A CASE STUDY ON FEEDING FIBER-DIGESTING ENZYMES TO DAIRY CATTLE

J. D. Johnston
Ritchie Feed & Seed Ltd.

Gloucester, Ontario, Canada

INTRODUCTION

Successful dairy farming in the present age is a challenge as today's dairies face the universal problems of diminishing returns on equity and ever more pressing environmental rules. Solving these problems is not a matter of finding one magical answer, as success lies in understanding and utilizing the knowledge base of multiple disciplines. Nutrition is but one of these disciplines, however as ration expense is usually the largest cost component faced by producers consideration of any process or product that raises returns is worth looking at. Improvements in the engineering of dairy feeding systems and a better understanding of ration digestibility have shown obvious promise[i], as have harvesting equipment changes such as kernel processors.

Production increases of 10%[ii] and improvements in dry matter intake[iii] have been reported when kernel processing was employed. Responses have not always been positive nor has there been consistent improvements in milk protein or fat levels[iv]. The reasons for this variability in response are not entirely understood, but the stage of crop maturity, ruminal starch disappearance, and the dynamics of fiber disappearance are reasonable guesses. It must however be remembered that kernel processors are designed primarily to affect the rate of starch availability and their effect on fiber digestion per se does not appear to be large[v]. There use does, however, allow for harvesting corn silage with a longer cut length, and hence a higher effective NDF level. This in turn would suggest a longer ruminal residency time for the fiber fraction and as a result offers the opportunity to modify the digestion rate of the stalk and leaves. As the stalk and leaves represent 40-60% of most corn silages, the implications of improving their digestibility are significant and should therefore not be ignored.

The use of exogenous fibrolytic enzymes is another possible option that could be used to improve dietary fiber disappearance rates, and several authors have reported on their successful use[vi vii viii]. Most of the research reported to date involved the use of enzymes sprayed on to the forage before incorporation into the final diet. This paper will deal with the evaluation of a dry commercial enzyme supplement (Fibrozyme, Alltech, Inc.) through the measurement of its effects on In Vitro NDF digestion of corn silage and the production responses of a commercial dairy herd.

Measuring NDF Digestibility

Measurements of NDF digestibility rates can be achieved using In Vivo, In Situ, or In Vitro techniques. Of the three the In Vivo is undoubtedly the best, although it is very costly. In Situ techniques have been shown to offer an accurate measurement of rumen degradation rates over various outflow rates[ix], but the logistics of chemical analysis of the digested samples are time consuming and do not lend themselves to automation. In addition the technique suffers from concerns of overcoming the cow to cow variances implicit in the technique's use. On the other hand, In Vitro analyses have been greatly simplified with the marketing of new analytical systems[x], and their use for measuring 30hr IVNDF digestibilities has grown. This type of analysis is not without its problems, as their use relies on several assumptions for pH and residency times. They are however a tool which can be used to screen large numbers of samples in an effective and affordable manner.

Methodology is obviously of critical importance, as the technique used must demonstrate accuracy without interfering in fermentation in order to describe feed ingredients in a manner that can be used in rumen models. Grant and Mertens[xi] reported that lag times were an important concern as they can be lengthened by decreased pH levels or by the inclusion of non-structural carbohydrates. Recently reported work[xii] has shown that neither the lag time nor the extent of NDF digestion is affected at pHs in the 6.2 to 6.8 range, although the overall rate can be diminished. Several studies[xiii xiv] have shown that this range of 6.2-6.8 is the threshold for optimal NDF digestion, hence any In Vitro analyses should be conducted with innoculum pHs between those levels.

The In Vitro measurement of digestibility has progressed from the early two-stage technique[xv] to the more automated systems of today. Several authors have reviewed the newer techniques[xvi xvii] and have shown that the results obtained form the new systems are reliable and repeatable. A recent comparison between the filter bag and conventional tube technique reviewed 72 combinations of whole crop, leaf, or stem from two forage species measured over three grinding sizes for several measurements of digestibility including NDF. The results showed that while the standard errors and coefficients of variation were somewhat higher for the filter bag technique, the true and apparent digestibilities from the tube method[xviii] were accurately predicted by the respective estimates from the bag technique.

The experiment referred to above involved the use of the Ankom Daisy II equipment with which the NDF digestibility can be measured for forages, grains, or total mixed rations. The system utilizes miniature filter bags that are immersed in filtered rumen innoclum and buffer. The fermenting chamber holds up to four jars that can contain up to twenty-five filter bags each, hence the system offers a great deal of flexibility as far as experimental design is concerned. Analysis of results using the system has shown that similar samples can be successfully fermented within one jar or mixed with other samples within the same jar. As the samples are digested in separate filter bags, sequential ADF and NDF analyses can be performed with a minimum of problems once the fermentation is complete.

Results of In Vitro Forage Analyses

The use of In vitro systems to measure 30hr IVNDFD is certainly of value and it has been used as one method of ranking corn silage varieties[xix]. In this work, varieties from three different companies were compared over a variety of nutrients. In Vitro true dry matter disappearance (IVTDMD) was shown to be statistically ($P<0.07$) different, as was In Vitro NDF disappearance. Further work conducted at the Miner Institute showed IVNDFD rates for corn silages that ranged from 24.87 to 61.56% with a C.V. of 19.53%, for samples originating from farms throughout New England[xx]. Multivariate analysis of these samples demonstrated that rainfall and temperature also contributed significantly to the variation in IVTDMD[xxi]. Further data on forages and TMR's is given in Tables 1 and 2.

