NDF - MAKING SOMETHING OLD, NEW AGAIN

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INTRODUCTION

Fiber digestibility and indigestibility are critical factors when assessing forage quality and formulating diets. Digestion characteristics of NDF influence feeding and rumination behavior, rate of particle breakdown, ruminal turnover and fill, dry matter intake, and overall efficiency of milk component output. Traditionally, nutritionists have focused on measures of NDF digestibility at specific timepoints and assumed that NDF was a relatively homogenous fraction. However, recently the focus has included indigestible fiber as well because of the recognition of its importance establishing the digestible portion or pool of NDF which leads to the extent of digestion and influences the rate(s) of fiber fermentation in the rumen. For purposes of nutritional modeling, indigestible NDF is required as the end point for fermentation to allow accurate estimation of the potentially digestible NDF fraction and its rate(s) of digestion. Measuring true NDF indigestibility would require infinite time, especially in aerobic systems, so in the actual rumen of a dairy cow or in an artificial rumen system, true indigestibility is never achieved. The standard nomenclature throughout the literature is “indigestible NDF (iNDF)” (Mertens, 1993; Huhtanen et al., 2006); however, to improve the accuracy of the standard terminology used to describe fiber fermentation dynamics, Mertens (2013) coined the term “undigested NDF (uNDF)” as the laboratory measure (typically in vitro or in situ) of indigestible NDF at a specified fermentation time. You will see both terms used, and for the most part, they are interchangeable as long as you know the method and time point used to determine the NDF digestion endpoint. However, moving forward, we will standardize our terminology to uNDF. To achieve iNDF requires estimations out to infinite time and that estimated residue might not be consistent with the interactive behavior of the forage and feed with rumen function.

WHY SHOULD WE USE uNDF?

Determination of uNDF should be included in routine forage and feed analysis because indigestible NDF is a uniform feed fraction with a predictable digestibility (i.e. zero). By contrast, NDF is a non-uniform feed fraction; it contains multiple pools that digest predictably as a function primarily of lignification (Van Soest, 1994).

Undigested NDF is the functional fiber fraction that influences physical effectiveness, gut fill, and digestion/passage dynamics of forages. Undigested NDF is important biologically because:

- it can be used to estimate potentially digestible NDF(pdNDF) (NDF - uNDF),

- the uNDF fraction together with earlier time points of fermentation can be used to estimate the fast and slow pools of NDF digestion and their digestion rates (Raffrenato and Van Amburgh, 2010),
- measures of NDF pools and rates of digestion based on uNDF can help explain feeding and ruminating behavior, especially when chemical composition (i.e. ADL, NDF, ADF) are similar,
- chewing response to peNDF is likely influenced by forage uNDF,
- estimates of the slow pool of NDF and its rate of digestion plus the uNDF are related to dry matter intake and passage from the rumen,
- uNDF plays a critical role in maintaining the ruminal digesta load, and
- uNDF predicts forage quality because of the relationship between uNDF and OM digestibility (Nousiainen et al., 2003).

At any given time, rumen fiber fill is a function of dietary uNDF, slowly fermenting NDF, and undigested fast-pool NDF. The rumen space resulting from turnover of the fast fiber together with the slow fiber and uNDF allows for more dry matter intake. The more rapidly rumen space is made available (i.e. the greater the turnover), the higher the intake that can be attained. The total mass of uNDF within the rumen can be thought of as a "baseline" of fill which constrains the possible NDF flux. We propose that there is a maximum and minimum amount of ruminal uNDF to avoid limits on feed intake and to maintain proper ruminal health, respectively. Undigested NDF can improve the precision of estimating dry matter intake by telling us, for example, how much uNDF in a TMR that a cow can consume before filling her rumen, and conversely, how much uNDF must be consumed to maintain rumen fill and digestive efficiency.

In fact, there may be an optimal mass of digesting NDF within the rumen; above this amount, fill limits intake while below this amount, intake could increase further although possibly at the expense of feed efficiency (Weakley, 2011). Although the effect on dry matter intake of adjusting dietary NDF is 2 to 3 times greater than changing the NDF digestibility (Mertens, 2009), in many practical feeding situations where dietary NDF has reached the maximum fill potential in high-producing cows, then NDF digestibility (or indigestibility) becomes most important (Weakley, 2011). We believe that uNDF measured at 240 hours of in vitro fermentation ($uNDF_{240}$) is a forage fraction that accurately assesses the indigestible component of NDF.

