trials with lactating dairy cows to evaluate the effects of corn silage stage of maturity at harvest on lactation performance. The results of these trials are presented in Tables 1 and 2.

In Trial 1 (Table 1; Bal and co-workers, 1997a), 20 mature Holstein cows averaging 75 DIM at trial initiation were used in a replicated 4X4 Latin Square design with 28-day periods. Diets containing 50% forage (67% corn silage and 33% alfalfa silage) and 50% concentrate were formulated to 18% CP (DM basis) and fed as TMR. Cargill hybrid 4277 was chopped (1/4" TLC without rolling) at early dent (**ED**; half of kernels dented), quarter milkline (**1/4 ML**), two-thirds milkline (**2/3 ML**), and black layer (**BL**) maturity stages and stored in silo bags.

^a Prepared for Vita Plus Dairy Summit, December 10-11, 1998, Minneapolis, MN, Univ. of Wisconsin Arlington Dairy Day, December, 16, 1998, Arlington, WI, and WAPAC Seminar, December 3, 1998, Madison, WI.

Current Research in Forage Utilization at the UW-Dairy Science Department

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As cattle are fed for higher levels of production it becomes more important to define nutrient requirements in increasingly sophisticated terms. The National Research Council (NRC, 1989) has established requirements for NEL, and suggested optimal dietary guidelines for ruminally degraded organic matter for dairy cattle. Laboratory methods for directly measuring these parameters however, have not been established.

Diets for dairy cattle are also routinely balanced for ruminally degraded protein (RDP) and ruminally undegraded protein (RUP). The forages fed to dairy cattle typically provides half or more of the total protein intake in high producing dairy cattle. Requirements for RUP and RDP have been established by the NRC(1989) yet there no standard accepted procedures for routinely analyzing forages for RUP or RDP.

There is a need to re-evaluate how energy and protein components of forages are analyzed and a need to develop testing procedures that can provide estimates of ruminally available energy and protein that can be used in ration formulation. Over the past three years we have been developing methods to more accurately measure the energy value and ruminal protein degradability of forages for dairy cattle. The goal of the research has been to develop rapid, inexpensive and reliable methods for analyzing forages that can be readily adapted by commercial testing laboratories.

Predicting the energy value of forages

The digestibility of forages can be determined in at least four different ways. The most accurate and precise approach is by feeding trials (in vivo studies). In vivo studies

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Reproductive Management of Dairy Cows using Ultrasound

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INTRODUCTION

The use of transrectal ultrasound for reproductive research in dairy cattle has revolutionized our understanding of reproductive biology. Research using ultrasound has contributed to our understanding of ovarian physiology (Ginther et al., 1996), and aided in quantification of conception rates in lactating dairy cows and dairy heifers (Pursley et al., 1997). Insights into many reproductive disorders including pregnancy loss (Vasconcelos et al., 1997; Fricke et al., 1998), cystic ovaries (Bosu et al., 1987; Garverick, 1997), and uterine infections (Bosu et al., 1987; Peter and Bosu, 1989) also have been made. In addition, the dairy industry has directly benefited from basic research using ultrasound through development of a controlled breeding program for lactating dairy cows which combines synchronization of ovulation with the ability to conduct a timed AI without the need for estrus detection (Ovsynch; Pursley et al., 1997; Fricke et al., 1998).

It is clear that ultrasound has made a tremendous impact as a scientific tool, however, ultrasound holds much promise as a tool to improve reproductive management in a dairy operation. As ultrasound technology becomes more accessible to bovine practitioners, ultrasound will begin to be employed for routine herd visits. There are several reasons that transrectal ultrasound is not widely used among bovine practitioners at present. First, research-grade ultrasound machines are relatively expensive, costing between \$15,000 to \$20,000. Second, most ultrasound machines used for research are large and require an external power source, thereby making them cumbersome to use under field conditions. Because of these factors, ultrasound has been restricted to specialized on-farm procedures such as fetal sexing. Recently, several manufacturers have developed newer ultrasound machines that are cheaper, smaller, and battery operated. Continuation of this trend will foster future use of this technology by bovine practitioners for routine reproductive management in dairy operations.

ULTRASOUND APPLICATIONS

From a reproductive perspective, ultrasound has many applications including specialized procedures such as transvaginal oocyte recovery, follicular ablation, and embryo transfer. Other procedures using ultrasound include fetal sexing and early diagnosis of cows carrying twins. Although each of these procedures involves use of ultrasound, use of ultrasound for routine reproductive management holds the greatest potential for widespread use among bovine practitioners and for improving reproductive efficiency in dairy operations. Because use of ultrasound for fetal sexing and for early identification of cows carrying twins often generates excitement among those in the dairy industry, these applications will be discussed first.

Fetal Sexing

Transrectal ultrasound can be used to detect the sex of bovine fetuses in vivo. Sex is determined by evaluating the morphology of the genital tubercle using ultrasound and is a reliable and accurate method for sex determination beginning on day 55 to 60 of gestation (Curran et al., 1989). Because the reproductive tract and conceptus descend beyond the pelvic rim and into the abdominal cavity as gestation ensues, it becomes increasingly difficult to physically reach the fetus and make an accurate diagnosis during later stages of gestation. Generally, a greater level of operator experience and proficiency is required for sex determination using ultrasound compared with that required for early pregnancy diagnosis or examination of ovarian structures.

Determination of fetal sex is useful when combined with a management decision or strategy that justifies the expense of fetal sexing. In other words, a producer who pays for information regarding fetal sex must economically justify the usefulness of that information. In Wisconsin, fetal sexing services can cost between \$10 to \$15 per cow. Filling sales contract obligations regarding the sex of a calf carried by a pregnant cow to be sold is one scenario that may justify this expense. If the sex of a calf is a determining factor for culling decisions regarding a pregnant cow, fetal sexing might be justified. In contrast, the cost associated with fetal sexing is unwarranted if the information is not used to make a management decision.

Although many producers may initially consider routine fetal sexing and aborting all cows carrying male calves with the intent of rebreeding the cow, several factors would argue against such a strategy. First, the estimated average lactation length of cows subjected to induced abortion and rebreeding would exceed 500 days (~18.5 month calving interval) based on average reproductive performance and management indices for lactating cows (Table 2). Second, there would be a 50% chance that the second pregnancy also would result in a male calf. Based on these considerations and depending on the value of the dam and calf, culling or allowing the pregnancy to continue may be a better alternative to aborting the pregnancy. Because of the economic and management considerations associated with fetal sexing, routine fetal sexing of all pregnant cow in a herd will not likely become a standard reproductive management practice on most dairies.

Table 1. Estimated intervals and cumulative days in milk associated with events after a management decision to terminate a pregnancy after identification of a male calf.

Mean interval from:	Interval (Days)	Cumulative Days in Milk
Calving to fetal sexing and induction of abortion	204 ^a	204
Induction of abortion to second conception	84 ^b	288
Second conception to dry off	232 ^c	520

^aAverage days open (144 days; voluntary waiting period = 60 days, conception rate = 40%, and service rate = 40%)+ day of gestation at fetal sexing (60 days).

^bMedian days to second conception using AI breeding (84 days; conception rate = 40%, and service rate = 40%).

^cAverage gestation length (282 days) - average dry period (50 days).

Identification of Cows Carrying Twins

Twinning is an unavoidable outcome of reproduction in dairy cattle and is undesirable in a dairy operation because it reduces overall profitability and reproductive efficiency (Eddy et al., 1991; Beerepoot et al., 1992). Cows carrying twin pregnancies can be accurately identified at 40 to 55 days post AI using transrectal ultrasonography (Echternkamp and Gregory, 1991; Davis and Haibel, 1993; Dobson et al., 1993).

Several management scenarios could be considered upon identification of a cow carrying twins. Continued management of the cow could be avoided either by culling the cow or by aborting the twin pregnancy, usually through administration of an ecbolic agent such as PGF_{2α}. Several factors would argue against aborting a twin pregnancy with the intent of rebreeding the cow. First, the estimated average lactation length of cows subjected to induced abortion and rebreeding would approach 500 days (~18.5 month calving interval) based on average reproductive performance and management indices for lactating cows (Table 2). Second, the risk for a twin pregnancy during the subsequent gestation is increased because cows calving twins are at greater risk for subsequent twinning (Nielen et al., 1989). Based on these considerations and depending on the value of the dam and calf, culling may be a better alternative to aborting the pregnancy.

Table 2. Estimated intervals and cumulative days in milk associated with events after a management decision to terminate a pregnancy after diagnosis of twins.

Mean interval from:	Interval (Days)	Cumulative Days in Milk
Calving to twin pregnancy diagnosis and induction of abortion	184 ^a	184
Induction of abortion to second conception	84 ^b	268
Second conception to dry off	232 ^c	500

^aAverage days open (144 days; voluntary waiting period = 60 days, conception rate = 40%, and service rate = 40%)+ day of gestation at diagnosis of twins (40 days).

^bMedian days to second conception using AI breeding (84 days; conception rate = 40%, and service rate = 40%).

^cAverage gestation length (282 days) - average dry period (50 days).

Early Pregnancy Diagnosis

Pregnancy loss contributes to reproductive inefficiency in lactating dairy cows because fertility assessed at any point during pregnancy is a function of both conception rate and pregnancy loss. Conception rates at 28 to 32 days post-AI in lactating dairy cows range from 40 to 47% (Pursley et al, 1997; Fricke et al., 1998), whereas conception rates in dairy heifers are nearly 75% (Pursley et al., 1997). Similarly, pregnancy loss in lactating dairy cows is greater than that in dairy heifers (20% vs. 5%; Smith and Stevenson, 1995). Although the specific factors responsible for early embryonic loss in dairy cows are not known, they may be similar to those factors responsible for reduced conception rates.

