Laboratory Measurements of NDF and Starch Digestibility

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In vitro/in situ analyses are common among research institutions and have become more prevalent in commercial laboratories. As these research methods become more commercialized, there needs to be an understanding among users of what the analysis do and do not tell us.

Background on In Vitro Analysis

Most in vitro analysis today can be traced back to the Tilley and Terry (1962) 2 stage method. The authors used this method to measure in vitro dry matter digestibility **(IVDMD)** and successfully related it to in vivo DM digestibility at maintenance levels of intake. Later Van Soest et al. (1991) modified the procedure to estimate true DM digestibility and NDF digestibility **(NDFD)**.

In vitro "artificial rumen" digestibility can improve assessment of nutritive value, but caution is advised as in vitro digestibility "does not" equal in vivo digestibility. The Van Soest et al. (1991) method was designed to measure the maximum potential digestibility of a feedstuff. In order to achieve this, Van Soest et al. (1991) optimized a mixture of enzymes, buffer solution, and rumen fluid so that the only factor that would limit digestion would be the inherent characteristics of the feed and time. From a feed laboratory perspective, this makes sense because as soon as other limitations are put into the system, we are no longer evaluating the feed but feed and system interactions.

This approach does pose challenges to nutritionists and dairy producers as it still requires them to interpret the results in the context of a commercial dairy farm. Factors which have been shown to have an affect on NDF digestibility in vivo but are not accounted for in any in vitro system are provided in Table 1.

In vivo digestion is specific to the diet and the animal being fed. Trying to mimic in vivo digestibility is neither the goal of, nor a realistic expectation, of an in vitro system. At their best, in vitro analysis can evaluate the potential digestibility of a feedstuff. Using models or programs in combination with in vitro analysis "may" get you closer to understanding how your individual in vitro analysis will respond to the animal and diet being fed.

Methods

Primary methods commercially available for evaluating fiber digestibility are procedures related to the Goering and Van Soest (1970) procedure using fermentation vessels or filter bags. A new method (Goeser and Combs, 2009) using preincubation **(PI)** or standardized rumen fluid and filter bags is also available on a commercial basis. Ring tests conducted by Hall and Mertens (2012) reveals the Goering and Van Soest (1970) based methods were able to reliably rank sample data in order of 30 hr-NDFD (Spearman correlation coefficient = 0.93) with 80% of the ranking correct

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or different by only 1 ranking. The standardized PI did not correctly rank corn silage and overall NDFD values were significantly lower than using the Goering and Van Soest (1970) method. Neither procedure showed an advantage in repeatability of measurements.

Basic Understanding of NDFD and Lignin

There are "abundant data" available that show negative correlations between lignin concentration and DM and NDF digestibilities. (Jung et al., 1997). As the industry has moved toward using NDFD, instead of lignin, this concept has sometimes been overlooked by some laboratory managers and nutritionists. A simple, but effective, tool that anyone can use to evaluate a laboratory's ability to measure NDFD is to run a correlation between lignin as percentage of NDF and NDFD. While the relationship may not be strong (R^2 of 0.40 in some data sets) within a forage type, there should be a relationship. Failure to show a relationship, most commonly on corn silage, should alert lab managers and users of NDFD that there is an analytical problem and corrective action is needed.

Digestion kinetics and indigestible NDF (iNDF)

Understanding digestion kinetics is relevant because time and potential extent of digestion are becoming crucial limitations for high-producing animals. As dairy cows produce more milk, more feed is required and consequently feed moves through the animal faster. Faster rates of passage through the digestive tract results in shorter retention times, which means less time is available for digestion (Forage Facts 2, Mertens Innovation and Research, LLC, Belleville, WI).

Numerous papers describe iNDF and its important role in reliable determination of digestion kinetics, as well as its use as an internal marker. We typically measures undigested NDF **(uNDF)** (what has not been digested after a specified amount of time) to estimate iNDF. According to the Lucas (1964) principle, iNDF is an ideal fraction since by definition it is digested at a predictable rate of zero. Ellis et al. (1999) stated the determination of iNDF should be included in all basic feedstuff analysis because it has a predictable digestibility; it can be used for the estimation of the potential digestible NDF **(pdNDF)** as NDF-iNDF, and it has an important role in contributing to rumen digesta load. With the growing popularity of iNDF, it is important to understand that the "time point" used to determine iNDF is highly dependent on the analytical system.