Table 1. Variation in NDF and Digestibility of Northeastern Forages

Feed	Number of Samples	Analysis	Average	Minimum	Maximum
Alfalfa Silage	35	NDF%	50.6	35.88	69.71
		DNDF%	51.6	36.93	65.26
		DMD%	75.15	56.03	85.67
Corn Silage	50	NDF%	46.65	32.09	63.42
		DNDF%	43.45	24.87	61.56
		DMD%	73.87	61.79	85.00
Grass Silage	49	NDF%	61.35	47.61	72.55
		DNDF%	48.21	35.10	65.72
		DMD %	67.59	53.37	83.68

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Table 2. In Vitro Digestibility Results

Feed	Analysis	Average	Standard Deviation	Low Value	High Value
Hay	IVTD %a	67.5	11.2	45.3	85.8
	dNDF % b	41	9	24	62
	IVTD-Nel Mcal/kg c	1.21	0.24	0.70	1.54
Haylage	IVTD %	70.8	10.5	40.9	83.8
	dNDF %	48	8	28	61
	IVTD-Nel Mcal/kg	1.25	0.24	0.61	1.56
Corn Silage	IVTD %	72.5	10.5	47.3	87.4
	DNDF %	43	7	28	54
	IVTD-Nel Mcal/kg	1.38	0.26	0.79	1.78
TMR	IVTD %	81.7	3.3	74.5	87.1
	dNDF %	51	7	41	62
	IVTD-Nel Mcal/kg	1.60	0.08	1.40	1.78

Source Dairy One Lab Ithaca New York
a In vitro true digestibility
b Digestible NDF

c Nel calculated from dNDF results

A similar use of In vitro analyses was begun at Ritchie Feed and Seed in the summer of 1997. The initial thought was to try and categorize forage samples by variety and geographic area. This effort soon proved to be all but futile, as large intra and intervarietal differences were observed both within and between sampling locations. Some of this variability was anticipated given the soils of Eastern Ontario, but the CV was not expected to be as large. Upon realizing the degree of variability (Table 3), work was begun to try and further quantify the differences between forage samples. Production differences between herds of similar DIM fed TMR's based on corn silages with like 30hr IVdNDFs led us to believe that the answer perhaps lay in the degree and extent of digestion expressed at various time points. The problem then becomes one of which time points should be selected, and whether the choice remains constant. Our experience has shown that time point selection is not constant as the effect of maturity at harvest requires varied time point selection.

Table 3. In vitro NDF Analyses for Corn Silage

N	100	100	100	100	
Mean	44.598	40.489	38.415	24.526	20.249
SD	5.9941	4.6858	4.8739	4.5757	3.6616
SE Mean	0.5689	0.4448	0.4626	0.4343	0.3475
C.V.	13.440	11.573	12.687	18.656	18.083
Maximum	56.800	52.400	50.800	37.900	

THE IMPLICATIONS OF VARIATIONS IN NDF DIGESTIBILITY

The dairy industry is all about dry matter intake. The more dry matter a cow consumes, the more milk and milk components the cow will produce. It is therefore important to understand the quality and digestibility of the forage being fed in order to maximize intake. Unfortunately many predictive equations and computer programs currently used in North America yield unsatisfactory results as they are based on static rather than dynamic analytical techniques. The basic assumption in these approaches is that all fibers are digested at the same rate and are passed from the rumen at the same rate. These techniques may have worked initially, but newer data has shown that it is time to move on, be it with the CNCPS or MOLLY type models or the Japanese developed Oa Ob system[xxii], which delineates fast and slow fiber fractions. The Oa fraction is the amount of fiber that disappears in a 4-6 hour digestion with a fiber digesting enzyme system, while the Ob fraction represents the slower fraction.

With the release of the feeding programs that include predictions of rumen yield we have been able to see how alterations in the NDF disappearance rate can influence the level of milk production through changes in rumen pH and the extent of rumen microbial protein synthesis. The technique that was found to be most successful was that of using time point analyses to estimate rapid and slow NDF digestion rates. Results from this approach are given in Table 4, which shows how a swing of 6 points in the soluble NDF fraction can have a dramatic effect on milk production and on predicted NEL levels. The data in Table 4 was generated from In vitro analyses using the CNCPS model and corrected 48hr NDF levels. The carbohydrate B2 (C:B2) fraction is equivalent to the rate of NDF disappearance, and while it is accepted that NDF digestion rates of 2%/hr would not occur that often, the table does demonstrate how predicted milk yield and Nel levels can change with varying NDF digestibilities. Another view was offered by Michigan researchers who proposed that a 1 unit increase in NDF digestibility would result in a 0.16kg and a 0.24 kg improvement in dry matter intake and milk production respectively.[xxiii]

C:B2 Rate %/hr	Milk Yield kg CPM	Predicted NEL
2	31.8	1.43
4	34.0	1.60
6	35.6	1.70
8	36.7	1.77

ECONOMIC IMPACT OF CHANGES IN NDF DISAPPEARANCE

Reviews of the diets fed high producing dairy herds have shown that they usually include a large percentage of highly fermentable NDF, and that these types of ingredients result in both increased microbial yields from the rumen[xxiv] and improved milk production. The improvements occur for many reasons, but an overall improvement in rumen health would appear to be the most logical explanation. The economic potential of harnessing improvements in fiber digestion can be significant, and in today's tight market it can be a lifesaver. As an example of just how large savings in production costs can be, data was collected from a 150-cow free stall herd producing approximately 30 liters per cow per day. The farm has two silos for corn silage. One silo had corn silage stored at 36% DM with a C:B2 rate of 3.07 %/hr and the other silo had corn silage at 32% DM with a C:B2 is at 7.0%/hr. Switching from one silo to the other resulted in a 0.75 liter in increase in milk[xxv] and a saving of $0.69 Cdn/hd/day.