At present, there is no practical way to reduce early embryonic loss in lactating dairy cows. However, recognizing the occurrence and magnitude of early embryonic loss may actually present management opportunities by taking advantage of new reproductive technologies that increase AI service rate in a dairy herd. One such technology is the use of transrectal ultrasonography for early pregnancy diagnosis. If used routinely, transrectal ultrasonography has the potential to improve reproductive efficiency within a herd by

reducing the period from AI to pregnancy diagnosis to 26 to 28 days with a high degree of diagnostic accuracy (Pierson and Ginther, 1984). Furthermore, use of ultrasound could minimize embryonic loss that may occur after manipulation of the reproductive tract and conceptus during pregnancy diagnosis using rectal palpation (Paisley et al., 1978; Vaillancourt et al., 1979).

There are two main caveats to using ultrasound for routine early pregnancy diagnosis in a dairy herd. First, when using ultrasound for early pregnancy diagnosis, emphasis must be given to identifying nonpregnant rather than pregnant cows. Of cows diagnosed pregnant at 28 days post AI, 14 to 16% experience early embryonic loss by 56 days post AI (Vasconcelos et al., 1997; Fricke et al., 1998). Therefore, cows diagnosed pregnant at 28 days post AI using ultrasound should be scheduled for reexamination around 56 days post AI, when the rate of embryonic loss per day begins to decrease (Vasconcelos et al., 1997). Second, a management strategy must be developed to return the nonpregnant cows to service as quickly as possible after pregnancy diagnosis. Such strategies include administration of PGF_{2α} to cows with a responsive CL, use of estrus detection aids, or a combination of both methods. Unfortunately, the service rate was only 58% when using a system combining PGF_{2α} and Kamar heat mount detectors (Britt and Gaska, 1998), probably due to the inherent inefficiencies of estrus expression and detection in lactating dairy cows.

Routine Reproductive Management

An attractive strategy for managing reproduction in a dairy herd would combine use of synchronization of ovulation and timed AI (Ovsynch), an estrus detection aid, and early pregnancy diagnosis using ultrasound. Every two weeks, groups of cows past the voluntary waiting period would receive their first postpartum insemination after synchronization of ovulation using Ovsynch. This would dramatically reduce median days to first AI by eliminating estrus detection for the first postpartum breeding. At the time of AI, an estrus detection aid such as a Kamar device or estrus detection tail paint would be applied to the cow. This would aid in detection of cows that return to estrus between 18 to 28 days post AI due to failure of conception or early embryonic loss. Cows detected in estrus during this period could then be inseminated based on the detected estrus. At 28 days post AI, a veterinarian using ultrasound would identify any nonpregnant cows, which would be scheduled for resynchronization using Ovsynch along with the next group of cows. This would eliminate reliance on estrus detection for the next breeding, thereby reducing the interval from pregnancy diagnosis to rebreeding. All cows diagnosed pregnant at 28 days post AI would be scheduled for a second ultrasound examination at 56 days post AI to determine if pregnancy loss had occurred.

This is an aggressive reproductive management system that would improve reproductive efficiency by maximizing AI service rate in the herd. This is accomplished through use of early pregnancy diagnosis using ultrasound. Although estrus detection would not be completely eliminated using this system, it would be minimized through the use of Ovsynch and timed AI.

CONCLUSION

As a research tool, transrectal ultrasound has revolutionized our understanding of reproductive biology. As a management tool, transrectal ultrasound may provide a diagnostic management tool for improving reproductive efficiency in dairy operations. Although there are many potential applications of ultrasound for use in reproduction, combining ultrasound for early pregnancy diagnosis with an estrus detection aid and synchronized breeding will likely result in the most widespread use of this technology. As ultrasound machines improve, more veterinarians will incorporate this technology for use in routine reproductive management.

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are not practical as a routine analysis because of the obvious limitations of expense, time and labor. In vivo studies are, however, the 'gold standard' by which alternative methods are compared. Alternative methods of estimating forage digestibility are based on: (1) empirical relationships between forage fiber and digestibility, (2) summative equations or (3) in vitro digestion of forages. Each alternative method has unique advantages and disadvantages, and more importantly, the alternative methods do not necessarily predict the same digestibility or even rank forages in the same order.

Empirical approach. The energy values (TDN or NEI) reported on most forage test reports are derived from empirical equations. The underlying principles behind the empirical method is that the energy value of forages (i.e. forage quality) is a function of its digestibility and intake potential. As the concentration of forage fiber increases, intake potential and digestible energy concentration decrease. Forage digestibility is most commonly predicted from a regression equation based on ADF ($DDM = 88.7 - .779 * ADF$; Rohweder et al., 1978). Intake potential is predicted from NDF ($DMI = 120/NDF$). Forage intake potential and digestibility are combined and reported as Relative Feed Value (RFV). The RFV is used to rank forages according to their potential to provide energy to high producing ruminants.

In dairy nutrition, the RFV can not be used directly in ration formulation, but the digestibility values derived from the empirical equations are used to balance diets for energy. Since energy is often the most limiting nutrient for high producing animals, estimates of forage digestibility must be accurate and precise. There are two major factors that affect the accuracy and precision of the predicted energy values of forages.

The first concern is how accurate and precise are the laboratory methods used to measure NDF and ADF content of forages. Most commercial labs use near infrared reflectance spectroscopy (NIRS) to predict fiber content of forages. Over the past 20 years, improvements have been made in NIRS instrumentation and calibration techniques that have significantly improved the accuracy and precision of this technique. The overall result is that the nutritive value of forages can be predicted by NIRS more rapidly and

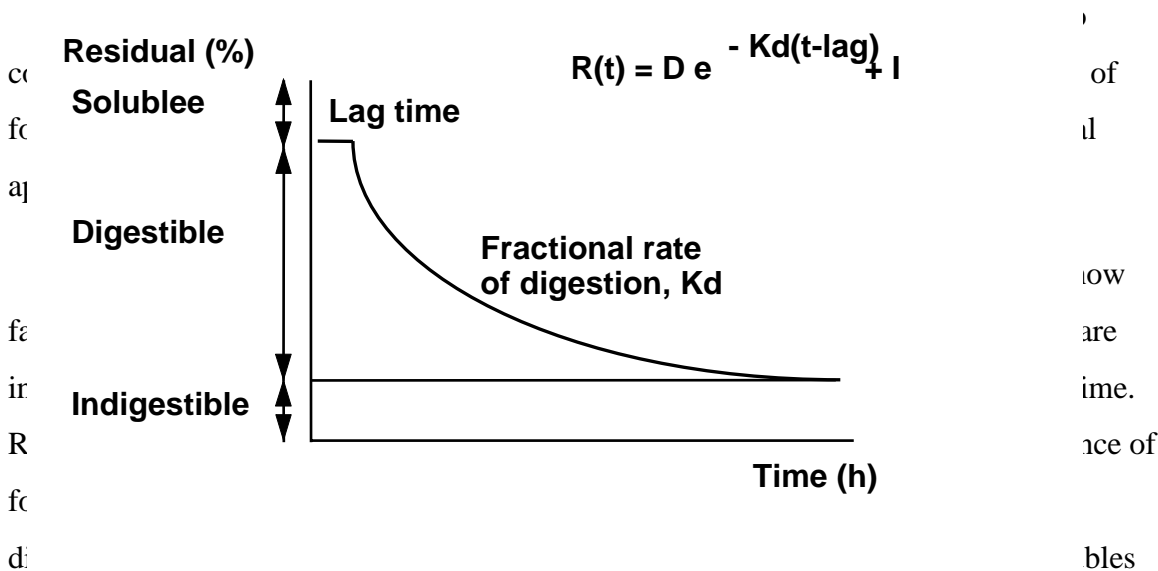
with less expense than with wet chemistry procedures. There is little question that the ADF and NDF values predicted by NIRS are as reliable as ADF and NDF values measured by wet chemistry.

The second concern is how reliable are ADF or NDF as predictors of digestibility. This is a problem that affects both wet chemistry and NIRS analysis. The regression equations used to predict forage digestibility are population specific (Weis, 1994). Thus, estimating digestibility of a forage from regression equations will only produce acceptable values if the forage sample is represented by the population of forages used to generate the regression.

It is well documented that factors such as environment in which the forage was grown (temperature, moisture, and light intensity), cutting number and year affect the relationship between forage fiber and digestibility. Therefore, data sets used to develop empirical equations must be carefully defined and constantly updated. Another problem is that samples sent for analyses are frequently mixes of legumes and grass that are not well defined. The precision of the energy value estimated from applying single variable regression equations in this case becomes questionable. Even under the best conditions, the correlation between fiber levels of alfalfa and grass/legume mixtures to dry matter digestibility are typically .8 or less (Weis, 1994). This indicates that the regression equations used to predict forage digestibility can under or overestimate the digestibility of any single forage by as much as 25 to 30%. The accuracy of estimates of TDN or NEL based on fiber analyses will continue to be the major concern if single variable regression equations are used as the reference for developing calibration equations of mixed samples in NIRS.