A study conducted by Boyd and Mertens (2011) looked at factors affecting the results of iNDF, such as grind size, fermentation times, and fermentation methods: in vitro flasks, rotating jar systems, and in situ bags (Tables 2 and 3).

The purpose of the information in Tables 2 and 3 is not to select a method that is best suited for all iNDF applications, but rather to educate the industry that debates over "the right time point" are secondary or trivial if the user has not accounted for which system (in vitro methods; bags, vessels, blank corrected, and ash corrected) are being used.

Mertens et al. (2012) documented that extending fermentation time from 120 to 240 hr reduced uNDF by 5 to 15% using a Goering and Van Soest (1970) crucible method. In addition, the use of blanks and ash correction were critical for the measurement of iNDF.

Significant data exist that support longer fermentation times will yield lower, more accurate determinations of iNDF. This is true regardless of the type of fermentation vessel used. However, long fermentation times present an obstacle for commercial adaptation of iNDF. This limitation could be overcome if reliable near infrared **(NIR)** calibrations can be developed. Preliminary work done within our lab indicates that NIR can accurately

predict uNDF 240 h using the Goering and Van Soest (1970) method with continuously gassed fermentation vessels.

Take home messages:

1. In vitro analysis can measure the potential digestibility of a feedstuff. In vivo measurements are specific to each diet and animal.
2. There is an inverse relationship between lignin as percentage of NDF and NDFD.
3. The iNDF can be the most important analysis when describing digestion kinetics.
4. The iNDF analysis is a function of the system, method, fermentation vessel, and time used in the assay.

Starch Digestibility

In the current economy of high feed prices and consequently high corn or starch prices, there is an ever increasing desire to maximize the feed utilization of starch. The primary components affecting starch digestibility are: 1) particle size, 2) moisture of the corn kernel, 3) length and intensity of fermentation during the ensiling process, and 4) genetic difference.

Despite the widespread understanding of the factors that affect starch digestibility (i.e., particle size), there are a limited number of approaches to integrating the physical characteristics and nutrient composition of the feedstuffs. It is this author's opinion that any measure of starch fermentation or starch kd rates should include an assessment of particle size to have any chance of being able to relate to what is happening in the animal. Figure 1 provided the distribution of particle size in corn grain and high moisture corn of samples submitted to Dairyland Laboratories. As the industry is gaining a better understanding of the importance of particle size, the number of requests for this analysis within Dairyland Laboratories has grown from 249 samples in 2009 to over 1100 samples in the 2012 crop year.

Fecal starch and University of Wisconsin Grain 2.0 (Hoffman et al., 2012) are two commercially available options that account for particle size of the material as being fed.

Fecal starch (FS)

Fecal starch has been gaining acceptance in the dairy and beef industries as a practical method for evaluating the feeding of starch. The system and interpretation of the results are easily understood and producers seem to "relate" to the concept. The correlations of FS to apparent or total tract starch digestibilities are provided in Table 4.

University of Wisconsin Grain 2.0

This system (Hoffman et al., 2012) provides an integrated approach combining the physical characteristics, nutrient composition including ammonia N as a marker for extent of fermentation in corn grain and snaplage, and prolamin for corn grain. The report output consists of effective mean particle size, starch fermentation rate (as fed; kd/hr) with variable rumen passage rate, ruminal starch digestibility and total tract starch digestibility.

In corn grain, ammonia N is a reliable indicator of starch digestibility because dry corn and freshly harvested high moisture corn (>15% moisture) contains little to no ammonia N. In addition, ammonia N helps define the intensity and duration of fermentation on high moisture corn during ensiling (Figure 2). For commercial laboratories, NIR calibrations for ammonia N can be developed that are very reliable and robust. Ammonia N calibrations from Dairyland Laboratories developed from samples selected over several years (2003 through 2013) have 1-VR (correlation of NIR and chemistry values) of 0.953, SECV (standard error of crossn validation set) of 0.017, and mean of 0.240 (739 samples).

