

In Vitro Digestibility of Forages

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Introduction

Forages are a necessary component of diets for lactating dairy cows because they provide coarse fiber needed to optimize rumen function. However, forages alone provide insufficient nutrients to achieve high milk yield, and they must be supplemented with other feed ingredients. Because forage quality is highly variable, their quality must be assessed before diets are formulated. Forages have been traditionally analyzed for crude protein and fiber concentrations because of their direct effect on diet formulation. More recently, in vitro neutral detergent fiber digestibility (**IVFD**) has been identified as an important quality parameter that is highly variable among forages and has consistent effects on productivity of dairy cows. However, it is important to understand the unique characteristics and limitations of in vitro measurements of forage NDF digestibility to maximize the benefit of enhanced IVFD. This paper will answer some frequently asked questions regarding the interpretation and utilization of IVFD data of forages.

Why is In Vitro Fiber Digestibility Important?

In vitro NDF digestibility of forages is extremely variable; 30-hour IVFD ranged from 35.6 to 69.9 % and from 23.2 to 59.2 %, respectively, for corn silage and legume hay analyzed at Dairy One Forage Lab (Ithaca, NY) from 2000 to 2004 (95% confidence interval adapted from

www.dairyone.com; Table 1). In addition, wet chemistry forage analyses performed at the Cumberland Valley Analytical Services (Maugansville, MD; www.foragelab.com) during the last two years indicated that IVFD is poorly related to the concentration of NDF, ADF, or CP for corn silage and legumes (Table 2), indicating that IVFD is an additional and independent measure of forage quality. In vitro digestibility has become widely used; in 2004, 13.1, 24.2, and 36.8% of forage samples analyzed for NDF content (for mixed forage hay, mixed forage haylage, and corn silage, respectively) were also evaluated for IVFD at the Dairyland Laboratories, Inc. (Arcadia, WI; www.dairylandlabs.com). This indicates that nutritionists and dairy producers believe that IVFD as an important quality parameter of forages.

While many parameters of forage quality affect diet formulation and possibly diet cost, few actually affect feed intake and milk yield when diets are properly formulated. The IVFD of forages has consistent effects on productivity of dairy cows, making this analytical value a very important quality parameter of forages. Several years ago, we reported that a one-unit increase in in-vitro or in-situ digestibility of NDF was associated with 0.37 and 0.55 lb/day increase in dry matter (**DM**) intake and 4% fat-corrected milk yield, respectively (Oba and Allen, 1999b). This relationship was developed by statistical analysis of treatment means from experiments reported in Journal of Dairy Science. To validate this finding, 12 forage comparisons

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reported in 9 recent articles of the Journal of Dairy Science were reviewed (Table 3). These recent publications compared different corn or sorghum hybrids except for one article (Neylon and Kung, 2003), in which effects of cutting height of corn silage were evaluated. Nine comparisons out of twelve reported that milk yield significantly increased when diets containing corn silage with enhanced IVFD was fed, and the remaining three comparisons reported that milk yield numerically increased without statistical significance. All experiments except for one (Ballad et al., 2001) reported significant differences in IVFD (30-hour) for the forages compared and were used for further statistical evaluation. The average difference in IVFD for those forages was 7 units, and this was associated with the difference of 1.8 lb/day of DMI and 3.3 lb/day of 4% FCM yield; one unit increase in IVFD was associated with 0.26 lb/day of DMI and 0.47 lb/day of 4% FCM yield. These values are reasonably close to the benchmark that we established previously (Oba and Allen, 1999b). It is important to note that effects of enhanced IVFD were not confounded by different dietary NDF contents for the 12 comparisons in Table 3; mean dietary NDF contents were 33.2 and 33.4%, respectively, for diets containing forages with greater IVFD and those with lower IVFD. This is important because feed intake is negatively related to dietary concentration of forage NDF (Allen, 2000). Thus, this more recent literature also strongly supports the idea that the quality of NDF, determined by IVFD measurements, is positively related to animal performance.

What is In Vitro Digestibility?