DIETARY RESPONSE OF ENZYMES IN RUMINANTS

The inclusion of enzymes in the diets of mongastrics is quite common. Their use in ruminant diets has not been as prevalent, as the fibrolytic activity of a rumen is such that the inclusion of further exogenous enzymes in the diet was felt to be unwarranted. Additional concerns have been that these enzymes cannot survive proteolysis in the rumen or that their activity would be diminished by delivery systems such as pelleting. Recent research has proven that these concerns may be unwarranted, as positive effects have been noted in both dairy and beef cattle[xxvi xxvii]. Production responses have varied from feed efficiency improvements of 10% in beef cattle[xxviii] to milk increases of 2.5 kg when lactating cattle were fed a TMR diet treated with a fibrolytic enzyme product[xxix]. A recent two year study of examining the use of a cellulase/xylanse mixture fed to lactating Holsteins demonstrated that the productive improvements in milk yield and 3.5% FCM were most likely due to improved NDF digestion. These authors reported a significant ($P<0.05$) increase in 48hr. NDF digestion but only a numerical increase at 12hr. Other authors have shown a significant ($P<0.05$) increase in NDF digestion within six hours while the effect was lost when digestion times of 30 or 48 hours were looked at[xxx](Table 5). This time difference is important, as it would appear that productive responses do occur if NDF digestion improvements take place within the first six hours after ingestion.

FIELD TRIALS

As the mode of action of the various enzyme products appears to vary according to their make up, generalized conclusions cannot really be drawn. With this in mind a field trial was undertaken to see if the product Fibrozyme affected In Vitro NDF disappearance rates, and if so in what manner. The study involved the use of four corn silage hybrids planted at three densities of 22K, 27K, and 32K per acre. Samples were taken following a 90-day fermentation, with NDF disappearance rates being calculated from the results of In Vitro analyses. Fibrozyme was added to the treatment groups at a rate that would be equivalent to 15gm/hd/day. The hybrids selected represented a reasonable cross section of those available to today's producers and included both leafy and grain type corn plants. Neutral detergent fiber levels were determined using equipment produced by Ankom Technologies. Rumen fluid was taken from lactating Holsteins producing in excess of 30 l per day. The fluid was strained through four layers of cheese cloth and added to a Kansas State buffer system. The samples were fermented for 0, 3, 6, 30, and 48 hours at a constant temperature of 39.1 C. The hybrids used in the study were grown with three replicates per hybrid for all planting densities and were analyzed with three replicates. A summary of the trial is given in Table 5

	Initial rate of digestion (6 h)			Extended rate of digestion (30 h)			NDF remaining (48 h)	
All hybrids	Control	Fibrozyme	% Increase	Control	Fibrozyme	% Increase	Control	Fibrozyme
22K	1.39	2.13	72.8	13.08	12.86	19.9	20.10	19.27
27K	2.49	3.32	69.3	14.83	12.81	-11.7	19.89	19.86
32K	2.57[a]	4.66[b]	196.7[c]	14.35	15.17	13.3	21.20	20.37
Overall	2.15[a]	3.38[b]	112.9[c]	14.09	13.61	7.1	20.39	19.83

The results demonstrate that Fibrozyme does give rise to a significant ($p<0.05$) improvement in the disappearance of NDF across corn silage hybrids and planting rates within the first 6 hours of in vitro fermentation. This effect is of particular interest as field reports on the success of enzymes have been mixed. Use in the field has no doubt been based on "a one size fits all strategy" and the fact that there appeared to be a significant hybrid effect within the overall Fibrozyme effect may explain the variation in field response. Correlations between the use of fibrozyme and plant characteristics or digestion rates were calculated (Table 6). Several relationships between ADF and NDF were also examined as it was hypothesized that the success of Fibrozyme could be predicted by NDF-ADF differences. This theory did not prove to be statistically significant, but the analyses did show that there is a window of opportunity for use of the product based on the level of NDF within the first six hours of In vitro fermentation. The window lies within the 37 to 42% range as may be seen in Figure 2. The practical importance of this information lies in the fact that Fibrozyme would not appear to alter the NDF disappearance of high quality corn silage nor will it improve that of poor quality silage. It would however significantly impact the corn silages that fall in between, and as is shown in Table 1 this includes a wide range of corn silages. The data for this table was drawn from the analyses of corn silage samples grown in similar environmental conditions during the summer of 1998 in the Ottawa Valley.

Table 6. The Relationship between Fibrozyme and the Corn Silage Characteristics

Factor	Correlation
Corn Hybrid	0.077
Planting Density	0.233*
NDF Content	-0.259*
ADF Content	-0.389*
Ratio NDF/ADF	-0.024
NDF-ADF	-0.136
Initial NDF Digestion	-0.406*

*P<0.05

Figure 1.
Effects of ADF Content of Corn Silage on In Vitro Activity of Fibrozyme