In vitro starch digestibility (7hr)

The basis for most all commercially available in vitro starch digestibility derives from adaptations of Richards et al. (1995). This has all the same advantages and disadvantages of in vitro fiber analysis, with added complications of grind size when performing the analysis. Most common grind sizes are 2 to 6 mm and incubation lengths from 6 to 12 hr. Equations for determining kd rates are available using IVSD 7hr. (Sniffen et al., 2009).

Emerging Technologies

Gas production – Fermentrics - allows for the direct measurement of digestion rates for both fast (primarily starch) and slow (primarily fiber) pool nutrients. The use of curve peeling techniques and equations published in the literature are used to estimate the carbohydrate pool Kd values (e.g., carbohydrate fractions B1, B2 and B3) and allow for measured rates to be used for feedstuffs rather than relying on book values.

Calibrate® technologies is a patented feed ingredient analysis program that provides insights into ruminal starch and fiber digestibilities in dairy rations. The Calibrate program utilizes a rapid NIR test to measure ruminal starch and fiber digestibilities of ration ingredients. Ingredient starch digestibility is then ranked on a proprietary GPN™ scoring index from 1 (slow) to 11 (fast) for maximum accuracy. Fiber digestibility is ranked on a proprietary FPN™ scoring index from 60 (slow or low ruminal digestion) to 180 (fast or high ruminal digestion). The NIR calibrations have been developed based on the analysis of over 16,000 in vitro rumen digestible test samples. The GPN™ and FPN™ results combined with a Calibrate® nutritional calculator allows for precise information on the starch and fiber digestibilities of the feedstuff on individual farms.

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Table 1. Factors affecting in vivo digestibility.

Limitation	Reference
Particle size	Shaver et al., 1986; Woodford and Murphy, 1988; Bal et al., 2000; Yasari et al., 2004
Starch content	Burroughs et al., 1949; Chappell and Fontenot, 1968; Joanning et al., 1981; Miller and Muntifering, 1985; Grant and Mertens, 1992; Visser et al., 1998; Valadares Filho et al., 2000
Starch degradability	Cooke and Bernard, 2005
pH	Grant and Mertens, 1992
Passage rate	Oba and Allen, 1999
Parity	Keuhn et al., 1999
Stage of lactation	Keuhn et al., 1999

Summary

1. For the measurement of iNDF, time was more important for the IV-Cr Sand method - unexplained was why cutter grind is less than cyclone grind.
2. Grind was more important for Daisy-F57- unexplained was why 96 is < 144 hr.

Table 2. Effects of in vitro (IV) system, time, method, and grind on results for indigestible NDF (iNDF).[1]

Time (hr)	Method[1]	Grind	iNDF Estimate (%)	P < 0.05
144	IV-Cr Sand	Cutter 1mm	19.0	a
144	IV-Cr Sand	Cyclone 1mm	19.5	ab
96	IV-Cr Sand	Cutter 1mm	20.6	ab
96	IV-Cr Sand	Cyclone	21.4	b
96	Daisy F57	Cyclone 1mm	24.9	c
144	Daisy F57	Cyclone 1mm	25.0	c
96	Daisy F57	Cutter 1mm	26.3	cd
144	Daisy F57	Cutter 1mm	26.9	d

[1]IV-Cr Sand = In vitro analysis using the Gooch crucible and sand for the NDF analysis on the residue and Dairy F57 = Ankom In Vitro Daisy System and the F57 bag.

Summary

1. For the measurement of iNDF, time was more important for the IV-Cr Sand method - unexplained was why cutter grind is less than cyclone grind.
2. Grind was more important for Daisy-F57 - unexplained was why 96 is < 144 hr.

Table 3. Effects of time, method and grind comparison for corn stalks and grass hay on results for indigestible NDF **(iNDF)**.