Summative equations. An alternative approach for predicting forage digestibility is to analyze forages for energy yielding components (i.e. the protein, fat, non-structural carbohydrate and fiber) and sum the digestible parts of each component together to predict forage digestibility. This approach has been refined by Ohio State University scientists (Weis, 1994). To calculate forage digestibility, total fiber (NDF), lignin

(ADL), total protein (CP), cell wall bound protein (ADIN), fat, and ash contents of feeds are measured. Forages are analyzed for each nutrient by either wet chemistry or NIRS. Each energy yielding component of the feed (fiber, protein, fat, NSC) is then multiplied by a digestibility coefficient and the products are summed together. The summative equation's main advantage over empirical equations is that the summative approach is population independent. Therefore, the summative method can be used to predict energy values of grasses, legumes, corn silage and mixtures of forages. The disadvantages with this procedure are cost and time associated with analyzing the components. Several forage testing laboratories report forage digestibility based on the summative equation



(fraction A); slowly digested NDF (fraction B) ; and indigestible NDF (fraction C). Fraction B is also defined by its rate (kd) of degradation.

Figure 1. Kinetics model for estimating forage digestion

The advantage of this approach over the empirical approach is that this is a direct measurement of forage digestibility. Digestibility is not predicted from fiber content. Advantages of the kinetic approach over the summative equation methods are: (1) digestion coefficients are derived from direct measurements rather than empirical coefficients and (2) forage digestibility can be adjusted to compensate for the effects of intake on forage digestibility. The major disadvantage of this approach is that is time consuming, labor intensive and requires more highly trained personal, equipment and facilities than the other two approaches.

Although most university or industry research labs have facilities to conduct these analyses, only a few commercial labs in the United States offer in vitro analysis as a method to estimate forage digestibility. The only practical way for commercial labs to adapt this approach is to develop a NIRS procedure that will predict forage digestibility based on a database of forages that have been analyzed by the in vitro procedure. The NIRS calibration equations from this database could then be downloaded into the NIRS units of the commercial labs. We have been working for the last three years to determine if it is feasible to directly predict the rate and extent of digestion parameters of forages by NIRS. This research involved three objectives.

1. Determine if different methods for predicting forage digestibility result in different estimates of digestibility.
2. If estimates of forage digestibility derived from the in vitro 'kinetic' approach are more accurate and precise than the currently used empirical equations, develop NIRS equations that will predict digestion kinetics.
3. Validate the new NIRS procedure to ensure that the estimates of digestibility are accurate and the procedure is robust enough to be practical for commercial application.

Results- Predicting forage digestibility by NIRS-kinetic approach

Comparing estimates of digestibility derived by empirical, summative or kinetic methods. The data in Table 1 demonstrates some of the potential problems with the current system of forage analysis. Hay and silage samples were collected at random from forages that were submitted to the UW Soil and Plant Analysis Laboratory at Marshfield. Each sample was analyzed in our laboratory for ADF and NDF by wet chemistry and RFV and DDM were calculated. We also directly measured dry matter digestibility of each forage by an in situ-kinetic procedure (Beyer-Neumann, 1998).

Results of this preliminary study show that in general, as the fiber level of forages increase, the RFV declines and the digestibility estimated by either the empirical approach or the kinetic approach decline. There are several instances, however, where individual forage samples deviate substantially from the trends. The three highlighted forage samples are examples. The digestibilities of these three forages were similar when measured by the kinetic approach even though they ranged in RFV from 182 to 116. Digestibility of the forage with the highest RFV (182) and the lowest ADF (24.9%) differed by 10 units when estimated by either the empirical or kinetic procedures. The RFV and empirically derived digestibility suggest that these three forages would be utilized quite differently by high producing dairy cows but the kinetic estimates of digestibility suggest that there would be little difference in utilization of these three forages by high producing dairy cows.

Although this is not conclusive proof that one approach to estimating digestibility is superior to another, it is evidence that forage digestibility estimates based on a kinetic approach could be substantially different than estimates derived by the empirical approach that is routinely used by forage testing laboratories.

Table 1. Relative Feed value, fiber content¹ and in Situ dry matter digestibility of forages routinely submitted to the Marshfield Soil and Plant Analysis Laboratory

Forage type	RFV	ADF	NDF	Empirical	in Situ
				DDM ²	DMD ³
				-----% of DM -----	
leg silage	233.5	25.4	27.5	69.1	71.7
leg-grass	187.2	29.9	32.6	65.6	70.5
leg-grass	182.0	24.9	35.5	69.5	59.5
leg silage	161.5	30.1	37.7	65.5	62.0
leg silage	159.9	31.8	37.3	64.1	63.8
leg-grass	158.3	32.3	37.5	63.7	69.1
leg silage	149.3	35.1	38.4	61.6	65.1
leg-grass	137.7	35.0	41.6	61.6	65.1
leg silage	136.5	34.9	42.1	61.7	59.0
leg-grass	134.3	35.1	42.7	61.6	61.4
leg-grass	128.3	34.0	45.2	62.4	61.9
leg silage	125.7	36.7	44.7	60.3	55.0
leg-grass	121.5	36.9	46.1	60.2	54.8
leg-grass	116.5	34.6	49.4	61.9	59.1
leg-grass	115.5	40.0	46.5	57.7	57.0
leg-grass	100.7	41.4	52.3	56.6	54.9

¹Fiber analysis done by wet chemistry analysis.

²DDM = 88.9 - (.779*ADF), Rohweder et al. (1978).

³In situ-kinetic procedure (Beyer-Newmann, 1998).

In a second study, we collected samples (n=108) submitted to the Marshfield station for routine analysis and analyzed them in our lab for fiber (NDF, ADF, lignin), protein, fat, and ash. From the chemical analysis we were able to estimate forage digestibility from several empirical or summative approaches. We also directly measured forage digestibility by in vitro-kinetic procedure (Rodrigues, 1998). Results,

partially summarized in Table 2, suggest that on average the empirical estimates of forage digestibility were significantly different than digestibilities calculated by the summative or in vitro-kinetic procedures. The summative approach and the in vitro kinetic approaches gave similar estimates of forage digestibility. Further analysis also revealed that the empirical approaches ranked forages differently from the most digestible to the least digestible, than either the summative or in vitro-kinetic procedures. The summative and in vitro kinetic approaches ranked forages similarly. Results of this study confirm that empirical equations will give different estimates of forage digestibility than either summative or in-vitro kinetic approaches. Diets prepared by using the forage digestibility from the empirical equations are expected to produce different outcomes in terms of animal response than if available energy of forages had been predicted by in vitro kinetics or the summative equations.

Table 2. Average digestibility of 108 forages as predicted by empirical, summative or invitro approaches. (Rodrigues, 1998)

<u>Method</u>	<u>Average digestibility</u>	<u>Minimum</u>	<u>Maximum</u>	<u>Std. Dev.</u>
Empirical ¹	61.1 ^b	50.5	69.1	3.4
Summative ²	58.6 ^a	39.0	67.8	4.9
In vitro ³	58.8 ^a	42.1	68.9	5.3

¹Rohweder et al 1978

²Weis et al 1994.

³Rodrigues, 1998

^{a,b} Means in the same column with different superscripts are different (p<.05)

Calibration of the NIRS to in vitro-kinetics. The second phase of this project was to develop an NIRS calibration equation that can predict digestion kinetics of forages. Beyer-Neumann (1998) found that it was possible to develop a NIRS calibration equation to predict in situ digestibility. In this initial study, a NIRS calibration equation was developed with 30 forage samples. NIRS predictions of the rate and extent of forage digestion were as accurate and precise as predictions of the CP, NDF and ADF content of

the forages. The number of samples used in this study was not large enough to develop a commercially viable equation, but this preliminary experiment demonstrated that digestion kinetics of the forage can be predicted by NIRS.

Rodrigues (1998) then calibrated the NIRS to predict *in vitro* digestibility of forages. The goal of this experiment was to develop a database that could serve as the basis for developing a NIRS calibration equation that can be adapted to commercial testing laboratories. Forage samples (n=182) submitted to commercial testing labs for routine analysis were collected. The samples were pre-scanned with an NIRS instrument and 108 samples that were spectrally different from one another were selected. Each sample was analyzed for CP, NDF, ADF, lignin, fat, and ash. Fiber digestibility was then determined by an *in vitro* procedure on each sample. Dry matter digestibility, the fractions of digestible and indigestible fiber and the rate of fiber digestion were then determined from the *in vitro* assay. NIRS calibrations were then developed to predict the CP, NDF, ADF and digestion kinetic parameters of the forages. The NIRS calibration statistics are summarized in Table 3. The mean concentrations of CP, NDF and ADF of the sample indicate that the database generally represents high quality forages. The low standard errors of calibration and high R^2 for the calibration equations that predict CP, NDF and ADF indicate that these forage components can be predicted with a high degree of accuracy and precision. The calibration statistics are similar to calibration statistics for NIRS equations currently used by commercial testing labs.

The higher SEC and lower R^2 for the digestion kinetics parameters indicate that these components are predicted with less accuracy and precision than CP, NDF or ADF. The calibration statistics for *in vitro* digestibility are still acceptable however. The database is still quite small relative to the databases used by commercial labs to predict CP and fiber. It is likely that as more samples are added to our database, the calibration statistics for digestion kinetic parameters will improve.

Table 3. NIRS calibration statistics for protein, fiber and *in vitro*

digestion kinetics parameters of 108 forage samples

<u>Item</u>	<u>Mean</u>	<u>SEC</u>	<u>R²</u>
Crude protein, % of DM	19.7	0.58	0.97
NDF, % of DM	43.4	1.4	0.95
ADF, % of DM	35.3	0.69	0.97
Fraction B, % of DM	22.6	2.4	0.80
Fraction C, % of DM	20.5	1.9	0.82
<u>Rate of degradation, %/hr</u>	<u>8.3</u>	<u>1.6</u>	<u>0.64</u>

(Rodrigues et al. 1998)

Summarized in Table 4 are calibration statistics for directly predicting forage digestibility by empirical, summative and in vitro-kinetic approaches. These data suggest that the summative and in vitro-kinetic methods for predicting forage digestibility are adaptable to NIRS analysis and the results will be predicted with nearly the same accuracy and precision as measurements of fiber and protein.