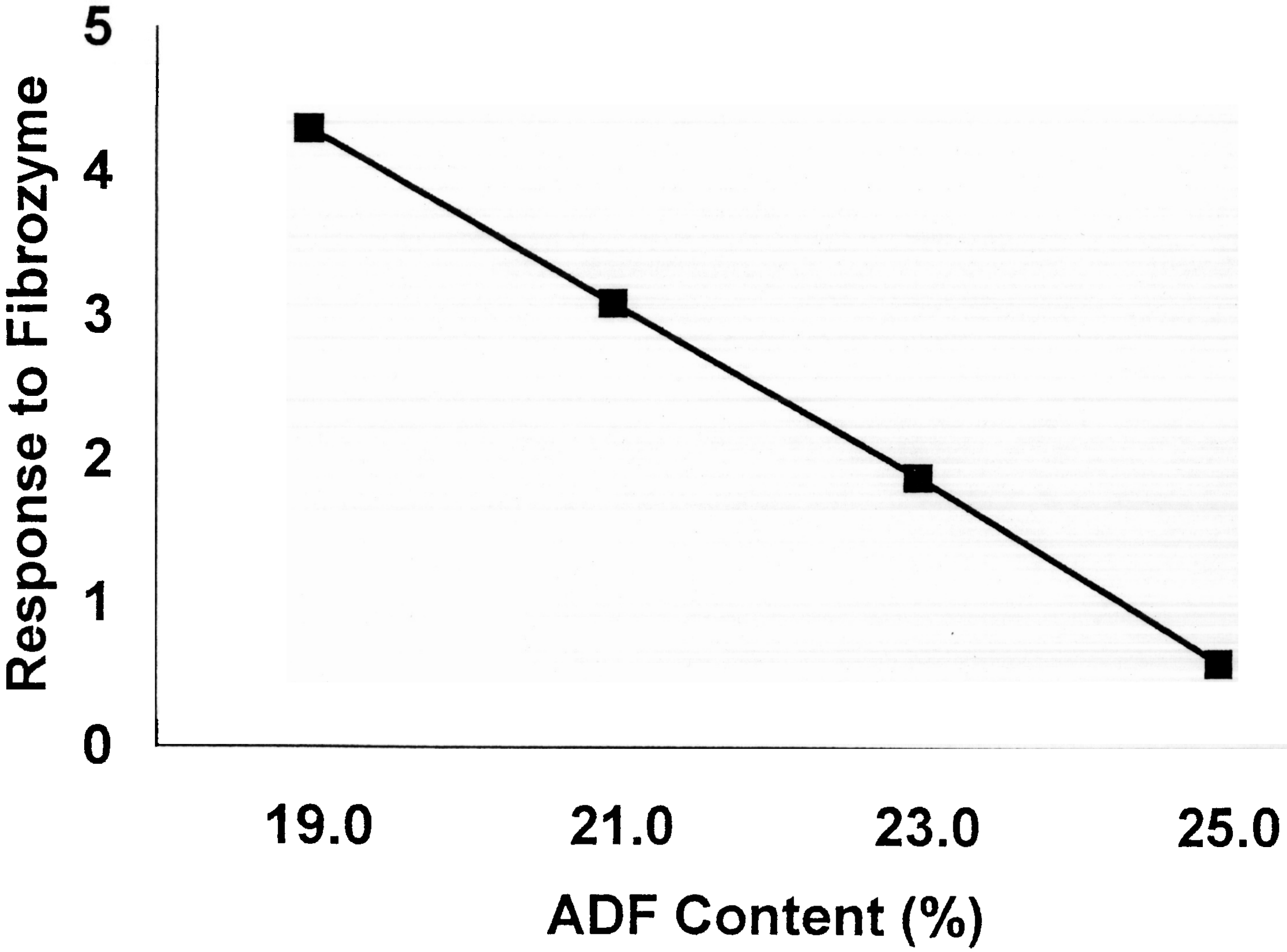
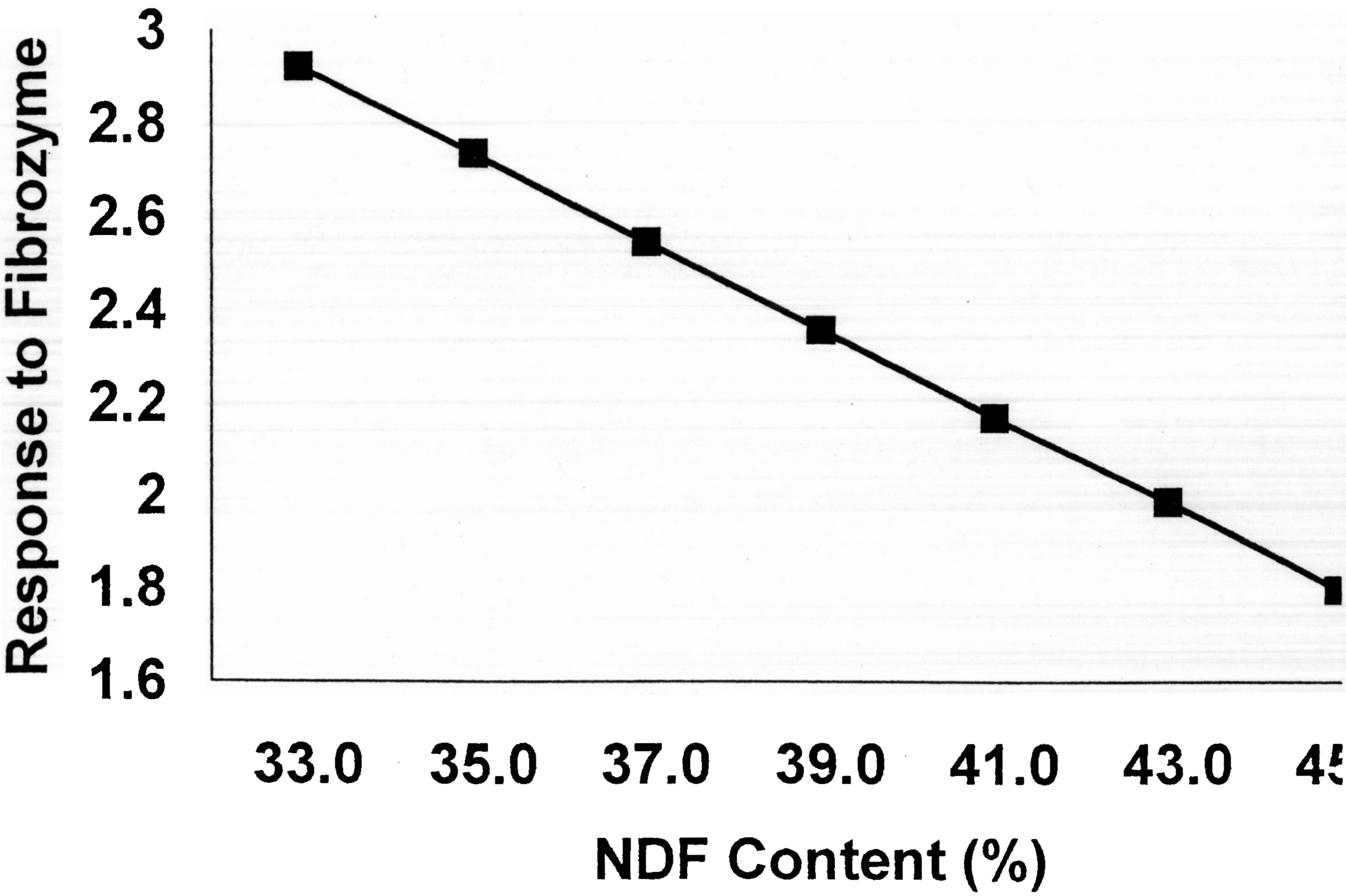