UPDATING THE ANALYSIS OF NDF TO aNDFom

One other related aspect of uNDF and NDF in general is the use of organic matter correction. Biogenic ash (ash integral to plant development) is soluble in NDF solution, so that is properly accounted for during the assay, however, soil ash is not soluble in NDF solution and if not removed or accounted for will falsely inflate the NDF values and the same is true for the uNDF. Moving forward, both the NDF and the uNDF should be ash corrected to remove any potential confounding by soil contamination. Management approaches that take advantage of practices like "hay in a hurry" along with large, high horsepower choppers will impact the amount of soil that is found in the forages. In addition, based on region of the country that forage is produced or sourced

will also affect the level of contamination. More sandy soils and irrigation practices such as flood irrigation can cause soil to be adhered to the plant. The easiest way to account for the contamination is to ash the residue after both the NDF and uNDF to correct the value. This also reduces bias in the estimation of rates of digestion since organic matter correction provides a more correct value for the true available NDF content. Thus, aNDFom analyses (NDF with sodium sulfite, amylase and ash correction) will provide nutritionists with more accurate information and in some cases significantly lower values.

There are no changes in the targets for aNDFom intake and in many cases, under reformulation, the amount of forage fed will increase 2-3% once the ash content of the NDF is accounted for. Under conditions where there was significant ash contamination, the amount of forage required to meet the typical dietary levels (e.g. 32%) can be increased by over 10% to maintain adequate aNDFom levels for normal rumen health. It is possible in certain situations, that inconsistent intakes, changes in rumination and rumen pH along with manure scores that are inconsistent can be an outcome of underfeeding forage and fiber because the NDF content of the diet was underestimated due to ash contamination. This most likely happens in the regions of the country where flood irrigation and sandy soils are more prevalent but it is still a possibility in the Northeast due to larger equipment, wide-swathing and variable field conditions.

HOW DO WE MEASURE uNDF?

The approach for estimating iNDF within the structure of the Cornell Net Carbohydrate and Protein System (CNCPS; Tylutki et al., 2008) has been through the use of acid detergent lignin (ADL) and a fixed factor of 2.4 calculated as ADL*2.4/NDF (Chandler et al., 1980). For other applications the approach most often used is that of Conrad et al. (1984) where a surface area relationship is described by a power function ((1- $lignin^{0.67}/NDF^{0.67}$) was used to describe the relationship between lignin and NDF to characterize the unavailable NDF. This approach is used in many of the net energy equations by commercial laboratories and the 2001 NRC (NRC, 2001).

More recently, iNDF has been estimated through long-time in vitro or in situ fermentations. The method recommended by the Cornell group requires 240 hours of in vitro fermentation using a Tilley-Terry system with modifications described by Raffrenato and Van Amburgh (2010). The fermentation end point *per se* is not important – it will vary with fermentation system. For example, the in situ approach published by Huhtanen et al. (2007) uses 288 hours to reach a similar fermentation endpoint to measure iNDF. The goal is to reach a point where the residue weight does not change significantly with additional hours of fermentation – this will be a measure of uNDF and the estimate of indigestible NDF for estimation of rates and extent of digestion. For commercial laboratory application and routine model inputs, we prefer the use of an in vitro approach which allows for sample submission from nutritionists and development of an adequate-sized database to develop NIR equations that will reduce the cost and increase the speed of sample analysis.

Examples of the chemistry related to NDF and NDF digestibility in four corn silages along with the calculated indigestibilities based on Chandler et al., and Conrad et al., are found in Table 1. The data in the table demonstrate the subtle differences that can be observed when analyzing for aNDFom compared with aNDF. The average difference among this very small sampling is 0.9 units of NDF, a very modest amount. However, we have analyzed or dealt with samples that were up to 10 units different after ashing, so again, it depends on where the sample is from and the agronomic an harvest conditions it is under. The uNDF as measured at 240 hr averages 24.8 %NDF whereas the lignin (%NDF)*2.4 value averages 41.9% and the power function of Conrad et al. (1984) averages 20.7%. The differences between the actual measurement and the calculations are significant and will result in biased estimations of total digestibility, rates of digestion and energy predictions. The Conrad et al. calculation average is biased because there is one sample that is very high compared to the rest, and that sample has the lowest measured uNDF of the four silages presented. Overall, this small example demonstrates that the values estimated by the previous methods using fixed factors as a function of the chemical measurement of lignin miss the potential interaction (cross-linking) between lignin and carbohydrate that actually impact the digestion capacity of the plant.

Table 1. Corn silage fiber chemistry, 240 in vitro indigestibilities (uNDF), and estimations of indigestible fiber by Chandler et al. (1980) (lignin (%NDF) x 2.4) and Conrad et al., 1984.

Corn silage	aNDF, %DM	aNDFom, %DM	Lignin, %NDF	uNDF, %NDF	Chandler et al. 1980	Conrad et al., 1984
1	38.1	37.5	6.61	23.6	42.3	16.4
2	39.5	38.9	6.46	25.6	39.2	16.89
3	41.5	40.9	7.47	27.3	43.4	17.7
4	43.7	41.9	7.51	22.8	42.8	31.8

Similar observations have been made for the non-forage fiber sources. Byproducts like beet pulp and citrus pulp that have good nutrient value and can be routine sources of energy for lactating dairy cattle have digestion behavior that is not dissimilar from forages. Data were generated to better understand when the uNDF is identified in non-forage fiber sources and that is in Table 2. For most non-forage feeds, the uNDF can be measured after 120 hr of in vitro digestion provided the samples are filtered on the appropriate filter paper (Whatman AH934 or equivalent). The only feed that had behavior more similar to forages was citrus pulp where the uNDF of the sample represented below was only identified at 240 h of fermentation.