Table 4. NIRS calibration statistics for estimates of forage digestibility
determined by empirical, summative and in vitro approaches (n = 108 samples)

<u>Method</u>	<u>Mean</u>	<u>SEC</u>	<u>R²</u>
Empirical ¹	59.4	1.37	0.96
Summative ²	48.26	1.52	0.87
<u>In vitro kinetic³</u>	<u>51.74</u>	<u>1.67</u>	<u>0.87</u>

¹Rohweder et al, 1978.

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Validation The third phase of this project was been to confirm that the estimates of forage digestibility derived from the NIRS-in vitro kinetics approach truly reflect in vivo digestibility. An experiment was conducted with 12 lactating dairy cows to evaluate the *in vivo* digestibility of three alfalfa hays. The *in vivo* digestibility of the hays was then used to validate alternative systems that indirectly predict forage digestibility. Digestibility coefficients for hays were estimated by an empirical method (Rohweder et al 1978), a summative method (Weis et al., 1994) and by the *in vitro*-NIRS method developed in our lab (Rodrigues, 1998). The feeding trial measured production, feed intake, rumen environment, rate of passage, and digestibility in cows fed diets containing one of three alfalfa hays. The hays used in this study were selected based on their ADF contents and the digestibilities predicted from the empirical and *in vitro* NIRS methods. The compositions of the hays are summarized in Table 5. Hay L-ADF₁ and L-ADF₂ were similar in NDF and ADF composition. Digestibilities of these two hays were also similar when calculated by the empirical method (63.8%) or analyzed by the *in vitro* NIR procedure (63.8%). Hay H-ADF was approximately 6 units higher in ADF than the other two forages and as a result the empirical regression would suggest that it would be approximately 5 units lower in digestibility (58.9%) than the other two hays. The results of the *in vitro*-NIRS procedure, however, suggest that this hay was similar in digestibility to the other two hays. Results of the feeding trial suggested that digestibilities predicted by the *in vitro* NIR approach and the summative approach were similar to the digestibilities observed *in vivo* (Table 6). In the context of this study, the empirical approach was less accurate than the summative and *in vitro* NIR approaches.

Table 5 Composition of the alfalfa hays used in the *in vivo* study.

<u>Hay</u>	<u>OM</u>	<u>CP</u>	<u>NDF</u>	<u>ADF</u>	<u>RFV</u>
L-ADF1	85.3	17.3	42.1	32.3	141
L-ADF2	83.2	18.7	42.6	32.1	140
H-ADF	87.5	15.9	49.2	38.5	111

Table 6. Dry matter digestibility measured *in vivo* and predicted by four different approaches for three alfalfa hays.

<u>Item</u>	<u>L-ADF₁</u>	<u>L-ADF₂</u>	<u>H-ADF</u>	<u>S.E.M.</u>	<u>P (trt)</u>
<i>In Vivo DM digestibility</i>					
Total diet	66.6	65.8	65.1	1.58	0.63
Hay ¹	59.1	57.1	56.5	--	--
<i>Predicted digestibility</i>					
Empirical equation ²	63.7	63.9	58.9	0.65	<.01
Summative approach	50.6	48.0	46.4	1.04	.08
<u>In vitro-NIRS approach</u>	<u>64.2</u>	<u>63.0</u>	<u>61.1</u>	<u>0.78</u>	<u>.09</u>

¹Digestibility of hay calculated by correcting total diet digestibility for grain.

²Rohweder et al., 1978.

³Weis et al., 1994.

Predicting bypass protein

Legume and legume-grass silages often supply the majority of CP, ruminally degraded protein (RDP) and ruminally undegraded protein (RUP) in dairy cow and heifer diets. Requirements for RDP and RUP have been established (NRC, 1989), but no commercial test is available to directly estimate RDP or RUP. We have developed a NIRS procedure, based on ruminal in situ degradation of forage proteins to predict the RUP content of forages. Although results are preliminary, it appears that the RUP content of legume, grass or grass-legume mixtures of silage can be predicted by NIRS. The calibration statistics (Table 7) suggest that the kinetics parameters that are used to calculate RUP and the direct estimate of RUP by NIRS are comparable to the calibration statistics for predicted crude protein.

Table 7. NIRS calibration statistics for protein fractions in legume-grass silage samples.

<u>Item</u>	<u>R²</u>	<u>SEC</u>
CP, % DM	0.96	0.80
Fraction A	0.96	1.80
Fraction B	0.96	1.51
Fraction C	0.92	0.69
kd, 1/hr	0.87	1.42
<u>RUP, % CP</u>	<u>0.94</u>	<u>1.23</u>

Hoffman et al., 1998

The procedure is sensitive enough to detect differences in RUP caused by different maturities and forage moisture content at ensiling. The RUP content of 300 legume-grass forage samples were evaluated with this newly developed equation. The NIRS procedure suggests that the RUP content of forages was not affected by moisture of ensiling when legume-grass silages are ensiled at less than 50% DM, but when forages contained more than 50% DM at ensiling, RUP contents of forages increased as forage DM increased.

The predicted RUP content of legume-grass silages appear to be within industry norms and are consistent with known pre-established relationships between other nutrients in legume-grass silages and RUP.

Conclusions

An NIRS prediction of forage dry matter and protein digestion kinetics would make it possible to value the energy and protein in hay more accurately than is currently possible with the RFV system. By knowing the kinetics of forage digestion, it would be possible to adjust the digestible energy of hay for animals of different levels of production and value forages according to the production level of the animal. Forage pricing formulas could also be developed that would value the digestible energy and the bypass protein.

Ration formulation software is available that predicts nutrient flows based on feed ingredient and digesta kinetics (Fox et al. 1992). The digestion kinetics of most feeds are however estimated since methods to directly measure the kinetics of digestion are too costly and slow to do on a routine basis. The proposed NIRS method would greatly facilitate the use of kinetics-based ration software.

In vitro methods are currently used by most forage breeders to evaluate digestibility of new forage varieties. More emphasis is being placed today on new corn silage and alfalfa varieties that are either a) higher in digestibility at a given maturity or b) decline in digestibility more slowly over time. Most plant breeders use either relative feed value or a single time point in vitro digestibility to assess forage digestibility. Development of this NIRS technique would provide plant breeders with an alternative means of forage selection. With our approach, four distinct characteristics (A, B, C pools and Kd) of forages could be identified in forages and selection could be based on these parameters.

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are not practical as a routine analysis because of the obvious limitations of expense, time and labor. In vivo studies are, however, the 'gold standard' by which alternative methods are compared. Alternative methods of estimating forage digestibility are based on: (1) empirical relationships between forage fiber and digestibility, (2) summative equations or (3) in vitro digestion of forages. Each alternative method has unique advantages and disadvantages, and more importantly, the alternative methods do not necessarily predict the same digestibility or even rank forages in the same order.

Empirical approach. The energy values (TDN or NEI) reported on most forage test reports are derived from empirical equations. The underlying principles behind the empirical method is that the energy value of forages (i.e. forage quality) is a function of its digestibility and intake potential. As the concentration of forage fiber increases, intake potential and digestible energy concentration decrease. Forage digestibility is most commonly predicted from a regression equation based on ADF ($DDM = 88.7 - .779 * ADF$; Rohweder et al., 1978). Intake potential is predicted from NDF ($DMI = 120/NDF$). Forage intake potential and digestibility are combined and reported as Relative Feed Value (RFV). The RFV is used to rank forages according to their potential to provide energy to high producing ruminants.

In dairy nutrition, the RFV can not be used directly in ration formulation, but the digestibility values derived from the empirical equations are used to balance diets for energy. Since energy is often the most limiting nutrient for high producing animals, estimates of forage digestibility must be accurate and precise. There are two major factors that affect the accuracy and precision of the predicted energy values of forages.