Figure 2.
Effects of NDF Content of Corn Silage on In Vitro Activity of Fibrozyme



3
2.8
2.6
2.4
2.2
2
1.8
1.6
Response to Fibrozyme
33.0
35.0
37.0
39.0
41.0
43.0
NDF Content (%)

APPLICATIONS AT A COMMERCIAL DAIRY LEVEL

Having shown that a window of opportunity existed for the use of the Fibrozyme product, the next step was to ascertain whether the In Vitro analysis screening technique could be applied at the field level. A farm whose 1999 corn silage fell within the correct window of opportunity was selected and Fibrozyme was added to the diet in a commercial supplement. The silage was fermented for eighty days prior to its being fed, with pH and visual assessments being made to ensure a good fermentation had taken place. The diet was balanced using the CPM model with considerations being made for rate and extent of fiber digestion, and was approached on a commercial basis in order to review whether laboratory results were directly applicable to field. The DHIA results for the farm are given in Table 7, with the 1999 corn silage usage beginning two weeks prior to the October test date. As may be seen the herd dropped 2 liters once the 1999 corn silage crop was fed.

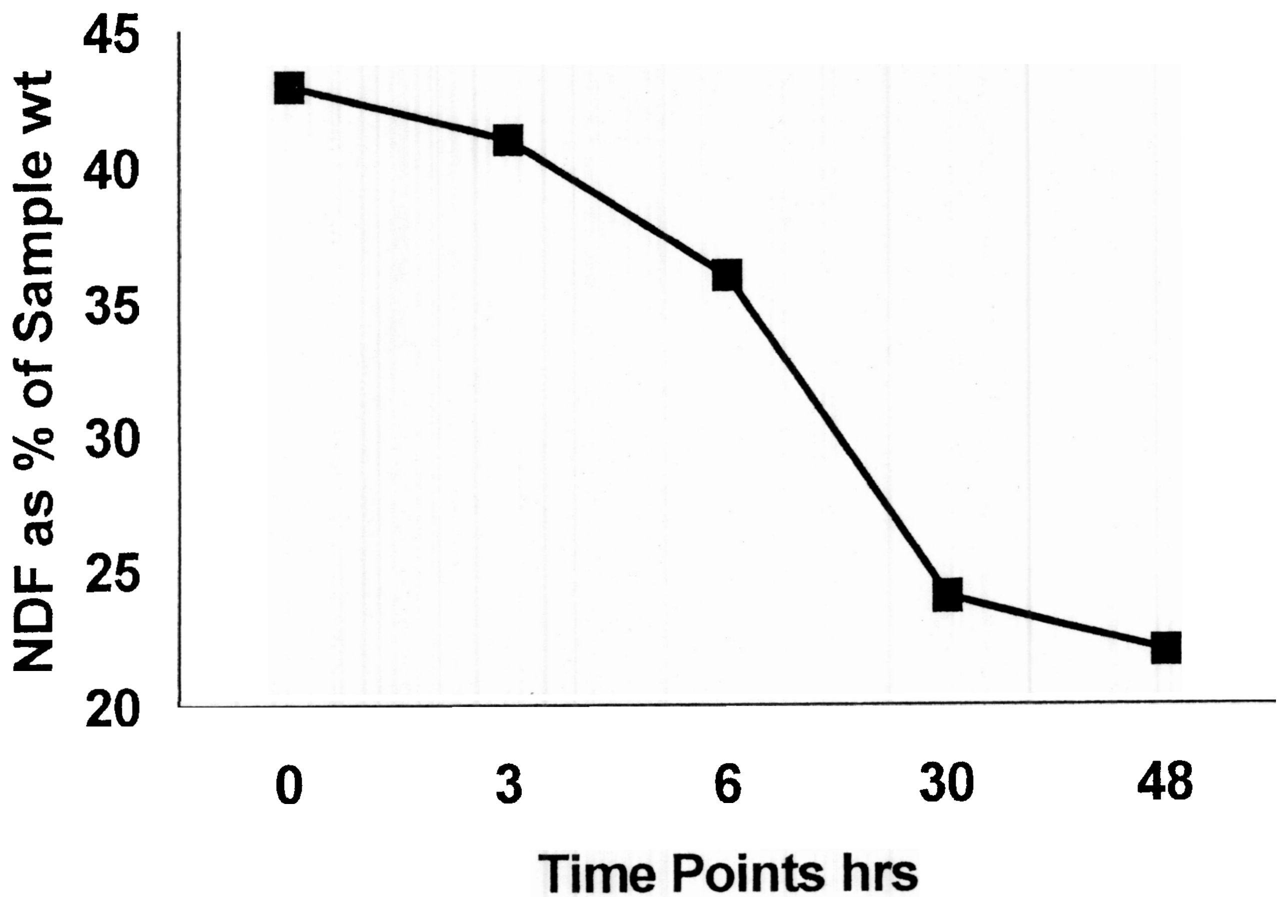
The response to the inclusion of the 1999 corn silage, when the other diet components were left similar, was a loss of milk within five days. The effect of the inclusion of Fibrozyme was that the milk returned to the previous levels. As luck would have it these dietary changes fell in line with monthly DHIA testing and the milk response was recorded with similar days in milk. This was extremely encouraging as it appeared to demonstrate that diet formulation based on the corn silage parameters given above did in fact work. Fortunately few cows freshened or were dried off during this period, and while this was due more to good luck than good experimental management, the production improvements did lead to the conclusion that it would be worthwhile to run a more controlled experiment that removed cow day effects while also trying to explain if the effect of the enzyme was due to intake improvements.