The IVFD of forages is determined by incubating dried ground forages in flasks with rumen microbes for a given period of time. Forages are dried and ground (usually to pass through a 1-mm screen) so that a representative sample can be taken. The ground forage samples are placed in individual flasks and incubated with rumen fluid

containing rumen microbes collected from cows with rumen cannula. The flask also contains buffers, macro-minerals, trace-minerals, nitrogen sources, and reducing agents to maintain pH and provide nutrients required for growth of rumen bacteria. Because oxygen is toxic to rumen bacteria, flasks are gassed with carbon dioxide to maintain anaerobic conditions, and temperature is held at 104°F (body temperature) during the incubation. A variation of this method is when forage samples are sealed in porous dacron bags which are incubated in groups in jars containing rumen fluid and media.

Every effort is made to provide the optimum environment for survival and growth of fiber-digesting bacteria in the incubation media. This is extremely important because digestion is a function of both enzyme activity and structural characteristics of substrates. If enzyme activity is limiting because of inadequate buffering or lack of essential nutrients, IVFD will be reduced, and more importantly, differences in IVFD among forages will be compressed and not reflective of the true differences among forages. Forages are rarely fed as a sole ingredient to dairy cows but are supplemented with other ingredients to enhance ruminal fermentation and nutrient supply to the animal. Therefore, it is important to use an in vitro system that measures the maximum IVFD of forages, not one that limits IVFD because of lack of buffering or essential nutrients.

It is important to recognize that IVFD is a biological evaluation rather than chemical evaluation of forage quality; microbial activity in rumen fluid of cows can vary with diet and over time relative to feeding which affects the results. Thus, measurements of in vitro digestibility are associated with greater intrinsic variation compared with chemical measurements, such as CP and NDF. This variation can be reduced by feeding the donor cows a high forage diet, sampling rumen fluid at the same time relative to feeding, and blending rumen fluid from several cows for each incubation.

In vitro digestibility is not necessarily the same as in vivo digestibility because the environment in the rumen is often less than optimum for fiber-digesting bacteria. For example, rumen pH is often lower than optimum for the fibrolytic bacteria because highly fermentable diets are typically fed to high producing cows. In addition, forage fiber particles in the rumen are longer than those of ground forages used for in vitro measurements of digestibility. Longer particle size limits the surface area for microbial degradation per unit of fiber mass. Therefore, in general, in vitro digestibility of forages should be greater than in vivo digestibility as long as an optimum fermentation environment, such as pH, temperature, and anaerobic conditions, is carefully maintained in the incubation media. In addition, the range in NDF digestibility of forages measured in vitro is greater than the range measured in vivo (Oba and Allen, 1999b) because the same retention time is used across samples, although actual retention time of forages likely varies with rate of digestion (Allen, 2000).

What is In Situ Digestibility?

Some researchers evaluate in-situ NDF digestibility of forages rather than IVFD. What are the differences between in-vitro and in-situ measurements? Is one superior than the other as a tool for evaluation of forage quality? Our opinion is that for ranking forages for NDF digestibility as a proxy for intake potential, IVFD is best. For the in-situ digestibility measurement, ground forage samples are placed in small porous dacron bags and inserted into the rumen through rumen cannula. Although in-situ measurements evaluate forage samples directly in the rumen of live animals, enzyme activity might be limited by low pH, decreasing differences among forages. In addition, although dacron is available with different pore sizes, a pore size must be selected (usually ~50 μm) that allows entry of microbes but retains feed particles, a challenge at best.

Can IVFD be Used to Predict Energy Concentration of Forages?

The recent Nutrient Requirements of Dairy Cattle (NRC, 2001) suggests that 48-hour in vitro digestibility can be used as a measure of digestible NDF at maintenance. The NRC (2001) discounts the energy content of forages based on actual intake level of animals which a forage is fed to and total digestible nutrients (TDN) concentration of diets (i.e., diets with greater TDN content discount energy content of feeds at a greater rate as intake increases). Thus, the dairy NRC (2001) appears to do a better job conceptually in estimating energy density of forages compared with previous editions. Indeed, the energy content of forages is lower if fed to cows with greater feed intake. In addition, forages fed in high grain diets likely have lower digestibility compared with those fed in low-grain diets because of sub-optimal enzymatic capacity for fiber digestion in the rumen. However, these changes made in the current NRC (2001) did not solve the intrinsic problem that limits the use of in-vitro digestibility for estimation of energy content of forages: inconsistent measurements.