The first concern is how accurate and precise are the laboratory methods used to measure NDF and ADF content of forages. Most commercial labs use near infrared reflectance spectroscopy (NIRS) to predict fiber content of forages. Over the past 20 years, improvements have been made in NIRS instrumentation and calibration techniques that have significantly improved the accuracy and precision of this technique. The overall result is that the nutritive value of forages can be predicted by NIRS more rapidly and

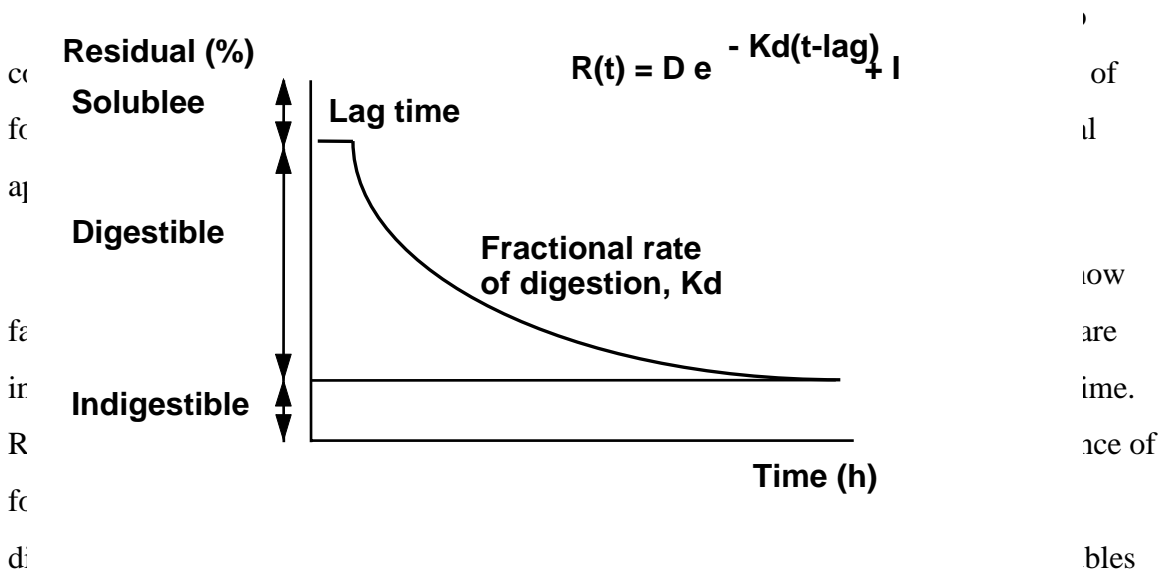
with less expense than with wet chemistry procedures. There is little question that the ADF and NDF values predicted by NIRS are as reliable as ADF and NDF values measured by wet chemistry.

The second concern is how reliable are ADF or NDF as predictors of digestibility. This is a problem that affects both wet chemistry and NIRS analysis. The regression equations used to predict forage digestibility are population specific (Weis, 1994). Thus, estimating digestibility of a forage from regression equations will only produce acceptable values if the forage sample is represented by the population of forages used to generate the regression.

It is well documented that factors such as environment in which the forage was grown (temperature, moisture, and light intensity), cutting number and year affect the relationship between forage fiber and digestibility. Therefore, data sets used to develop empirical equations must be carefully defined and constantly updated. Another problem is that samples sent for analyses are frequently mixes of legumes and grass that are not well defined. The precision of the energy value estimated from applying single variable regression equations in this case becomes questionable. Even under the best conditions, the correlation between fiber levels of alfalfa and grass/legume mixtures to dry matter digestibility are typically .8 or less (Weis, 1994). This indicates that the regression equations used to predict forage digestibility can under or overestimate the digestibility of any single forage by as much as 25 to 30%. The accuracy of estimates of TDN or NEL based on fiber analyses will continue to be the major concern if single variable regression equations are used as the reference for developing calibration equations of mixed samples in NIRS.

Summative equations. An alternative approach for predicting forage digestibility is to analyze forages for energy yielding components (i.e. the protein, fat, non-structural carbohydrate and fiber) and sum the digestible parts of each component together to predict forage digestibility. This approach has been refined by Ohio State University scientists (Weis, 1994). To calculate forage digestibility, total fiber (NDF), lignin

(ADL), total protein (CP), cell wall bound protein (ADIN), fat, and ash contents of feeds are measured. Forages are analyzed for each nutrient by either wet chemistry or NIRS. Each energy yielding component of the feed (fiber, protein, fat, NSC) is then multiplied by a digestibility coefficient and the products are summed together. The summative equation's main advantage over empirical equations is that the summative approach is population independent. Therefore, the summative method can be used to predict energy values of grasses, legumes, corn silage and mixtures of forages. The disadvantages with this procedure are cost and time associated with analyzing the components. Several forage testing laboratories report forage digestibility based on the summative equation



(fraction A); slowly digested NDF (fraction B) ; and indigestible NDF (fraction C). Fraction B is also defined by its rate (kd) of degradation.

Figure 1. Kinetics model for estimating forage digestion

The advantage of this approach over the empirical approach is that this is a direct measurement of forage digestibility. Digestibility is not predicted from fiber content. Advantages of the kinetic approach over the summative equation methods are: (1) digestion coefficients are derived from direct measurements rather than empirical coefficients and (2) forage digestibility can be adjusted to compensate for the effects of intake on forage digestibility. The major disadvantage of this approach is that is time consuming, labor intensive and requires more highly trained personal, equipment and facilities than the other two approaches.

Although most university or industry research labs have facilities to conduct these analyses, only a few commercial labs in the United States offer in vitro analysis as a method to estimate forage digestibility. The only practical way for commercial labs to adapt this approach is to develop a NIRS procedure that will predict forage digestibility based on a database of forages that have been analyzed by the in vitro procedure. The NIRS calibration equations from this database could then be downloaded into the NIRS units of the commercial labs. We have been working for the last three years to determine if it is feasible to directly predict the rate and extent of digestion parameters of forages by NIRS. This research involved three objectives.

1. Determine if different methods for predicting forage digestibility result in different estimates of digestibility.
2. If estimates of forage digestibility derived from the in vitro 'kinetic' approach are more accurate and precise than the currently used empirical equations, develop NIRS equations that will predict digestion kinetics.
3. Validate the new NIRS procedure to ensure that the estimates of digestibility are accurate and the procedure is robust enough to be practical for commercial application.

Results- Predicting forage digestibility by NIRS-kinetic approach

Comparing estimates of digestibility derived by empirical, summative or kinetic methods. The data in Table 1 demonstrates some of the potential problems with the current system of forage analysis. Hay and silage samples were collected at random from forages that were submitted to the UW Soil and Plant Analysis Laboratory at Marshfield. Each sample was analyzed in our laboratory for ADF and NDF by wet chemistry and RFV and DDM were calculated. We also directly measured dry matter digestibility of each forage by an in situ-kinetic procedure (Beyer-Neumann, 1998).

Results of this preliminary study show that in general, as the fiber level of forages increase, the RFV declines and the digestibility estimated by either the empirical approach or the kinetic approach decline. There are several instances, however, where individual forage samples deviate substantially from the trends. The three highlighted forage samples are examples. The digestibilities of these three forages were similar when measured by the kinetic approach even though they ranged in RFV from 182 to 116. Digestibility of the forage with the highest RFV (182) and the lowest ADF (24.9%) differed by 10 units when estimated by either the empirical or kinetic procedures. The RFV and empirically derived digestibility suggest that these three forages would be utilized quite differently by high producing dairy cows but the kinetic estimates of digestibility suggest that there would be little difference in utilization of these three forages by high producing dairy cows.

Although this is not conclusive proof that one approach to estimating digestibility is superior to another, it is evidence that forage digestibility estimates based on a kinetic approach could be substantially different than estimates derived by the empirical approach that is routinely used by forage testing laboratories.

Table 1. Relative Feed value, fiber content¹ and in Situ dry matter digestibility of forages routinely submitted to the Marshfield Soil and Plant Analysis Laboratory

Forage type	RFV	ADF	NDF	Empirical	in Situ
				DDM ²	DMD ³
				-----% of DM -----	
leg silage	233.5	25.4	27.5	69.1	71.7
leg-grass	187.2	29.9	32.6	65.6	70.5
leg-grass	182.0	24.9	35.5	69.5	59.5
leg silage	161.5	30.1	37.7	65.5	62.0
leg silage	159.9	31.8	37.3	64.1	63.8
leg-grass	158.3	32.3	37.5	63.7	69.1
leg silage	149.3	35.1	38.4	61.6	65.1
leg-grass	137.7	35.0	41.6	61.6	65.1
leg silage	136.5	34.9	42.1	61.7	59.0
leg-grass	134.3	35.1	42.7	61.6	61.4
leg-grass	128.3	34.0	45.2	62.4	61.9
leg silage	125.7	36.7	44.7	60.3	55.0
leg-grass	121.5	36.9	46.1	60.2	54.8
leg-grass	116.5	34.6	49.4	61.9	59.1
leg-grass	115.5	40.0	46.5	57.7	57.0
leg-grass	100.7	41.4	52.3	56.6	54.9

¹Fiber analysis done by wet chemistry analysis.

²DDM = 88.9 - (.779*ADF), Rohweder et al. (1978).

³In situ-kinetic procedure (Beyer-Newmann, 1998).

In a second study, we collected samples (n=108) submitted to the Marshfield station for routine analysis and analyzed them in our lab for fiber (NDF, ADF, lignin), protein, fat, and ash. From the chemical analysis we were able to estimate forage digestibility from several empirical or summative approaches. We also directly measured forage digestibility by in vitro-kinetic procedure (Rodrigues, 1998). Results,

partially summarized in Table 2, suggest that on average the empirical estimates of forage digestibility were significantly different than digestibilities calculated by the summative or in vitro-kinetic procedures. The summative approach and the in vitro kinetic approaches gave similar estimates of forage digestibility. Further analysis also revealed that the empirical approaches ranked forages differently from the most digestible to the least digestible, than either the summative or in vitro-kinetic procedures. The summative and in vitro kinetic approaches ranked forages similarly. Results of this study confirm that empirical equations will give different estimates of forage digestibility than either summative or in-vitro kinetic approaches. Diets prepared by using the forage digestibility from the empirical equations are expected to produce different outcomes in terms of animal response than if available energy of forages had been predicted by in vitro kinetics or the summative equations.

Table 2. Average digestibility of 108 forages as predicted by empirical, summative or invitro approaches. (Rodrigues, 1998)

<u>Method</u>	<u>Average digestibility</u>	<u>Minimum</u>	<u>Maximum</u>	<u>Std. Dev.</u>
Empirical ¹	61.1 ^b	50.5	69.1	3.4
Summative ²	58.6 ^a	39.0	67.8	4.9
In vitro ³	58.8 ^a	42.1	68.9	5.3

¹Rohweder et al 1978

²Weis et al 1994.

