

APPLICATIONS OF uNDF IN RATION MODELING AND FORMULATION

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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 primarily on measures of fiber digestibility, but recently the focus has included indigestible fiber as well because of the recognition of its importance in setting the extent and influencing 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, so in the actual rumen of a dairy cow or in an artificial rumen system, true indigestibility is never reached. The standard nomenclature is “indigestible NDF (iNDF)” throughout the literature (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.

The current approach for estimating iNDF within the structure of the Cornell Net Carbohydrate and Protein System (CNCPS; Sniffen et al., 1992; Fox et al., 2004; Tylutki et al., 2008) is through the use of acid detergent lignin (ADL) and a fixed factor of 2.4 calculated as $ADL \times 2.4 / NDF$ (Chandler et al., 1980). 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 modeling purposes. 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 the speed of sample analysis.

BIOLOGICAL IMPORTANCE OF 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.

It is also important to point out that other measures of undigested NDF are currently being used in the field to predict dry matter intake of various forages. One published example is uNDF measured at 30 hours of fermentation (termed NDF_{u30}) as described by Jones (2014). This measure of rumen fill appears to be working well in the field as a means to predict dry matter intake, especially for cattle consuming high-forage diets.

CURRENT RESEARCH ON uNDF, RUMEN FIBER DYNAMICS AND FILL

An informal “Fiber Group” has been working on improving the modeling of ruminal fiber turnover within CNCPS. The Fiber Group is comprised of researchers from Cornell University, Miner Institute, University of Bologna, Fencrest LLC, Mertens Innovation & Research LLC, and other industry scientists. Specifically, research groups at Bologna, Cornell, and Miner Institute have focused on evaluating uNDF_{240} and its relationship with chewing behavior, rumen fill, dry matter intake, and lactational performance over a number of feeding trials with various forage and non-forage sources of NDF. Subsequent sections of this paper will summarize current research findings from each of these groups.

MINER INSTITUTE RESEARCH ON uNDF: FOCUS ON LEVEL AND DIGESTIBILITY OF CORN SILAGE FIBER

Details of the projects conducted at Miner Institute have been previously published by Grant and Cotanch (2012) and details may be found there. The objectives of the initial study were to measure the passage kinetics of large, medium, and small particle pools for diets differing in amount and (in)digestibility of NDF from corn silage when lactating cattle are fed a total mixed ration consisting of either conventional or brown midrib (bmr) corn silage. To complement our studies, researchers at the University of Bologna have focused on evaluation of alfalfa and grass as major forage sources and Cornell researchers are focusing on non-forage sources of fiber.

The experimental diets are shown in Table 1. The major differences among the four diets were the forage content (~50% versus ~65%) and the forage NDF source (conventional versus bmr corn silage).

In general, cattle responded to these forage treatments as we would have predicted based on previous research with bmr corn silage and diets of varying forage content. At both lower and higher dietary forage contents, dry matter intake was increased with bmr silage at higher forage levels, but not at lower dietary forage. Hence, it appears that the more highly digestible bmr NDF allowed for greater intake related to greater ruminal turnover. The intake of NDF as a percentage of body weight was high for all diets, but was increased specifically for cows fed the bmr corn silage in a high-forage diet. Similar to feed intake, solids-corrected milk production was unaffected by source of corn silage at the lower forage content, but it was significantly increased by bmr corn silage when

fed in a higher forage diet. Efficiency of milk production was unaffected by source of corn silage at either level of dietary forage, but was lower for the higher forage diets.

Table 1. Composition of diets used in uNDF study at Miner Institute.

| Ingredient | % of ration DM | Diet | | | |
|-----------------------------|----------------|----------------|-----------------|-----------------|------------------|
| | | LF-LD (Low CS) | HF-LD (High CS) | LF-HD (Low BMR) | HF-HD (High BMR) |
| Conventional corn silage | | 39.2 | 54.9 | --- | --- |
| Brown midrib corn silage | | --- | --- | 36.1 | 50.2 |
| Hay crop silage | | 13.4 | 13.4 | 13.3 | 13.3 |
| Corn meal | | 17.3 | 1.6 | 20.4 | 6.3 |
| Grain mix | | 30.1 | 30.1 | 30.2 | 30.2 |
| <u>Chemical composition</u> | | | | | |
| Crude protein, % of DM | | 17.0 | 17.0 | 16.7 | 16.7 |
| NDF, % of DM | | 32.1 | 35.6 | 31.5 | 35.1 |
| Starch, % of DM | | 28.0 | 21.2 | 27.8 | 23.8 |
| 24-h NDF digestibility, % | | 56.3 | 54.0 | 62.0 | 60.3 |
| peNDF, % of DM | | 17.3 | 23.1 | 18.5 | 21.5 |