Because of the biological nature of in vitro digestibility measurements, it is challenging to get a same “absolute” value among several analytical laboratories. Consistency of measurements within a laboratory may be improved by adopting the best procedures and careful training of technicians. But, rumen fluid required for determination of IVFD is collected from different animals fed different diets at each analytical laboratory and variation in enzyme activity potentially affects the results to a great extent; IVFD might be 50% for a sample analyzed in one lab and 40% in another. It is not likely to get one consistent value for IVFD across several laboratories. This is one limitation for use of IVFD data for energy value. If you want to use IVFD to estimate energy content of forages, you need to have a consistent standard for enzymatic capacity used for the in-vitro measurements across all laboratories.

In addition, an incubation time of 48 hours is too long to estimate actual NDF digestibility even at maintenance level (as discussed below), and compensatory digestion of NDF in the large intestine make predicting energy concentration from IVFD a challenge. Therefore, in-vitro digestibility does not provide an “absolute” value that can be used for diet formulations. Chemical measurements, such as lignin content (% of NDF), eliminate intrinsic variation associated with biological assays. Use of commercial enzymes with a known activity may be another choice in the future. These alternative options raise other types of questions, but this further discussion is beyond the scope of this paper.

So How can IVFD be Used?

Even though we cannot get an absolute energy value from in-vitro digestibility measurements, IVFD still provides very useful data for nutritional management of dairy herds. For instance, IVFD is a powerful tool to rank forages by their quality. As discussed earlier, diets containing forages with different IVFD consistently affect animal performance. Positive effects of enhanced IVFD are greater for cows yielding more milk. This is likely because their maximum feed intake is limited by physical fill in the rumen to a greater extent compared with lower-yielding cows. Milk production responses to brown midrib corn silage, which has enhanced IVFD, were positively correlated with milk yield (Oba and Allen, 1999a). Lower producing cows had little response in DMI and milk yield to the corn silage with greater IVFD, while higher yielding cows responded by increasing feed intake and milk yield. Lower production responses for low producing cows is likely because their feed intake is not limited by physical fill of the diets. Thus, forages with greater IVFD should be allocated to higher yielding cows that will benefit the most. If a farm can feed different lots of forage to 2 or more groups of lactating cows, there is an opportunity to increase the benefit of enhanced IVFD by feeding the forage with greater IVFD only

to cows that will benefit the most. Because forages with enhanced IVFD might cost more to buy or produce (greater seed cost, lower yield), animals must respond enough to justify the investment for enhanced IVFD.

The IVFD data may also affect how you formulate the diets. When grain is less expensive than forages, dairy diets are normally formulated to include the maximum amount of grain without causing any digestive disorders, such as rumen acidosis or laminitis. On the other hand, when grain price increases, feed costs can be reduced by increasing the forage concentration in the diet. Because forage NDF is filling and often limits feed intake, forages with greater IVFD will allow more forage to be fed without compromising milk production. In a previous experiment (Oba and Allen, 2000), cows fed a corn silage with enhanced IVFD (55.9%) in a high forage diet without supplemental corn grain, produced as much milk as cows fed a corn silage with lower IVFD (46.5%) in a diet which contained dry ground corn at 29.2 % of dietary DM (33.7 versus 33.5 kg/day). Similarly, Weiss and Wyatt (2002) compared high-fiber corn silage with a dual-purpose corn silage. Although diets containing high-fiber corn silage had greater forage NDF content, they supported similar milk production as those containing corn silage with high starch concentration probably because of the greater IVFD. Identification of forages with greater IVFD will allow greater forage to be fed and decrease feeding costs when grain is costly without reductions in milk yield. This creates significant flexibility in diet formulation, especially because grain costs relative to forages are highly variable.