³Rodrigues, 1998

^{a,b} Means in the same column with different superscripts are different (p<.05)

Calibration of the NIRS to in vitro-kinetics. The second phase of this project was to develop an NIRS calibration equation that can predict digestion kinetics of forages. Beyer-Neumann (1998) found that it was possible to develop a NIRS calibration equation to predict in situ digestibility. In this initial study, a NIRS calibration equation was developed with 30 forage samples. NIRS predictions of the rate and extent of forage digestion were as accurate and precise as predictions of the CP, NDF and ADF content of

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L-ADF1	85.3	17.3	42.1	32.3	141
L-ADF2	83.2	18.7	42.6	32.1	140
<u>H-ADF</u>	<u>87.5</u>	<u>15.9</u>	<u>49.2</u>	<u>38.5</u>	<u>111</u>

Table 6. Dry matter digestibility measured *in vivo* and predicted by four different approaches for three alfalfa hays.

<u>Item</u>	<u>L-ADF₁</u>	<u>L-ADF₂</u>	<u>H-ADF</u>	<u>S.E.M.</u>	<u>P (trt)</u>
<i>In Vivo DM digestibility</i>					
Total diet	66.6	65.8	65.1	1.58	0.63
Hay ¹	59.1	57.1	56.5	--	--
<i>Predicted digestibility</i>					
Empirical equation ²	63.7	63.9	58.9	0.65	<.01
Summative approach	50.6	48.0	46.4	1.04	.08
<u>In vitro-NIRS approach</u>	<u>64.2</u>	<u>63.0</u>	<u>61.1</u>	<u>0.78</u>	<u>.09</u>

¹Digestibility of hay calculated by correcting total diet digestibility for grain.

²Rohweder et al., 1978.

³Weis et al., 1994.

Predicting bypass protein

Legume and legume-grass silages often supply the majority of CP, ruminally degraded protein (RDP) and ruminally undegraded protein (RUP) in dairy cow and heifer diets. Requirements for RDP and RUP have been established (NRC, 1989), but no commercial test is available to directly estimate RDP or RUP. We have developed a NIRS procedure, based on ruminal in situ degradation of forage proteins to predict the RUP content of forages. Although results are preliminary, it appears that the RUP content of legume, grass or grass-legume mixtures of silage can be predicted by NIRS. The calibration statistics (Table 7) suggest that the kinetics parameters that are used to calculate RUP and the direct estimate of RUP by NIRS are comparable to the calibration statistics for predicted crude protein.

Table 7. NIRS calibration statistics for protein fractions in legume-grass silage samples.

<u>Item</u>	<u>R2</u>	<u>SEC</u>
CP, % DM	0.96	0.80
Fraction A	0.96	1.80
Fraction B	0.96	1.51
Fraction C	0.92	0.69
kd, 1/hr	0.87	1.42
<u>RUP, % CP</u>	<u>0.94</u>	<u>1.23</u>

Hoffman et al., 1998

The procedure is sensitive enough to detect differences in RUP caused by different maturities and forage moisture content at ensiling. The RUP content of 300 legume-grass forage samples were evaluated with this newly developed equation. The NIRS procedure suggests that the RUP content of forages was not affected by moisture of ensiling when legume-grass silages are ensiled at less than 50% DM, but when forages contained more than 50% DM at ensiling, RUP contents of forages increased as forage DM increased.

The predicted RUP content of legume-grass silages appear to be within industry norms and are consistent with known pre-established relationships between other nutrients in legume-grass silages and RUP.

Conclusions

An NIRS prediction of forage dry matter and protein digestion kinetics would make it possible to value the energy and protein in hay more accurately than is currently possible with the RFV system. By knowing the kinetics of forage digestion, it would be possible to adjust the digestible energy of hay for animals of different levels of production and value forages according to the production level of the animal. Forage pricing formulas could also be developed that would value the digestible energy and the bypass protein.

Ration formulation software is available that predicts nutrient flows based on feed ingredient and digesta kinetics (Fox et al. 1992). The digestion kinetics of most feeds are however estimated since methods to directly measure the kinetics of digestion are too costly and slow to do on a routine basis. The proposed NIRS method would greatly facilitate the use of kinetics-based ration software.

In vitro methods are currently used by most forage breeders to evaluate digestibility of new forage varieties. More emphasis is being placed today on new corn silage and alfalfa varieties that are either a) higher in digestibility at a given maturity or b) decline in digestibility more slowly over time. Most plant breeders use either relative feed value or a single time point in vitro digestibility to assess forage digestibility. Development of this NIRS technique would provide plant breeders with an alternative means of forage selection. With our approach, four distinct characteristics (A, B, C pools and Kd) of forages could be identified in forages and selection could be based on these parameters.

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In Trial 2 (Table 2; Bal and co-workers, 1997b), 21 mature Holstein cows averaging 75 DIM at trial initiation were used in a replicated 3X3 Latin Square design with 28-day periods. Diets containing 50% forage (67% corn silage and 33% alfalfa silage) and 50% concentrate were formulated to 18% CP (DM basis) and fed as TMR. Pioneer hybrid 3563 was chopped (1/4" TLC without rolling) at ED, half milkline (1/2 ML), and BL maturity stages and stored in silo bags.

Table 1. Impact of stage of maturity at harvest on silage composition, intake, digestion, and milk production by dairy cows (Year 1; Bal and co-workers, 1997a).

Item	ED	1/4 ML	2/3 ML	BL	SE	(P<)
WP ^a Moisture %	69.9	67.6	64.9	58.0	--	--
WP NDF %	52.0	44.4	40.5	41.3	--	--
WP Starch %	18.2	28.7	37.2	37.4	--	--
DMI, lb/d	56.1	56.5	56.5	56.3	.9	NS
Milk, lb/d	71.3 ^b	71.7 ^{ab}	73.5 ^a	71.9 ^{ab}	.9	.07
Fat, lb/d	2.57	2.51	2.51	2.53	.04	NS
CP, lb/d	2.46 ^b	2.46 ^b	2.57 ^a	2.49 ^b	.02	.05
DOM ^b , %	65.2 ^a	62.1 ^a	61.4 ^a	58.5 ^b	.7	.05
DADF ^b , %	45.7 ^a	38.3 ^b	33.6 ^c	29.4 ^d	1.4	.05
Dstarch ^b , %	94.1 ^a	92.9 ^{ab}	92.2 ^b	87.7 ^c	.6	.05

^aWP=Whole plant.

^bTotal tract digestibility of organic matter, acid detergent fiber, and starch, respectively.

Table 2. Impact of stage of maturity at harvest on silage composition, intake, and milk production by dairy cows (Year 2; Bal and co-workers, 1997b).

Item	ED	1/2 ML	BL	SE	(P<)
Moisture %	71.3	64.3	48.1	--	--
NDF %	45.8	42.2	45.7	--	--
DMI, lb/d	57.9	58.3	59.2	.7	NS
Milk, lb/d	89.3 ^a	87.8 ^{ab}	86.5 ^b	.9	.05
Fat, lb/d	3.26	3.26	3.19	.04	NS
CP, lb/d	3.02 ^a	2.99 ^{ab}	2.93 ^b	.02	.05
MUN, mg%	14.6 ^c	15.2 ^b	16.9 ^a	.7	.05

Delaying harvest to the BL stage of maturity reduced milk and milk protein production in both trials. This was related to a corresponding decline in total tract digestibility of OM, ADF, and starch (Table 1).

Data in Table 3 shows the reduction in ruminal *In situ* macro-bag dry matter, NDF, and starch degradation that occurs when corn silage is harvested late (Bal and co-workers, 1998d). It is important to note from this data that we not only sacrifice ruminal starch degradation with late harvest, but also ruminal stover and NDF degradation.

Milk and milk protein production was highest for 2/3 ML and ED in Trials 1 and 2, respectively. This corresponded to 65% and 70% whole-plant moisture in Trials 1 and 2,

respectively. Trial 2 was conducted during a year when rates of corn fill and dry-down were more rapid than normal.

This resulted in a smaller improvement in silage quality from ED to ½ ML (4% units lower NDF) in Trial 2 than from ED to ¼ - 2/3 ML in Trial 1 (8-12% units lower NDF). There was no further improvement in silage quality seen with delaying harvest past 2/3 and ½ ML in Trials 1 and 2, respectively. This agrees with the report of Wiersma and co-workers (1993) where the highest quality corn silages were harvested at ½ - ¾ ML versus ED or BL. They (Wiersma and co-workers, 1993) also reported increased whole-plant DM yield per acre from ED to ½ ML with no further improvement from ½ ML to BL.

Table 3. Impact of stage of maturity at harvest on silage composition and ruminal *In situ* macro-bag dry matter, NDF, and starch degradation (Bal and co-workers, 1998d).

Item	Early Harvest	Late Harvest	(P<)
WP Moisture %	63.2	49.1	--
WP NDF %	41.7	41.3	--
Stover RDMD %	46.8	38.1	.01
WP RDMD %	62.6	57.4	.05
WP RNDFD %	31.4	23.3	.05
WP RStarchD %	75.4	65.8	.01

Taking into account silage composition, lactation performance, nutrient digestion, and silage DM yield per acre, a ½ ML harvest target with a range from ¼ ML to ¾ ML seems reasonable. There can be considerable variation in whole-plant moisture content at a specific kernel milkline position. Because of this, whole-plant moisture content is the best trigger of when to harvest corn silage. Taking into account the above factors, a 65% whole-plant moisture target seems best. This will likely mean harvesting some silage at 70% moisture so that the corn does not dry down too far by the end of the harvest. This is especially true during rapid dry-down years and when using custom harvesters.