Eating and ruminating behavior was lowest for cows fed the low forage diet with bmr corn silage. Regardless of the diet, interestingly the percentage of total chewing time spent eating and ruminating did not differ and averaged about 35 and 65%, respectively. From a time budgeting standpoint, the range in eating time was nearly one hour longer between cows fed a high corn silage diet and those fed a lower forage diet with bmr silage. We need to keep this in mind as we move to higher forage diets – what is the impact of forage amount and digestibility on the time required for a cow to process her feed? Details of lactational, chewing, and ruminal responses are found in Grant and Cotanch (2012).

Table 2 provides the NDF and uNDF composition of the three silages and four diets used in the study. The diets with bmr corn silage contained much less uNDF than the conventional corn silage diets.

Table 2. NDF and uNDF composition of forages and diets fed in Miner study.

| Item | BMR CS | Conv CS | HCS | LF-LD | HF-LD | LF-HD | HF-HD |
|----------------------------------|--------|---------|------|-------|-------|-------|-------|
| NDFom, % of DM | 34.8 | 36.1 | 46.2 | 30.8 | 33.7 | 30.7 | 33.5 |
| NDFD _{240m} , % of NDF | 62.1 | 48.6 | 57.7 | ... | ... | ... | ... |
| uNDF _{240om} , % of NDF | 21.9 | 30.5 | 30.3 | 26.7 | 28.5 | 22.5 | 22.6 |
| uNDF _{240om} , % of DM | 7.6 | 11.0 | 14.0 | 8.2 | 9.6 | 6.9 | 7.6 |

Table 3 shows the NDF and uNDF intake and rumen fill of each diet. From this information it appears that, for diets based on corn silage and haycrop silage, maximum NDF intake is approximately 10 kg/d or 1.5% of body weight. Likewise, maximum rumen mass of NDF is about 8.5 kg or 1.3% of body weight. The intake of uNDF appeared to be maximized at 2.6 kg/d or about 0.40% of body weight. The rumen mass of uNDF

ranged between 0.48 and 0.62% of body weight. Fecal output of uNDF balanced the uNDF intake for each diet. Interestingly, the ratio of rumen uNDF/intake uNDF was approximately 1.6 for all diets. We need to determine if this ratio is similar for diets based on other forage types and for cows at other stages of lactation including dry cows.

Table 3. 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 |

Table 4. Total tract fiber digestibility¹: Miner Institute study with conventional and BMR corn silage as low and high digestibility forages fed at high and low dietary forage level.

| Item | Diet ² | | | | SEM | P |
|--|--------------------|--------------------|--------------------|---------------------|------|-------|
| | HF-HD | LF-HD | HF-LD | LF-LD | | |
| TTD pdNDF, % of pdNDF | 71.9 | 68.3 | 71.9 | 69.5 | 1.9 | 0.16 |
| TTD NDF _{om} , % of NDF _{om} | 55.7 ^{ax} | 52.9 ^{ax} | 51.4 ^{ay} | 51.0 ^{by} | 1.5 | 0.04 |
| Fecal pdNDF, kg | 2.13 ^{xy} | 2.22 ^x | 1.86 ^y | 2.01 ^{xy} | 0.16 | 0.07 |
| Fecal ratio pdNDF:uNDF ₂₄₀ | 0.96 ^{by} | 1.10 ^{ax} | 0.70 ^{cz} | 0.84 ^{bcy} | 0.06 | <0.01 |

¹Calculated using uNDF_{240om} as an internal marker.

^{abc} $P \leq 0.05$.

^{xyz} $P \leq 0.10$.

²HF-HD: 64% forage, BMR CS; LF-HD: 49% forage, BMR CS; HF-LD: 68% forage, conventional CS; LF-LD: 53% forage, conventional CS.