Time (hr)	Method[1]	Grind	iNDF Estimate (%)	P < 0.05
288	IS-IS bag	Cutter 2 mm	19.65	a
240	IS-IS bag	Cutter 2 mm	20.35	ab
144	IS-IS bag	Cutter 2 mm	21.47	abc
288	IS Daisy F57	Cyclone 1 mm	21.81	bc
240	IS-IS bag	Cutter 2 mm	22.60	cd
144	IV-Cr sand	Cyclone 1 mm	22.92	cde
144	IV-Cr sand	Cutter 1 mm	23.02	cde
96	IV-Cr sand	Cyclone 1 mm	24.48	de
96	IS-IS bag	Cutter 2 mm	24.59	de
96	IV-Cr sand	Cutter 1 mm	25.12	e
144	IS Daisy F57 bag	Cutter 1 mm	27.53	f
96	IS F57 bag	Cutter 1 mm	29.69	g

[1]IS-IS = In situ using in situ bags, IS-F57 = in situ using F57 bags, and IV-Cr sand = in vitro analysis using the Gooch crucible and sand for the NDF analysis on the residue.

Summary

1. In Situ method at 240 to 288 hr generated lower iNDF than in vitro methods at 144 hr, suggesting that in vitro time was not adequate for these slowly digesting fibers.
2. Forage type may be an additional factor affecting the time requirement for measurement of iNDF.
3. All systems using Daisy F57 filter bags generated larger iNDF, regardless of grind or system and suggesting that Daisy F57 bags impede the digestion of iNDF.

Table 4. Correlation of fecal starch **(FS)** to apparent or total tract starch digestibility **(TTSD)**.

Author	Type of Collections	Correlation R^2
Ferguson, Univ. Pennsylvania (personal communication)	Farm TMR/Fecal	0.78 FS to Apparent Digestibility
Owens and Zinn, 2005	Literature Review	0.94 FS to TTSD (% intake; lactating cows)
Ferraretto and Shaver, 2012	Rectal Grab Samples (506); Used digesta markers	0.94 FS to TTSD