Table 7. Effect of Fibrozyme use on an 81 Cow Dairy

	1998 Corn Silage	1999 Corn Silage - Fibrozyme	1999 Corn Silage + Fibrozyme
Avg. DIM	183	184	184
Avg. Production l/d	27	25	27
Avg. Milk Fat %	4.1	4.1	3.9
Avg. Milk Protein%	3.5	3.5	3.5

The second feeding trial used a stall dairy with 100 cows and was run as a two-week switch back trial. The make up of the herd was such that the majority of the cows on test were in either their first or second lactations, but this was felt to be a positive as these animals would face the greatest challenge as far as intake was concerned (Table 8). Corn silage samples were collected prior to and during the course of the trial to identify the NDF characteristics, and the average of ten samples is shown in Figure 3. As may be seen the NDF fractions of the corn silage fell within the 37 to 42% window of opportunity and the CPM model was used to design the diet used in the trial. Fibrozyme was added to the diet at 15mg/hd/day by inclusion in a protein supplement that was pelleted at 165F and 137 kPa in order to minimize the possibility of enzyme denaturation. Once the trial began the diet proportions remained constant save for dry matter corrections. In order to minimize any production variability arising from incompletely fermented corn silage, the diet was based on silage harvested in the fall of 1998 and stored in a sealed bunker since harvest. The diet composition and trial results are given below (Table 9-12).

Figure 3.
NDF Disappearance in Trial Corn Silage N=10



As previous field experience had shown that forages with constant rates of NDF disappearance yield more productive milk responses in lactating cattle, efforts were made when formulating the diet to keep the disappearance rate of NDF in the TMR diet as constant as possible. This was done by matching the NDF rates of the ingredients to the theoretical ideal, and the results are shown in Figure 4. Of interest is that the predicted C:B2 from the CPM model was 7.6%/hr while the calculated rate from our results was 7.2 %/hr, hence some degree of comfort can be placed in the concept of designing rations along these lines. A similar exercise was run on the protein side, with the theoretical SIP being 41% while the calculated amount from the lab analyses was 49%.

Table 8. Lactational Status of Cows Used in the Trial

LACTATION	AVE DIM AT START	RANGE IN DAYS
1	86.1	21-171
2	133.7	13-388
3	132.0	14-262

Table 9. Ingredient and Chemical Composition of Total Mixed Ration

COMPOSITION	% OF DRY MATTER
Hay	1.74
Haylage	21.4
Corn Silage	26.73
HM Corn	31.09
Raw Soybeans	9.44
Protein Pellet	9.60
Chemical Analysis	
DM	43.1
CP	16.2
NDF	32.3
Starch	31.9
Nel Mcal/kg Calculated	1.61

Table 10. Changes in Milk Production (kg/hd and %) Associated with the Addition of Fibrozyme

Parity		Period 1 +Fibrozyme	Period 2 -Fibrozyme	Period 3 +Fibrozyme
1	N=50	29.92 a +5.2%	28.68 b -1.5%	28.31 b +0.31%
2	N=30	35.8 a +6.0%	34.1 b - 4.53%	33.5 b +0.20%
3	N=20	33.71 a +1.48%	32.63 ab - 1.18%	29.98 b -8.81%