BOLOGNA RESEARCH: ALFALFA AND GRASS DRY FORAGE DIETS IN PARMIGIANO REGGIANO REGION OF ITALY

Milk for making Parmigiano Reggiano cheese is produced by cattle fed rations without fermented silages, to avoid uncontrolled fermentations in the cheese due to possible clostridial contamination. According to the feeding recommendations of the Producer Consortium (www.parmigiano-reggiano.it) forages (primarily alfalfa and grass

hays) must be included in the rations at a minimum level of 50% of ration DM in order to preserve milk composition, cheese making properties, health and feeding welfare of the cows.

When rations are provided with a dry TMR technique (no water addition to avoid undesired fermentation in the feed bunk and problems with the cheese making) forages are very finely chopped (usually to a length of 2 cm) to avoid sorting activity and related problems. When a dry TMR is adopted, farm management is improved, as well as dry matter intake (5-10% higher) and cow performance, but the rations need to be constantly available at the feed bunk during the day. In such a TMR, peNDF is consistently lower (12-14%) than levels suggested by literature (22-24%), and diets are balanced with forages able to enhance rumen motility. Previous studies clearly demonstrated that straw, thanks to its particular characteristics, is able to improve rumination, even if very finely chopped (Fustini et al, 2011). For this reason dry TMRs based on alfalfa forage are implemented with straw and/or grass forages like wheat hay, *Lolium*, Italian Ryegrass, or mixed hays.

In order to avoid the risk of peNDF shortage, the free access to long hay in the manger is also recommended. In this case, if the rations are well balanced, cows would consume less than 1 kg/d of long hay on average.

Even if successful due to good practical results, the use of these strategies generates many nutritional questions about the possible negative impact on rumen fermentation dynamics, and on the total fiber digestibility, mainly due to the lack of long particles in the rations.

Therefore, based on discussions among the informal “Fiber Group” and considering the practical importance of this issue for the Parmigiano-Reggiano region, a trial was designed to test the effects of dry TMR differing in level of forage inclusion (High, Low) and forage (alfalfa hay) digestibility (High, Low) on: animal performance, ruminal pH, passage dynamics of medium, small, and fecal alfalfa particles, and estimation of total tract fiber digestibility using uNDF₂₄₀ as the internal marker.

Procedures for Bologna Study

Eight pluriparous Holstein cows (parity = 3.63±0.92) were assigned to a 4 x 4 Latin square. At the beginning of the trial, average milk production was 46.0±5.2 kg/d, 101±38 days in milk and 662 ± 42 kg average BW. Each period lasted for four weeks (3 wk adaptation), and all parameters were recorded during the fourth week of each period. The four experimental diets (HF-HD: High Forage-High Digestibility; LF-HD: Low Forage-High Digestibility; HF-LD: High Forage-Low Digestibility; LF-LD: Low Forage-Low Digestibility) were balanced for starch and crude protein (Table 5). Diets were differentiated in terms of forage NDF (High and Low) using soybean hulls and soybean meal. All the diets had a lower peNDF content compared to the common dry TMR formulated for Parmigiano Reggiano cheese production. However, this lower peNDF

was desired to better measure any effect due to inadequate long forage particles in the ration. We decided to use wheat straw in order to enhance and maintain rumination.

The chemical composition of the two alfalfa hays used in the trial are shown in Table 6. The IVNDFD estimated at 24 h was 9 points lower for LD alfalfa (~26% difference) compared to the HD alfalfa. Moreover, the uNDF/ADL ratio was different and in-line with that observed by previous studies (Palmonari et al., 2014).

Results and Discussion for Bologna Study

Dry matter intake and production data are reported in Table 7. As expected, high digestibility alfalfa led to an increased DMI, regardless the F:C ratio. However, observed differences were higher than expected and reported in literature. In the present study, dry matter intake was 0.63 kg higher for each point of alfalfa 24-h IVNDFD. Such differences could be in part a function of the fine particle size of the TMR used in this experiment. Dry matter intake was unaffected by daily uNDF intake, which was higher in the HF-HD diet. These data suggest that ruminal pdNDF rate of digestion drives DMI, rather than uNDF *per se*.

Thus, it seems that the filling effect of the diet could be related to the amount and rate of degradation of pdNDF fraction. These data confirm the ability of the rumen to retain potentially digestible particles of forages until they reach a critical dimension (due to chewing activity) and specific weight, which increases as the fermentation gas production decreases. Both of these characteristics are influenced by the uNDF:pdNDF ratio and the amount of pdNDF of forages.