Analysis of forage for IVFD is also an important troubleshooting tool when switching forages. For instance, milk yield sometimes decreases when switching from old corn silage to the new crop or from one lot of alfalfa to another. It is a good idea to sample the current forage before switching so that it can be sent to the lab for IVFD

analysis if production decreases. While a production decrease when switching to new crop corn silage might be from excessive kernel passage, if new corn silage is significantly lower in IVFD, physical fill might become a dominant factor limiting feed intake and decreasing milk yield as well. In addition, if new corn silage is significantly greater in IVFD than corn silage that you have been feeding, the new diet may depress milk fat content unless the diet is adjusted. If you open the silo a couple of weeks before you start feeding to high producing cows and feed it to the low group or heifers, you will have sufficient time to take a representative sample, analyze it for IVFD, and make necessary adjustments in diet formulation. Assessment of IVFD for new corn silage to compare with that from a previous year can help explain a production drop or prevent a potential problem before it occurs.

Although IVFD analysis provides useful data in nutritional management, it is important to know that you cannot compare IVFD between grasses and legumes. Although IVFD is in general greater for grasses compared with legumes, filling effects of legumes in the rumen are usually less than those of grasses, probably because of different physical characteristics such as fragility of fiber or buoyancy in the rumen (Allen, 2000). Many experiments evaluating legumes versus grasses reported that cows fed legumes had greater feed intake and milk production at similar IVFD (Oba and Allen, 1999a), suggesting that the comparison of IVFD across different forage families is not appropriate. But, if we have mixed forage samples with unknown ratio of legumes and grasses, how should we interpret the data? At first, you may want to check the ADF to NDF ratio of the forages because this value is greater for legume, averaging 80%, whereas it is about 50 to 60% for grasses. If you find a wide variation in the ADF to NDF ratio among forages of which you wish to compare the IVFD values, you should not use IVFD data to make any decisions in nutritional management because it implies a significant mixture of grasses and legumes. In

general, feeding grasses and legume-grass mixes to high producing cows should be avoided because the fiber is more filling and will limit feed intake to a greater extent.

What Should I Analyze?

When you receive in vitro digestibility data from a laboratory, you will see two types of digestibility: IVFD and in vitro true dry matter digestibility (**IVTDMD**). The IVTDMD is a calculated value from IVFD, assuming that everything except for fiber is hydrolyzed by the end of the incubation time. Although this is a reasonable assumption, you may not get additional information about the quality of forages from IVTDMD data. Wet chemistry forage analyses performed at the Cumberland Valley Analytical Services (Maugansville, MD) during the last two years indicated that IVTDMD are negatively related to NDF content and positively related to CP content for all forage types (Table 4). You may sometimes find that IVTDMD is greater for one sample and that IVFD is greater for the other when you send multiple samples for analysis. This occurs if one sample has lower concentration of NDF that is less digestible, and another sample has higher concentration of NDF that is more digestible. How should we interpret those data? The objective of in vitro digestibility measurements is to gain additional information which you cannot obtain from conventional chemical measurements. The IVFD data reflect the quality of forage fiber, which is difficult to determine by other analytical methods, while IVTDMD does not.

Similarly, you will not gain a lot of additional information from analyses of total mixed ration (**TMR**) digestibility. As discussed earlier, the in vitro procedure is not an appropriate method to estimate in vivo digestibility and will not give you additional and valuable information to make decisions in nutritional management. If you need to obtain a rough estimate for TMR digestibility, more

economical other measurements such as NDF or starch content can be used. In addition, it is extremely challenging to obtain a representative TMR sample because of the wide variations in particle size and DM concentration. So, the value obtained from TMR analysis needs to be interpreted with extreme caution.

What is the Optimum Incubation Time: 24, 30, or 48h?