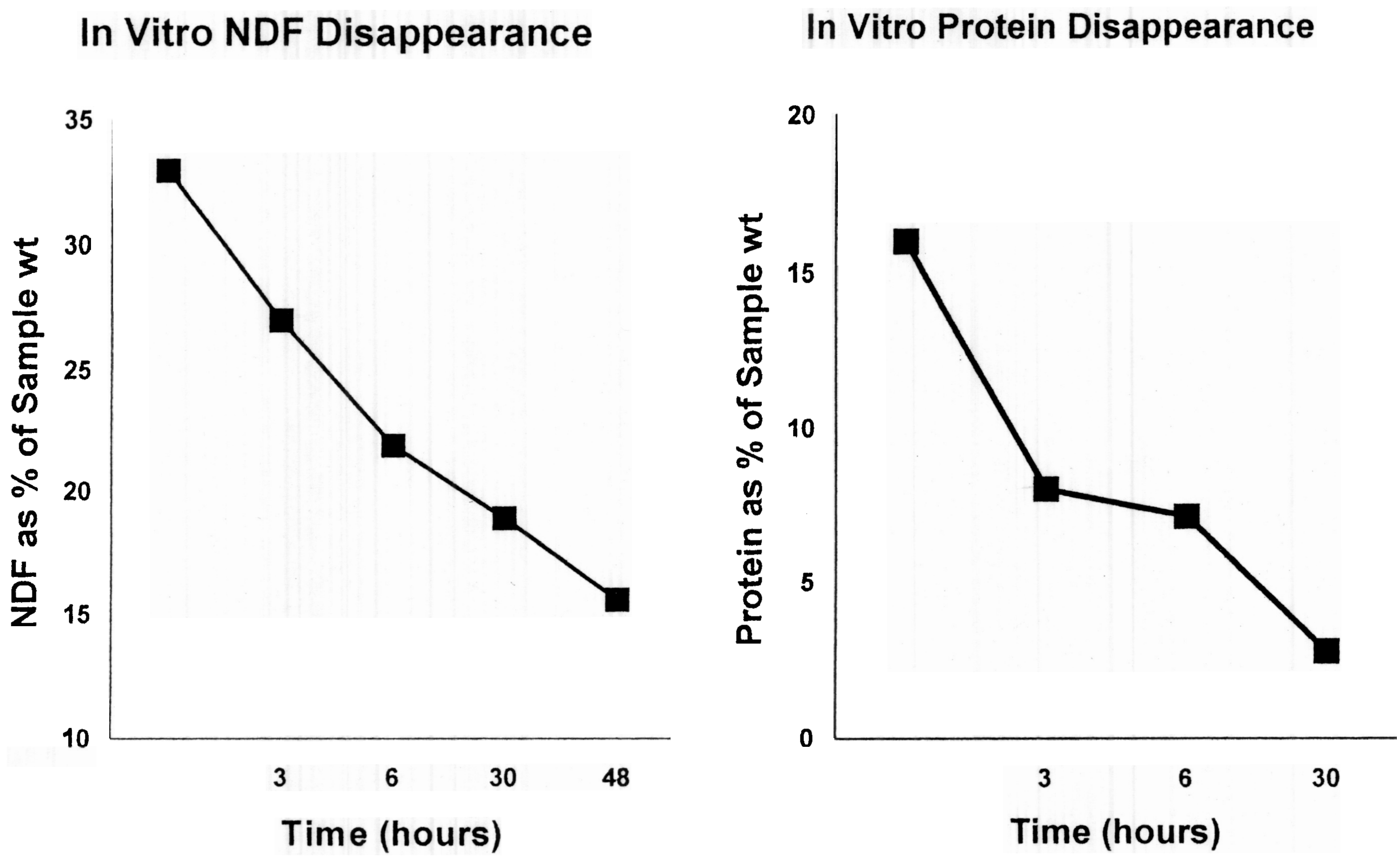
Table 11. Changes in Milk Fat (kg/hd/day) Associated with the Addition of Fibrozyme

Parity		Period 1 + Fibrozyme	Period 2 - Fibrozyme	Period 3 + Fibrozyme
1	N=50	1.13 a	1.05 a	1.05 a
2	N=30	1.46 b	1.34 a	1.34 a
3	N=20	1.23 a	1.23 a	1.18 a

Parity	Period 1 + Fibrozyme	Period 2 - Fibrozyme	Period 3 + Fibrozyme
1 N=50	0.93	0.86	0.91
2 N=30	1.07	1.05	1.06
3 N=20	1.04	1.03	0.95

Figure 4.