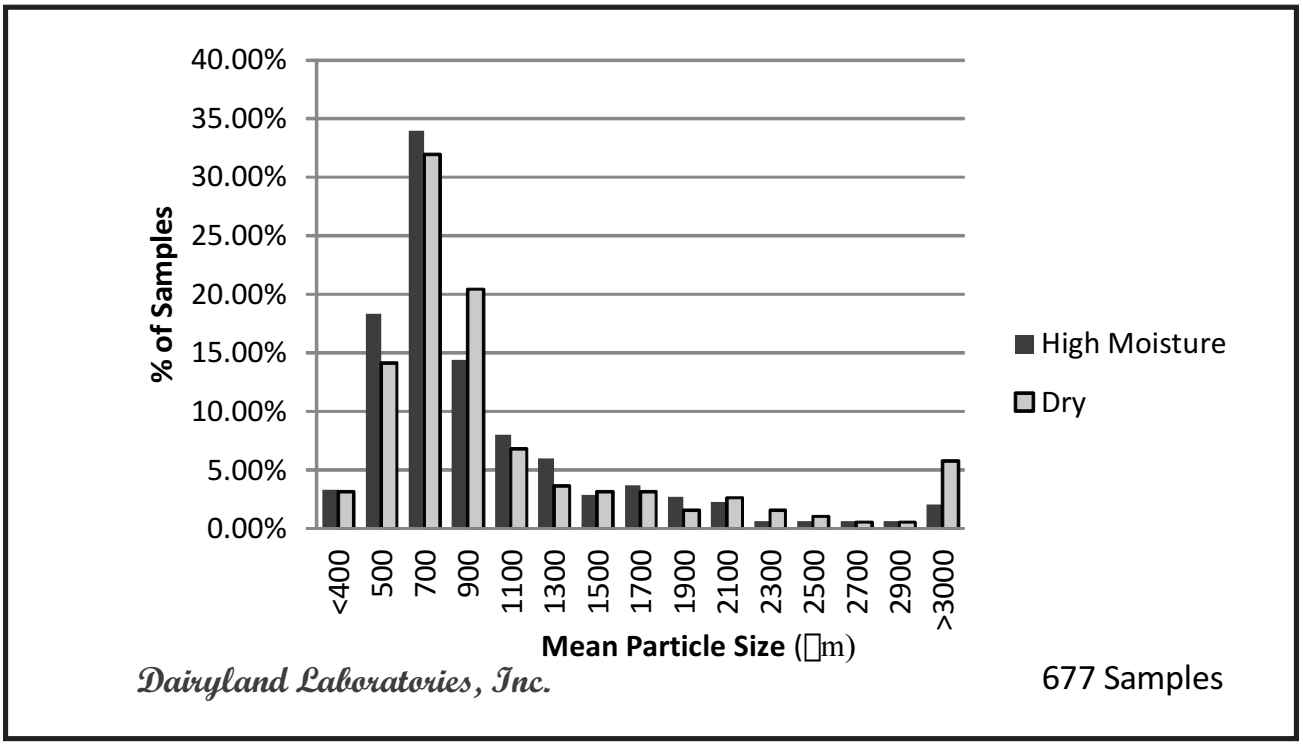

Figure 1. Particle size distribution of high moisture corn and corn grain samples.

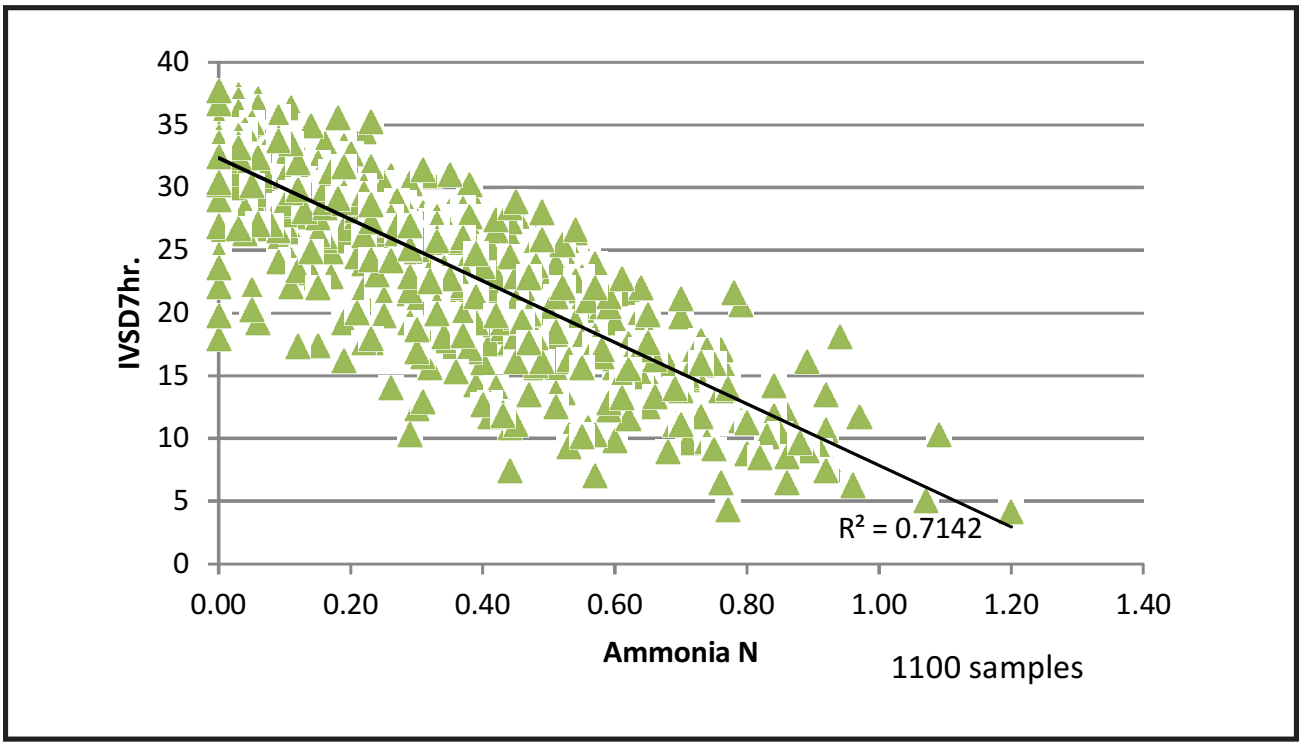

Figure 2. Ammonia N vs. in vitro starch digestibility **(IVSD** 7 hr; using near infrared predictions).