In fact, rate of rumen escape is higher for any fiber which is digested (uNDF) compared to the whole fiber (Krizsan et al., 2010). This could be explained by considering that forage particles rich in potentially digestible fiber can probably float in the rumen as long as they have to be almost entirely digested. In accordance to this, data collected in this trial suggest a more active role played by the rate of fiber digestion rather than simply the uNDF intake in explaining the variability in the dry matter intake. Milk production was not different, and the highest milk yield was obtained from the HF-HD diets, while similar results were observed among LD diets. As observed by others, when a higher amount of high digestibility forages are included and consumed we observed a reduced feed conversion when calculated using daily milk production.

Rumination time (Table 8) was continuously recorded by RuminAct (SCR- Israel). We observed significantly higher values for HD diets when compared to LD diets, regardless of forage amount. Rumination time was constant per unit of dry matter and differed when related to uNDF, aNDFom or peNDF.

The pH data were continuously recorded by SmaXtech device (Austria). No differences were found among treatments, even if higher values were observed for the HF-HD diet. The pH data suggest that rumination time *per se* is not able to drive and explain daily or individual pH fluctuations. In the LF-HD diet, we observed an increase of 109 min/d in

rumination compared to HF-LD. The amount of time with pH <5.8 was higher (+170 min/d), while for the pH <5.5 we observed an increase of 72 min/d.

Table 5. Composition of the experimental diets fed in Bologna study.

| Ingredients: % of ration DM | Diet composition | | | |
|----------------------------------|------------------|-------|-------|-------|
| | HF-HD | LF-HD | HF-LD | LF-LD |
| Alfalfa hay (high digestibility) | 46.8 | 36.8 | --- | --- |
| Alfalfa hay (low digestibility) | --- | --- | 38.8 | 30.1 |
| Wheat straw | 8.6 | 8.6 | 8.6 | 8.6 |
| Corn meal fine, 50%:flakes, 50% | 34.4 | 34.4 | 34.4 | 34.4 |
| Soybean hulls | 3.0 | 8.0 | 11.0 | 15.4 |
| Soybean meal, 44% CP | 4.0 | 6.0 | 7.0 | 8.3 |
| Cane-beet molasses blend | 0.5 | 0.5 | 0.5 | 0.5 |
| Vitamin and mineral premix | 1.9 | 1.9 | 1.9 | 1.9 |
| Forage content, % of DM | 55.4 | 45.4 | 47.4 | 38.7 |
| aNDFom forages,% | 23.2 | 19.5 | 23.3 | 19.4 |
| Chemical composition, % of DM | | | | |
| Crude protein | 14.1 | 14.2 | 14.5 | 14.9 |
| NDF | 31.6 | 33.6 | 35.8 | 37.0 |
| aNDFom | 30.7 | 32.3 | 34.4 | 35.2 |
| Starch | 23.1 | 22.6 | 22.7 | 22.9 |
| Sugar | 5.9 | 5.3 | 5.2 | 4.6 |
| peNDF | 12.9 | 12.8 | 11.6 | 11.2 |
| uNDF240om, % of NDF | 35.2 | 29.1 | 32.0 | 27.0 |
| uNDF240om, % of DM | 10.8 | 9.4 | 11.0 | 9.5 |
| IVNDFD24om, % of NDF | 46.2 | 48.9 | 43.8 | 44.6 |

Table 6. Alfalfa hay characteristics for Bologna study.

| Alfalfa hay | High Digestibility | Low Digestibility |
|------------------------|--------------------|-------------------|
| | (HD) | (LD) |
| Cutting | 5 th | 3 rd |
| Dry matter | 92.7 | 91.9 |
| Crude protein, % of DM | 20.5 | 18.5 |
| aNDFom, % of DM | 36.7 | 44.5 |
| ADF, % of DM | 30.1 | 36.1 |
| ADL, % of DM | 7.1 | 7.5 |
| IVNDFD-24h, % of NDF | 40.2 | 31.2 |
| uNDFom240h, % of DM | 15.7 | 18.5 |
| uNDF/ ADL | 2.21 | 2.47 |

Ruminal pH values demonstrate that HF-HD was the only diet that maintained acceptable ruminal conditions. This was not surprising, since this was the higher F:C ratio and in this diet the uNDF intake was 0.48% of BW.