The Dairy NRC (2001) stated “Digestible NDF can be obtained using a 48-hour rumen in vitro assay . . . to calculate digestible NDF at maintenance”. We think that 48 hours is too long to use for an incubation time for two reasons: 1) the retention time of indigestible NDF in cows at maintenance is likely less than 48 hours, and 2) grinding forages greatly increases their rate of digestion so the incubation time must be lowered to compensate.

The primary use of IVFD data is to rank forages by their potential to stimulate intake and milk production because IVFD of forages is an indicator of the filling effects of forage fiber in the rumen for a given forage type. Thus, we need to select the optimum incubation time, which allows us to detect the differences in filling effect of forage fiber in the rumen. To accomplish this goal, we need to know the length of time that fiber stays in the rumen. While total fiber leaves the rumen either by digestion or passage, indigestible fiber leaves the rumen by passage only. Therefore, the retention time of indigestible fiber reflects the maximum time that fiber stays in the rumen. The retention time of indigestible NDF, which is the reciprocal of its turnover rate in the rumen, ranged from 26.8 to 32.0 hours for cows producing 73.9 lb/day of milk (Oba and Allen, 2000) and from 27.0 to 30.3 hours for cows producing 79.6 lb/day (Oba and Allen, 2003). This retention time is expected to be shorter for cows producing more than 88 lb/day. If you are interested in the filling effects of forage when fed to

high producing dairy cows, they need to be estimated assuming a shorter retention time of digesta in the rumen. Therefore, the incubation time for IVFD should not be any longer than 30 hours, if you are interested in forage quality for high producing dairy cows.

You may think that a 24-hour IVFD is highly correlated with 30- or 48-hour IVFD, thus selection of a specific incubation time does not really matter. This argument may sound logical, but you may miss an essential part of data if you select an inappropriate incubation time. Let’s think about an example. You are comparing two samples of alfalfa silage. If you see 3 units of difference in 48-hour IVFD, you may think this difference is not significant. However, if the IVFD data obtained from the same samples but using 30-hour incubation shows a 10-unit difference, you expect that the forages you compared will cause significant difference in animal performance. You may see the opposite case: 10-unit difference for 48-hour incubation and 3-unit difference for 30-hour incubation. Although relative ranking between forages stays same, you may draw a wrong conclusion unless you select the right incubation time. So, why do you want to analyze 48-hour in vitro digestibility when you are interested in forage quality for high producing cows? If you are feeding these forages to high producing cows and wish to rank them by their filling effects in the rumen, a 24 or 30 hour of incubation is the right choice because it does not make sense to compare the filling effects of these forages assuming the retention time of 48 hours. However, if you are interested in forage quality for heifers or dry cows to rank them by its potential digestibility, you should choose a longer incubation time because it is closer to the retention time of digesta in the rumen of heifers or dry cows. Selection of the appropriate incubation time is important to make the right decision based on in vitro digestibility data.

How to Evaluate Analytical Laboratories?

Because the objective of forage analysis for IVFD is to rank forages, you should not compare samples analyzed across different laboratories. Procedures used at different labs vary widely as do the diets fed to cows used as rumen fluid donors and these factors can affect IVFD. It is best to send all samples that you wish to compare to a trusted lab and have them analyzed for IVFD in the same run to increase analytical precision. Precision and accuracy are two important criteria when you evaluate forage analytical laboratories.

Precision is a more important criterion than accuracy if the primary objective of your IVFD analysis is to rank forages. Precision can be defined as the ability of a measurement to be consistently reproduced, while accuracy can be defined as the ability of a measurement to match the actual value of the quantity being measured. However, the accuracy of measurement is also essential in IVFD analysis because the *in vitro* incubation environment needs to be optimal so that enzymatic capacity does not limit fiber digestion. So, the inaccurate but precise measurements indicate that a lab consistently fails to optimize the fermentation environment, which also is not desirable.