TEST DIET R



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[i] Spain, Jim. 1998. Profitable Forage Management Strategies. Western Canadian Dairy Seminar, Advances in Dairy Technology.
[ii] Straub, R.J., R.G. Kroegel, L.D. Satter, and T.J. Krause. 1996. Evaluation of a corn silage processor. ASAE paper no. 961033. American Society of Agricultural Engineers, St. Joseph. MI.
[iii] Schurig, M., and G. Rodel. 1993. Power consumption and the effect of corncrackers. ASAE paper no. 931586. American Society of Agricultural Engineers, St Joseph, MI.
[iv] Miller, C.N., C.E. Polan, R.A. Sandy, and J.T. Huber. 1969. Effect of altering physical form of corn silage on utilization by dairy cattle. J. Dairy Sci. 52:1955-1960.
[v] Paper on maceration
[vi] Rode, L.M., W.Z. Yang, and K.A. Beauchemin. 1999. Fibrolytic Enzyme Supplements for Dairy Cows in Early Lactation. J. Dairy Sci. 82:2121-2126.
[vii] Kung Jr., L., R.J. Treacher, G.A. Nauman, A.M. Smagla, K.M. Endres, and M.A. Cohen. 2000. Rhe Effect of treating Forages with Fibrolytic Enzymes on its Nutritive Value and Lactation Performance of Dairy Cows. J. Dairy Sci 83:115-122.
[viii] Zinn, R.A. and J. Salinas. 1999. Influence of Fibrozyme on digestive function and growth performance of feedlot steers fed a 78% concentrate growing ration. In "Under the Microscope - Proceedings of Alltech's 15th Annual Biotechnology in the Feed Industry Symposium" (T.P. Lyons and K Jacques, eds.). Nottingham University Press, UK.
[ix] Arieli, S.J., J. Mabjeesh, Z. Shabi, I. Bruckental, Y. Aharoni, S. Zamwel, and H. Tagari. In Situ Assessment of Degradability of Organic Matter in the Rumen of the Dairy Cow. 1996. J. Dairy Sci. 81:1965-1990.
[x] AnkomTechnologies
[xi] Grant, R. J., and D.R. Mertens. 1992. Development of buffer systems for pH control and evaluations of pH effects on fibre digestion In vitro. J. Dairy Sci. 75:1581-1587.
[xii] Piwonka, E.J., and J.L. Firkins. 1996. Effect of glucose fermentation on fibre digestion by ruminal microorganisms In vitro. J.Dairy Sci. 79:2196-2206.
[xiii] Grant. R.J., and S.J. Weidner. 1992. Digestion kinetics of fibre: influence of in vitro buffer pH varied within observed physiological range. J.Dairy Sci. 75:1060.
[xiv] Hiltner, P., and B.A. Dehority. 1983. Effect of soluble carbohydrates on digestion of cellulose by pure cultures of rumen bacteria. Appl. Environ. Microbiol. 46:642.
[xv] Tilley, J.M.A., and Terry, R.A. 1963. A Two-Stage Technique for the In Vitro Digestion of Forage Crops. Journal of British Grassland Society, 18:104-111.
[xvi] Garman, C.L., L.A. Holden, and H.A. Kane. 1997. Comparison of In Vitro Dry Matter Digestibility of nine feedstuffs using three different methods of analysis. J. Dairy Sci. Volume 80, Supplement 1, p 260.
[xvii] Cohen, M.A., H.E. Maslanka, and L. Kung, Jr. 1997. An Evaluation of Automated and Manual In Vitro Methods for Estimation of NDF Digestion. Conference on Rumen Physiology, Chicago, IL.
[xviii] Wilman D. and A. Adesogain.2000. A comparison of filter bag methods with conventional tube methods of determining the in vitro digestibility of forages. J. Dairy Sci. 83: Supplement 1, Abstract 1212.
[xix] Thomas, E.D., C.S. Ballard, C.J.Sniffen, D.S. Tsang, R.D. Allhouse, and P. Mandebvu. 1998. Effect of Hybrid on Corn Silage yield, Nutrient Composition, InVitro Digestion, Intake by Holstein Heifers, Intake and Milk Production by Lactating Holstein Cows. W.H. Miner Agricultural Research Institute, Chazy, NY. 98-13.
[xx] Allshouse, R.D., C.J. Majewski, and C.J. Sniffen. Investigations in Forage Quality. ! Variation in Forage Quality in the NorthEast. W.H. Miner Agricultural Research Institute, Chazy, NY. 98-8.
[xxi] C.J. Majewski, R.D. Allshouse, and C.J. Sniffen. Investigations in Forage Quality. !! Variability in Forage Quality Parameters for Corn Hybrids. W.H. Miner Agricultural Research Institute, Chazy, NY. 98-8.
[xxii] Protein and Amino Acid Nutrition of Lactating Cows – An Update. Charles J. Sniffen, W.H. Miner Agricultural Research Institute and William Chalupa, University of Pennsylvania. Dairy Professional Program, Cornell 1998.
[xxiii] Oba and Allen
[xxiv] CPM-Dairy version 1.0, Copyright 1998 by The Center for Animal Health and Productivity, School of Veterinary Medicine, University of Pennsylvania, Kennett Square PA; The Department of Animal Science, Cornell University, Ithaca, NY; and The William H. Miner Agricultural Research Institute, Chazy, NY.
[xxv] Dairy Farmers of Ontario, Milk Shipments, Feb 1999.
[xxvi] Beauchemin, K.A., L.M. Rode, and V.J.H. Sewalt. 1995. Fibrolytic enzymes increase fiber digestibility and growth in steers fed dry forages. Can. J. Anim. Sci. 75:641-644.
[xxvii] Beauchemin, K.A., W.Z. Yang, and L.M. Rode. 1999. Effect of grain source and enzyme activity on site and extent of nutrient digestion in dairy cows. J. Dairy Sci. 82:378-390.
[xxviii] Beauchemin, K.A., L.M. Rode, and V.J.H. Sewalt. 1995. Fibrolytic enzymes increase fiber digestibility and growth rate of steers fed dry forages. Can. J. Anim. Sci. 75:641-644.

[xxix] Stokes. M.R., and S.Zeng.1995. The use of carbohydrate enzymes as feed additives for early lactation cows. 23[rd] Biennial Conf. Rumen Function, Chicago, Il., 23:35 (Abstr.).
[xxx] Johnston, J.D. and I.C. Shivas.1999. Formulating the fourth diet. In "Under the Microscope - Proceedings of Alltech's 15[th] Annual Biotechnology in the Feed Industry Symposium" (T.P. Lyons and K Jacques, eds.). Nottingham University Press, UK.

INTERPRETATION OF RESULTS

The results given in Table 10 were positive and demonstrated that the addition of the Fibrozyme on the basis of the In Vitro NDF disappearance of corn silage during the first six hours is a valid tool when designing rations. The results do however pose as many questions as they answer, as the impact of the haylage fiber has to be considered. Preliminary In Vitro tests run to date on haylages have suggested that Fibrozyme alters the extent of digestion but not the rate. Drawing any firm conclusions from this preliminary data set has its problems, as it is very difficult to select a representative cross section of haylages. Efforts were made to segregate samples into legume versus grass haylages but in most cases the samples were not pure stands hence subjective judgments had to be made. It is hoped that the 2000 growing season will allow for the collection of a pool of samples that will help to explain the effect of Fibrozyme on both grass and legume haylages.

The unequal parity and wide DIM distribution of the cows in the trial made the statistical analysis a challenge. The average milk production change across parities amounted to 1.32 litres/hd/day, with the second lactation cows rising the most with a 1.88 litre/hd/day change. The statistically significant milk response was lost as expected during the withdrawal portion of the trial. Questions could be raised as to the measured effect when the Fibrozyme was reintroduced during the last part of the trial, and one possible answer is that there was a refractory response. If this were in fact what happened it would be of minimal significance on a commercial basis as producers would not add and remove the enzyme on such short notice. Of particular interest was the numerical increase in milk protein, which approached statistical significance ($p < 0.06$) in the first parity cows. This increase was presumably as a result of increased microbial protein production in the rumen. One could speculate as to whether this improvement was due to a direct enzymatic effect or changes in rumenal microbial populations, however drawing such a conclusion is far beyond the scope of this field trial.