Table 7. Intake and lactation performances for Bologna study.

| Item | Diet | | | | SEM |
|---------------------------|-------------------|--------------------|--------------------|-------------------|------|
| | HF-HD | LF-HD | HF-LD | LF-LD | |
| Dry matter intake, kg/d | 29.7 ^A | 29.2 ^A | 24.5 ^B | 24.5 ^B | 1.43 |
| DMI, % body weight/d | 4.42 ^A | 4.27 ^A | 3.68 ^B | 3.67 ^B | 0.14 |
| NDFom intake, % of BW/d | 1.36 | 1.38 | 1.26 | 1.29 | 0.05 |
| uNDF intake, % of BW/d | 0.48 ^A | 0.40 ^{AB} | 0.40 ^{AB} | 0.35 ^B | 0.01 |
| Water intake, lt/d. | 169 | 173 | 164 | 163 | 11.8 |
| Water intake, lt/kg DMI | 5.8 | 6.0 | 6.7 | 6.7 | 0.33 |
| Milk, kg/d | 41.2 | 40.0 | 39.1 | 39.2 | 2.27 |
| FCM ⁽¹⁾ , kg/d | 37.8 | 36.5 | 34.8 | 36.0 | 0.99 |
| FCM/DMI, kg/kg | 1.27 ^B | 1.25 ^B | 1.42 ^A | 1.47 ^A | 0.04 |

⁽¹⁾ FCM: fat-corrected milk.

^{AB}Means within same row without a common superscript differ ($P \leq 0.01$).

Table 8. Rumination, chewing behavior, and rumen pH for Bologna study.

| Item | Diet | | | | SEM |
|----------------------------|--------------------|-------------------|-------------------|--------------------|------|
| | HF-HD | LF-HD | HF-LD | LF-LD | |
| Rumination, min/d | 487 ^A | 499 ^A | 390 ^B | 410 ^B | 16.4 |
| Rumination, min/kg DM/d | 16.7 | 17.3 | 16.3 | 17.2 | 0.7 |
| Rumination, min/kg NDFom/d | 50.4 ^{AB} | 55.4 ^A | 43.7 ^B | 50.1 ^{AB} | 2.2 |
| Rumination, min/kg uNDF/d | 155 ^b | 184 ^a | 148 ^b | 181 ^a | 7.3 |
| Rumination, min/kg peNDF/d | 129 ^B | 135 ^B | 141 ^A | 156 ^A | 9.3 |
| Daily average pH | 5.81 | 5.72 | 5.77 | 5.71 | 0.07 |
| Time pH < 5.8, min/d | 674 | 903 | 733 | 904 | 135 |
| Time pH < 5.5, min/d | 122 | 329 | 257 | 323 | 107 |
| Area pH < 5.8 | 126 | 235 | 219 | 237 | 68.3 |
| Area pH < 5.5 | 14 | 50 | 77 | 52 | 34.5 |

^{ab}Means within same row without a common superscript differ ($P \leq 0.05$).

^{AB}Means within same row without a common superscript differ ($P \leq 0.01$).

Despite the recorded pH values, total tract NDF and pdNDF digestibility (Table 9) showed good results for all the experimental diets. Higher digestion was observed in the LD diets, in which a lower feed intake was also observed. This fact could suggest that the first limiting factor of ruminal fiber digestibility is the retention time. In fact, in preliminary data relative to the passage rate of fiber obtained in this study, (results not presented here), the highest values were observed in the HF-HD diet. Additionally, the differences in the pdNDF degradability, considering the absolute values on a nutritional and practical perspective, were not so relevant, and results of this study confirm the peculiar ability of cows to retain and digest the pdNDF.

Table 9. Total tract fiber digestibility in Bologna study.¹

| Item | Diet | | | | SEM |
|--------------------|-------------------|--------------------|--------------------|-------------------|------|
| | HF-HD | LF-HD | HF-LD | LF-LD | |
| TTD pdNDF, %pdNDF | 85.5 ^C | 86.1 ^{BC} | 87.6 ^{AB} | 88.9 ^A | 16.4 |
| TTD NDFom, % NDFom | 55.4 ^C | 61.1 ^B | 59.6 ^B | 64.9 ^A | 0.7 |

¹Calculated using uNDF as internal marker.

^{ABC}Means within same row without a common superscript differ ($P \leq 0.01$).

HF-HD: 23.2% forage aNDFom; LF-HD: 19.5% forage aNDFom; HF-LD:23.3% forage aNDFom;; LF-LD: 19.4% forage aNDFom.