It might be difficult to check the accuracy of analysis, but you can check the precision of analysis by inter-assay coefficient of variation (**CV**) and intra-assay CV. The CV is the expression of standard deviation as a percentage of a mean. For an example, if a standard sample is placed in three flasks within an incubation bath, the three measurements of IVFD are ideally identical but are slightly different in reality. This variation is referred to as an intra-assay CV. Thus, the lower CV is the better. When you try to compare two forages that differ in IVFD by 2 units (50 vs. 48%), you may wonder if the difference of 2 IVFD units or 4 % $[(50 - 48) / 50 \times 100]$ is meaningful. If the intra-assay CV is 1%, you may be able to say that the

difference is meaningful. But, if the intra-assay CV is 4%, the difference likely happens by chance, and you do not want to make any management decisions based on this analysis. Inter-assay CV is the variation observed among several different incubation runs. If this variation is too large, you may not want to compare a sample analyzed this year with the one analyzed in a previous year because the difference between two measurements likely happens by chance. Good laboratories should be able to provide you with their inter-assay and intra-assay CV if you ask. In any case, it is best to analyze any samples you want to rank or compare with each other in the same incubation bath to minimize potential confounding variations.

Several commercial labs provide service for IVFD analysis by near-infrared reflectance spectroscopy (**NIRS**). The NIRS is a technology that estimates chemical composition and bonds of forage samples by measuring reflectance of light with near infrared wavelengths and using that to predict IVFD. However, NIRS measurements still need to be calibrated with the data obtained from wet-chemistry, and different equations need to be used for each forage species and often for each growing environment of forages. Therefore, the accuracy of a measurement depends on the accuracy of analysis in wet-chemistry. One problem with NIRS that is common to all prediction methods is that the range of data is compressed. This means that a 5 unit difference in IVFD between two samples measured using traditional techniques is likely to be less using NIRS.

Summary

Fiber digestibility of forages is positively related to animal performance and varies greatly. The IVFD should not be used to adjust energy density of forages but is very useful to rank forages for their filling effects of NDF in the rumen. The IVFD analysis allows us to identify forages with greater potential to increase intake and milk

production so that we can allocate them to high producing cows which will benefit the most. Analysis of IVFD provides essential information to make good decisions in nutritional management and improve the profitability of dairy operations.

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Table 1. Mean and the 95% confidence interval for corn silage and legume hay in CP, NDF, and IVFD analyzed during 2000-2004 at Dairy One (Ithaca, NY; www.dairyone.com).¹

	n	Mean	Minimum	Maximum
Corn silage				
CP	77,401	8.3	6.2	10.4
NDF	80,894	44.8	32.2	57.4
30-hour IVFD	5,791	52.8	35.6	69.9
Legume hay				
CP	51,389	21.1	15.6	26.7
NDF	51,055	38.6	27.5	49.6
30-hour IVFD	770	41.2	23.2	59.2

¹CP = crude protein, NDF = neutral detergent fiber, and IVFD = in vitro fiber digestibility.

Table 2. Correlation coefficient of 30-hour IVFD (% of NDF) with NDF (% of DM), ADF (% of DM), CP (% of DM), and lignin (% of NDF). All samples were analyzed for 30-h IVFD, NDF, ADF, CP, and lignin by wet chemistry during the last two years (Courtesy of Cumberland Valley Analytical Services, Maugansville, MD).¹

	n	NDF	ADF	CP	Lignin/NDF
Legume	1864	-0.09	-0.20	0.11	-0.47
Mixed mainly legume	466	-0.49	-0.55	0.28	-0.64
Mixed	632	-0.43	-0.48	0.49	-0.58
Mixed mainly grass	501	-0.64	-0.63	0.62	-0.56
Grass	93	-0.43	-0.54	0.50	-0.63
Corn silage	5338	-0.06	-0.10	-0.16	-0.45

¹IVFD = In vitro fiber digestibility, DM = dry matter, NDF = neutral detergent fiber, ADF = acid detergent fiber, and CP = crude protein.