The trend in moisture content of corn silage samples analyzed by Dairyland Labs over the 90's is presented in Table 4. In the past we have harvested corn silage much too dry for good utilization. We are making good progress as the average moisture content through the lab has increased 4%-5% units over the decade, but we still can improve corn silage utilization simply by fine tuning harvest timing.

Table 4. Trend in moisture content of corn silage samples analyzed by Dairyland Labs, Arcadia, WI over the 90's.

Year	No. of samples	Average Moisture %	Range in Moisture %
1991	8, 673	58	48 - 69
1994	6, 225	59	49 - 70
1995	7, 433	59	49 - 70
1996	11, 578	63	54 - 72
1997	12, 672	63	43 - 83
1998	8, 223	63	40 - 84

CROP PROCESSING AND CHOP LENGTH EFFECTS

Feeding processed or rolled corn silage is gaining in popularity across the U.S., because of more widespread custom harvesting using self-propelled choppers and the marketing of pull-type choppers fitted with crop processors.

Johnson and co-workers (1996) reported that rolling increased milk yield 2.0 lb/cow/day, increased milk protein yield, and reduced corn kernel passage into the manure. An early trial at the U.S. Dairy Forage Research Center (Prairie du Sac, WI) showed about the same milk production response, but in a later trial (Bal and co-workers, 1998c) rolling was not beneficial.

We (Bal and co-workers, 1998b and 1999b) recently completed a feeding trial at our UW Arlington Dairy Cattle Center to evaluate corn silage crop processing and chop length effects on lactation performance. The results are presented in Table 5.

Pioneer hybrid 3563 was harvested as whole-plant corn silage at ½ ML stage of maturity. The control corn silage was chopped at 3/8" TLC without rolling using a pull-type chopper. Treatment corn silages were harvested at 3/8", 9/16", and 3/4" TLC and rolled using the same pull-type chopper fitted with a crop processor. The crop processor was set at less than one-millimeter roll spacing for all three chop lengths. Experimental silages were stored in silo bags, and averaged 33% whole-plant DM with little variation across treatments on feed-out.

Twenty-four mature Holstein cows averaging about 100 DIM at the start of the feeding trial were used in a replicated Latin Square design with monthly periods. Four of the cows were ruminally cannulated to facilitate rumen pH, fiber-mat formation, and digestion measurements. All diets were fed as TMR containing half forage of which two-thirds was one of the treatment corn silages and one-third was alfalfa silage (DM basis). All diets were formulated to 18% CP (DM basis) and to meet or exceed NRC guidelines for minerals and vitamins.

Table 5. Impact of crop processing and chop length on corn silage utilization by dairy cows (Bal and co-workers, 1998b and 1999b).

Item	3/8" TLC Unprocessed	3/8" TLC 1 mm Roll	9/16" TLC 1 mm Roll	3/4" TLC 1 mm Roll	SE	(P<)
WP MPL, mm	9.4	6.7	8.9	9.2	--	--
% Coarse WP	7.5	1.5	9.9	21.5	--	--
Rumination, h/d	7.8	8.0	8.0	8.2	.2	NS
DMI, lb/d	55.4 ^b	56.9 ^a	56.9 ^a	56.7 ^a	.4	.06
Milk, lb/d	98.6 ^b	102.2 ^a	99.7 ^{ab}	101.4 ^a	.9	.03
Fat, lb/d	2.95 ^c	3.17 ^a	3.06 ^b	3.13 ^{ab}	.03	.01
CP, lb/d	3.12	3.24	3.19	3.24	.05	NS

Mean particle length (MPL) of corn silage was measured using the Wisconsin Oscillating Screen Particle Separator. Rolling reduced MPL of the 3/8" TLC corn silage from 9.4 to 6.7 mm. Chopping rolled corn silage at 9/16" or 3/4" TLC increased MPL to near the length of the unrolled silage. Rolling reduce the percentage of coarse particles in the 3/8" TLC corn silage from 7.5% to 1.5%. This resulted in a lower percentage of coarse particles in the TMR for cows fed the 3/8" TLC-rolled silage (3% versus 6%).

Percentage of coarse particles was measured as the proportion of the silage or TMR retained on the top two screens of our separator. This is roughly comparable to the proportion that would be retained on the top screen of the Penn State – Nasco shaker box. Chopping rolled corn silage at 9/16" or 3/4" TLC increased the percentage of coarse particles to 10% and 21.5%, respectively. This resulted in a higher percentage of coarse particles in the TMR for cows fed these silages (6% and 10.5% for 9/16" and 3/4" TLC diets, respectively).

An interesting observation was that the coarse fraction in the unrolled silage contained a high proportion of whole and half cobs prone to sorting in the feed bunk. On the other hand, the coarse fraction in the coarsely chopped rolled silages had no cob material and was comprised primarily of precision-chopped stover. No unbroken corn kernels were found in the rolled silages.

Cows fed the rolled silages on average ate 1.5 pounds more diet DM each day than cows fed unrolled silage. Cows fed the rolled silages on average produced 2.5 pounds more milk and 3.5 more FCM each day than cows fed unrolled silage. Milk fat test was .10% units higher on average for cows fed the rolled silages. This improvement in milk fat test with rolling was unexpected, but may have resulted from less sorting of the cob fiber in the feed bunk for the rolled-silage diets. There were no differences in dry matter intake, milk yield, or milk composition among the rolled-silage diets.

Cows ruminated about eight hours per day and this did not differ among the four treatments. Ruminal pH did not appear to differ among the four treatments. Our measure of fiber-mat formation in the rumen revealed a drop off for the 3/8" TLC-rolled silage compared with the unrolled silage. Chopping the rolled silage at 3/4" TLC brought this

parameter back to what we observed for the unrolled silage. It is unknown whether this would have proved beneficial for preventing digestive disorders in fresh cows or in a longer-term study. Ruminant 24-hour macro-bag dry matter digestion was higher on average for the rolled silages.

Rolling corn silage that was harvested at ½ ML with 67% whole-plant moisture improved milk and fat production. Rolling corn silage chopped at a 3/8" TLC reduced its MPL and coarse particle fraction and tended to lower our measure of rumen fiber-mat formation. While this did not adversely affect rumination activity, feed intake or lactation performance in this trial, there may be cause for concern in fresh cows diets or in a longer-term lactation trial.

We recommend a 3/4" theoretical length of cut setting on the chopper for rolled corn silage at this time for several reasons. Feed intake and lactation performance were excellent for this coarsely chopped silage. Observed trends in particle length and rumen fiber-mat formation may prove beneficial in certain feeding situations. Power requirements for rolling and chopping will be lower and material throughput higher for the coarsely chopped silage.

Rolls should be set and maintained at a 1 mm spacing so that kernel and cob breakage is complete. In some cases, particularly with wet silages, the roll clearance may be expanded to 2 mm or 3 mm; Shinnars (personal communication) reported 91% broken kernels at 3 mm roll clearance with ½ ML silage chopped at ¾" TLC.

We can not support chopping at lengths greater than 3/4" TLC at this time for several reasons. We have no evidence that coarse chopping of corn silage greatly improves animal performance. There have been reports from the field about excessive equipment wear when chopping rolled silages at one-inch or greater TLC.

Also, we are concerned about adequate packing of coarsely chopped silage in the silo and the quality of the ensuing fermentation process. Data in Table 6 (Novak and co-workers, 1999) shows considerable variation in MPL and horizontal-silo packing density for processed corn silages on commercial dairies. It appears that we can improve upon the chopping/rolling currently being done commercially.

Table 6. Variation in mean particle length and horizontal-silo packing density for processed corn silages on 84 commercial Wisconsin dairies (Novak and co-workers, 1999).

Item	Average	Range	Standard Deviation
All samples, n=85			
DM %	35.5	25.5 – 57.3	6.9
MPL, mm	12.5	6.5 – 19.8	2.5
Coarse Particles, %	28.9	4.4 – 58.8	15.1
Subset, n=38			
DM %	34.2	25.5 – 47.7	6.7
MPL, mm	12.5	8.5 – 17.5	2.2
Coarse Particles, %	28.1	4.9 – 58.8	14.2
Packing density, lb DM/cu. ft.	11.9	8.6 – 18.3	2.0

It is often assumed in the field that crop processing is the salvation if BL corn silage is harvested, and at times it is even promoted that way. Data in Table 7 shows the impact of crop processing on ruminal *In situ* macro-bag dry matter, NDF, and starch degradation in early and late chopped corn silage (Bal and co-workers, 1998d).

While processing did improve ruminal starch degradation of late-chop corn silage, the greatest ruminal DM and starch degradation was observed for the processed early-chop silage. Further, processing had inconsistent effects on ruminal stover degradation and no effect ($P > .10$) on ruminal NDF degradation. In summary, processing can improve utilization of BL corn silage through greater starch degradation but it does not fully compensate for the losses incurred with late harvest. Also, processing does not reverse the reduction in ruminal stover and NDF degradation seen with late harvest. We do not recommend delaying harvest greatly when using a crop processor.

Table 7. Impact of crop processing on ruminal *In situ* macro-bag dry matter, NDF, and starch degradation in early and late chopped corn silage (Bal and co-workers, 1998d).