Once the uNDF was identified and understood, it was important to evaluate the measured values from these non-forage fiber sources in a similar manner to the forages to better understand if the static calculations for uNDF and the measured uNDF were similar. The data in Table 3 demonstrate that the measured uNDF is both over- and under-predict for the feeds represented in this table and these inconsistencies will impact the estimation of digestible NDF and will also affect energy predictions from this group of feeds. Static values as a function of the lignin to NDF relationship do not

adequately account for the digestibility and uNDF of non-forage fiber feeds in a similar manner as forages, however it is expected that the variation in non-forage fiber feeds will not be as great as the forages due to the lack of agronomic conditions affecting their development.

Table 2. The aNDFom (%NDF) residues of feeds after 96, 120, and 240h of fermentation

	Time (h)				
	96	120	240	SEM	P-value
Beet pulp	22[a]	19[b]	17[b]	0.01	0.004
Canola meal	40	41	41	0.01	0.79
Citrus pulp	21[a]	20[a]	16[b]	0.01	0.002
Corn Gluten feed	16[a]	14[ab]	13[b]	0.01	0.028
Corn distiller	16	16	14	0.01	0.50
Corn germ	34	29	27	0.03	0.74
Flaked corn	14	14	12	0.02	0.73
Rice hulls	94	93	93	0.01	0.61
Soybean meal	11	9	9	0.01	0.95
Soy hulls	10[a]	9[ab]	8[b]	0.01	0.022
Wheat distiller	28	26	25	0.01	0.20
Wheat middling	36[a]	31[b]	30[b]	0.01	0.001

[a,b]Values with different letters are statistically different

Table 3. The neutral detergent fiber, acid detergent lignin and comparison of three methods of estimation of uNDF based on 120 hr fermentation, the Chandler equation or the Conrad equation, respectively.

Feed	aNDFom (%DM)	ADL (%DM)	uNDF (%aNDFom)	2.4 x ADL (%aNDFom)	$ADL^{2/3}/NDF^{2/3}$ (%aNDFom)
Beet pulp	47	5.4	19	28	24
Canola meal	29	8.8	41	73	45
Citrus pulp	25	1.94	20	19	53
Corn gluten feed	37	2.27	14	15	4
Corn distiller	41	4.4	16	26	23
Corn germ	63	5.9	29	23	21
Flaked corn	13	1.4	14	26	23
Rice hulls	71	0.8	93	20	5
Soybean meal	9	0.85	1	23	21
Soy hulls	72	1.3	9	10	7
Wheat distillers	38	3.8	26	29	22
Wheat middlings	45	4.9	31	17	23

IMPLICATIONS AND APPLICATIONS

Data being generated on lactating dairy cattle indicate the cow can "identify" with the values related to the uNDF measurements along with the rest of the pools (fast and slow digesting NDF pools) and these measurements are in some manner related to rumen fill, eating speed and ultimately, dry matter intake. Data generated in a forage digestibility study at Miner Institute with high and low forage inclusion levels demonstrated that the cow consumes approximately the same amount of uNDF as she excretes in her feces every day. The precision of the relationship was surprising as showing in Table 4. The relationship between uNDF intake and uNDF excretion was 1:1 and coupled with the relationship between the rumen contents of uNDF and the intake of uNDF suggests that if we understand the uNDF, we can directly estimate the rumen fill of total NDF and further, we should be able to predict intake among differences in TMR uNDF values.

Table 4. Intake of NDF and uNDF and rumen fill for Miner study

Item	LF-LD	HF-LD	LF-HD	HF-HD
NDF_{om} intake				
kg/d	8.87	8.95	8.48	9.88
% of BW	1.32	1.33	1.27	1.47
Rumen NDF_{om}				
kg	8.50	8.58	7.82	8.48
% of BW	1.27	1.28	1.17	1.27
$uNDF_{240om}$ intake				
kg/d	2.39	2.63	2.03	2.21
% of BW	0.36	0.39	0.30	0.33
Rumen $uNDF_{240om}$				
Kg	3.82	4.16	3.20	3.46
% of BW	0.57	0.62	0.48	0.52
Fecal uNDF, kg/d	2.41	2.64	2.04	2.24
Ratio rumen/intake uNDF	1.60	1.58	1.58	1.57
Ratio intake uNDF/fecal uNDF	1.0	1.0	1.0	0.99

SUMMARY

Studies are underway to evaluate the concept of aNDFom pools, chewing and rumination and feed intake. The data generated to date suggests that predictions for energy, rates of digestion, microbial yield and dry matter intake will be improved through the application of uNDF and the pool approach to defining NDF digestion. This is exciting and gives new life to an old topic, and might help explain differences in feeding behavior that nutritionists and others have observed but never been able to quantify.

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