Table 3. Effects of enhanced 30-hour forage IVFD on DMI, milk yield, and 4% FCM yield in recent publications.¹

	30-hour forage IVFD (% of NDF)	Dietary NDF (% of DM)	DMI (lb/day)	Milk Yield (lb/day)	4% FCM Yield (lb/day)
Aydin et al., 1999 (JDS 82:2127-2135)					
Normal sorghum	40.1	32.3	47.3	47.3*	45.5*
BMR sorghum	49.2	31.6	49.9	53.5*	52.1*
Ballard et al., 2001 (JDS 84:442-452) ^a					
Mycogen (TMF TM corn silage)	28.2	35.3	...	68.4*	71.3*
Cargill (BMR corn silage)	45.7	34.7	...	73.5*	75.0*
Ebling and Kung, 2004 (JDS 87:2519-2527)					
Conventional corn silage	39.9	33.9	51.5*	91.1*	79.6
BMR corn silage	54.0	33.5	56.9*	97.5*	82.1
Ivan et al., 2005 (JDS 88:244-254)					
Corn silage with lower cell-wall content	50.7	30.8	53.2*	73.7*	69.7*
Corn silage with high cell-wall content	54.8	33.2	55.9*	78.5*	75.5*
Corn silage with lower cell-wall content	50.7	30.8	58.3	76.1	73.5*
Corn silage with high cell-wall content	54.8	30.8	59.6	78.1	76.8*
Neylon and Kung, 2003 (JDS 86:2163-2169)					
Corn silage with lower cut height	48.4	34.2	55.9	99.4*	88.4
Corn silage with higher cut height	50.7	33.5	56.3	102.7*	87.8
Oba and Allen, 1999a (JDS 82:135-142)					
Control corn silage	39.4	31.6	51.7*	85.6*	78.5*
bm3 corn silage	49.1	30.8	56.3*	91.7*	84.0*
Oba and Allen, 2000 (JDS 83:1333-1341)					
Control corn silage	46.5	29.1	50.2*	73.7*	69.9*
bm3 corn silage	55.9	28.7	51.9*	81.9*	72.4*
Control corn silage	46.5	38.4	45.1*	66.9*	65.8*
bm3 corn silage	55.9	37.5	48.4*	74.1*	72.6*
Thomas et al., 2001 (JDS 84:2217-2226)					
Dual-purpose corn hybrid	49.2	37.1	62.9	99.2*	97.7
Leafy corn silage hybrid	53.9	36.1	60.9	102.5*	100.8
Weiss and Wyatt, 2002 (JDS 85:3462-3469)					
Dual-purpose corn silage	35.4	28.9	52.6	73.3	73.3
High fiber corn silage	40.1	31.9	52.1	74.8	73.3
Dual-purpose corn silage	35.4	31.6 (18.1 ^b)	51.5	74.4	73.9
High fiber corn silage	40.1	27.6 (20.4 ^b)	52.1	78.1	73.7

¹IVFD = In vitro fiber digestibility, DMI = dry matter intake, FCM = fat-corrected milk, JDS = *Journal of Dairy Science*, and BMR = brown midrib.

* Significant effects of treatment ($P < 0.05$)

^aData were not used for the statistical analysis as P -value for IVFD was not reported.

^bForage NDF (% of dietary DM)

Table 4. Correlation coefficient of 30-hour IVTDMD % of DM with NDF (% of DM), ADF (% of DM), CP (%DM), and 30-hour IVFD (% of NDF). All samples were analyzed for 30-hour IVFD, NDF, ADF, CP, and lignin by wet chemistry during the last two years (Courtesy of Cumberland Valley Analytical Services, Maugansville, MD).

	n	NDF	ADF	CP	30-hour IVFD
Legume	1864	-0.81	-0.84	0.55	0.65
Mixed mainly legume	466	-0.78	-0.82	0.41	0.92
Mixed	632	-0.74	-0.76	0.55	0.92
Mixed mainly grass	501	-0.82	-0.80	0.69	0.96
Grass	93	-0.65	-0.69	0.61	0.96
Corn silage	5338	-0.60	-0.60	0.31	0.82

¹NTDMD = In vitro true dry matter digestibility, DM = dry matter, NDF = neutral detergent fiber, ADF = acid detergent fiber, CP = crude protein, and IVFD = in vitro fiber digestibility.