Item	Early Chop - Unprocessed	Early Chop - Processed	Late Chop - Unprocessed	Late Chop - Processed	Processing Effect (P<)
WP Moisture %	64.0	62.4	46.8	51.5	--
Stover RDMD ^a %	44.6	49.0	39.6	36.6	.01
WP RDMD ^a %	58.1	67.1	52.4	62.2	.01
WP RNFDF ^a %	30.2	32.6	21.5	25.1	NS
WP RStarchD ^a %	66.4	84.4	52.5	79.0	.01

^a Ruminal DM, NDF, and starch degradation, respectively.

HYBRID QUALITY EFFECTS

There has been extensive testing of corn hybrids for differences in silage quality, but most of this work has been *in vitro* with relatively few feeding trials (Allen and Oba, 1998). We conducted feeding trials with lactating dairy cows to evaluate “leafy” (Bal and co-workers, 1998a) and brown-midrib (Bal and co-workers, 1999a) corn silage hybrids. Results of the “leafy” and brown midrib trials are presented in Tables 8 and 9, respectively.

The “leafy” hybrid evaluated was Mycogen TMF 106, and the control hybrid was Pioneer 3563. Both hybrids were planted at populations of 24,000 and 32,000 plant per acre. Silages were chopped at ½ ML (3/8 “ TLC without rolling) and stored in silo bags. Twenty-four mature Holstein cows averaging 75 DIM at trial initiation were used in a replicated 4X4 Latin Square with 28 day periods. Main effects in the 2X2 factorial treatment arrangement were hybrid (“leafy” vs “grain”) and plant population (low vs high). All diets were fed as TMR containing half forage of which two-thirds was one of the treatment corn silages and one-third was alfalfa silage (DM basis). All diets were formulated to 18% CP (DM basis) and to meet or exceed NRC guidelines for minerals and vitamins.

Silage made from the “leafy” hybrid contained more whole-plant moisture than silage made from the “grain” hybrid, even though kernel milkline position at harvest was similar. Silage composition was similar for the two hybrids. Lactation performance did not differ between the treatment silages. Total tract digestibility of organic matter and ADF were lower and starch was higher for the “leafy” silage treatments. We observed a 15% unit increase in 24-hour ruminal starch degradation for the “leafy” silage. This is presumed to be related to the softer kernel texture for the “leafy” hybrid, and may be an important characteristic when silage is harvested too dry and a crop processor is not used. Under the harvest conditions of this study, the “leafy” hybrid did not improve lactation performance. Minnesota workers (Kuehn and co-workers, 1998a and 1998b) reported similar results for a “leafy” silage hybrid. It appears, that with regard to “leafy” corn silage, hybrid selection can be based on yield of DM per acre and other agronomic factors.

Table 8. Impact of a “leafy” corn silage hybrid on intake, digestion, and milk production by dairy cows (Bal and co-workers, 1998a).

Item	Pioneer 3563 24,000/acre	Pioneer 3563 32,000/acre	TMF 106 24,000/acre	TMF 106 32,000/acre	SE	(P<)
Moisture %	64.6	63.6	67.1	68.1	--	--
NDF %	44.6	45.7	45.5	46.5	--	--
Starch %	28.5	28.6	29.6	26.2	--	--
DMI, lb/d	60.4	61.1	59.8	59.2	.8	.10
Milk, lb/d	88.7	89.9	89.2	88.5	1.0	NS
Fat, lb/d	3.16	3.11	3.17	3.15	.05	NS
CP, lb/d	3.03	3.02	3.05	2.96	.04	NS
DOM, %	65.1	63.1	62.4	62.3	.6	.01
DADF, %	35.8	31.1	29.0	29.9	1.4	.01
DStarch, %	92.3	92.4	94.2	94.4	.6	.01

The brown midrib hybrid evaluated was Cargill FullTime™, and the control hybrid was Pioneer 3563. Silages were harvested at ½ ML (3/8” TLC without rolling) and stored in upright stave silos. Both hybrids were planted on the same day, but the BMR was harvested 5 days later than the control to achieve the same kernel milkline position at harvest.

Twenty-six Holstein cows averaging 120 DIM at the start of the trial were in a crossover design with 8 wk periods. Because of lower pretrial NDF content of the BMR and its reported (Allen and co-workers, 1997) higher NDF digestibility, control silage was fed in a low-forage diet and BMR silage was fed in a high-forage diet. Rations were formulated with 2/3 corn silage and 1/3 alfalfa silage in the forage DM to 18% CP using ground dry shelled corn, expeller soybean meal, and urea. The control diet contained 47% forage (DM basis). The BMR diet contained 60% forage (DM basis). Whole cottonseed and sodium bicarbonate were included in both rations at 7% and .8% of ration DM, respectively. Rations were fed as TMR individually to cows twice daily following once daily mixing.

Table 9. Impact of brown midrib corn silage on lactation performance by dairy cows (Bal and co-workers, 1999a).

Item	Pioneer 3563 Low Forage	Cargill BMR High Forage	SE	(P<)
Diet Forage ^a , % DM	47	60	--	--
DMI, lb/d	62.6	62.7	.4	NS
Milk, lb/d	98.2	95.1	.2	.001
Fat, %	3.18	3.46	.05	.001
Fat, lb/d	3.06	3.24	.05	.05
CP, %	3.27	3.20	.01	.001
CP, lb/d	3.14	2.97	.02	.001
BW Change, lb/d	1.16	1.25	.1	NS

^a67% corn silage and 33% alfalfa silage (DM basis).

In situ DM disappearance at 24 hours measured in three ruminally-cannulated cows was 58.4% and 62.4% for control and BMR silages ($P < .02$), respectively. This allowed for similar DM intakes for control and BMR diets, even though the BMR diet contained 13% units more forage DM. Milk production was lower for BMR fed in a high-forage diet. Milk fat yield was higher for BMR because of an increase in milk fat test, but milk protein percentage and yield were lower.

Oba and Allen (1997) reported that BMR increased milk production 6.0 lb./cow/day over its isogenic counterpart in 56% forage diets (DM basis). Oba and Allen (1998) reported that BMR increased milk production 7.5 lb./cow/day over its isogenic counterpart in both 29% and 38% NDF diets (DM basis). Feeding BMR in the low NDF diet reduced milk fat test .4% units. Comparison of control silage-low NDF versus BMR silage-high NDF diets shows similar milk yield, higher milk fat percent and yield, and lower milk protein percent for BMR silage-high NDF diet.

Results from this comparison are similar to our findings with BMR fed in a high-forage diet. More research with various types of diets is needed to optimize the utilization of BMR corn silage in dairy cattle diets. With regard to brown midrib corn silage, hybrid selection criteria should include the potential for lactation response and feed cost savings weighed against the extra seed cost and yield drag.

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UW Arlington Dairy Day

Wednesday, December 16, 1998

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- 9:00 - 10:00 a.m. **Registration**
- 10:00 - 10:40 a.m. **Update on UW-Madison Corn Silage Feeding Trials**
Randy D. Shaver, Ph.D.
Department of Dairy Science
- 10:40 - 11:20 a.m. **Current Research in Forage Utilization at the UW-Dairy Science Department**
David K. Combs, Ph.D.
Department of Dairy Science
- 11:20 - 12:00 Noon **The Prospects for Robotic Milking in Wisconsin**
Douglas J. Reinemann, Ph.D.
Department of Biological Systems Engineering
- 12:00 - 12:40 p.m. **Lunch**
- 12:40 - 1:30 p.m. **Breakout Sessions**
- Reproductive Management of Dairy Cows Using Ultrasound*
Paul M. Fricke, Ph.D., Department of Dairy Science
- Dairy Hoof Health - "Is it Really Laminitis, Doc?"*
Garrett R. Oetzel, D.V.M, M.S., Department of Dairy Science &
School of Veterinary Medicine
- Kernel Processing Demonstration*
Kevin Shinnars, Ph.D.
Department of Biological Systems Engineering
- Bunker Silage Management Demo*
Brian Holmes, Ph.D., Department of Biological Systems Engineering
Ken Bolton, Jefferson County, UW-Extension
- 1:40 - 2:20 p.m. **Emerging Mastitis Pathogens - Are these "new" bugs in your future?**
Pamela L. Ruegg, D.V.M., M.P.V.M.
Department of Dairy Science
- 2:20 - 3:00 p.m. **Future Employment of Cows in Wisconsin**
Robert Bremel, Ph.D.
Department of Dairy Science
- 3:00 p.m. **Adjourn/Tour of Facilities (Optional)**



UW Arlington Dairy Day

Wednesday, December 16, 1998

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Table of Contents	Page
Update on UW-Madison Corn Silage Feeding Trials	7
Randy D. Shaver, Ph.D. Department of Dairy Science	
Current Research in Forage Utilization at the UW-Dairy Science Department	19
David K. Combs, Ph.D. Department of Dairy Science	
The Prospects for Robotic Milking in Wisconsin	33
Douglas J. Reinemann, Ph.D. Department of Biological Systems Engineering	
Breakout Sessions	
<i>Reproductive Management of Dairy Cows Using Ultrasound</i>	39
Paul M. Fricke, Ph.D., Department of Dairy Science	
<i>Bunker Silage Management Demo</i>	
Brian Holmes, Ph.D., Department of Biological Systems Engineering Ken Bolton, Jefferson County, UW-Extension	45
Emerging Mastitis Pathogens - Are these “new” bugs in your future?	51
Pamela L. Ruegg, D.V.M., M.P.V.M. Department of Dairy Science	
Future Employment of Cows in Wisconsin	59
Robert Bremel, Ph.D. Department of Dairy Science	