Practical Remarks and Guidelines from Bologna Study

The results of the Bologna study suggest the following conclusions:

- Dry matter intake is very much influenced and improved by forage fiber digestibility (when it represents up to 50% of the ration) and not simply by the uNDF intake *per se*.
- HF – HD diet (High level of highly digestible alfalfa hay) allowed the higher milk production and the best ruminal pH values.
- When dietary peNDF % is low, high rumination time can be maintained only with high dry matter intake.
- When feeding a dry TMR diet including finely chopped alfalfa and straw, the suggested level of uNDF intake, in order to maintain the healthier rumen condition, is to be 0.48% of live BW, while and the minimum accepted amount to avoid feeding behavior disturbances should be over 0.40%.
- The use of rations with very low peNDF levels could be acceptable only when forage aNDFom remains up to 24 to 25% of DM;
- The inclusion of soybean hulls to balance the aNDFom supply does not look like the proper way to reduce risk of sub-clinical acidosis (as assessed by ruminal pH), even if the starch content of the diet is low (< 24% DM);
- The total amount of potentially digestible fiber (pdNDF) “utilized” by the cow is higher and better digested compared to what we are currently predicting, and the main factor influencing this function is not related to the forage fiber digestibility, but to the ability of the rumen to retain that fiber.
- The ability to retain the fiber in the rumen is very high even for fine forage particles.

CORNELL RESEARCH ON uNDF: NON-FORAGE FIBER SOURCES OF uNDF

The focus of the work at Cornell has been to extend the data of Raffrenato et al. (2011) into the non-forage fiber/byproduct feeds to better describe uNDF for application in diet formulation and incorporation into the CNCPS. Data from Huhtanen et al. (2007) and Raffrenato et al. (2009) demonstrated that the relationship between lignin and NDF as a fixed factor as has been used in the CNCPS is not appropriate and does not describe the digestible pool of NDF in forages.

As suggested by Mertens and Ely (1979) forage NDF digestion behaves in a heterogeneous manner with two digestible pools and the uNDF determined from a 240-h in vitro digestion (Raffrenato et al. 2011). To investigate this concept in byproduct feed sources, the methodology needed to be investigated and if necessary, adapted to determine the appropriate time points to understand if the NDF component of non-forage feeds behaved in a similar manner as forages. To our knowledge, this concept had never been evaluated in feeds such as beet pulp, soybean hulls, wheat middlings and other high-NDF byproduct feeds.

Eight byproduct or commercial feeds were evaluated using the in vitro approach of Raffrenato (2010) with multiple time points from 0 to 240 h of fermentation. The initial experiment reported that fermentable NDF was exhausted by 96 h for most of the feeds and by 120 h for soybean hulls (Table 10). Furthermore, the study was conducted on twelve feeds measuring digestibility every 3 h for the first 24 h, at 30, 48, 72, 96, and 120 h. This approach was repeated in triplicates and samples of each feed were obtained from 2 different providers.

Table 10. Residues of NDF after 96, 120, and 240 h of in vitro fermentation.

| Feed | Residues (%NDF remaining) | | | RMSE |
|---------------|---------------------------|-------|-------|------|
| | 96 h | 120 h | 240 h | |
| Beet pulp | 0.17 | 0.23 | 0.20 | 0.02 |
| Canola meal | 0.41 | 0.42 | 0.43 | 0.04 |
| Citrus pulp | 0.18 | 0.20 | 0.20 | 0.07 |
| Corn gluten | 0.15 | 0.13 | 0.12 | 0.04 |
| Soybean meal | 0.08 | 0.13 | 0.09 | 0.04 |
| Soybean hulls | 0.07 | 0.07 | 0.06 | 0.02 |
| Soy Plus | 0.09 | 0.06 | 0.06 | 0.03 |
| Wheat midds | 0.32 | 0.32 | 0.28 | 0.03 |

Overall, digestibility was very good for all of the feeds analyzed. The lag time was generally less than 3 h and for all of the feeds, the digestion was complete by 120 h, which is half the time necessary to identify the uNDF in forages.

Degradation curves were plotted on a semi-logarithm scale to evaluate the partition of NDF into fast degrading pool, slow degrading pool, and undigestible pool according to the method explained by Van Amburgh et al. (2003) and Raffrenato and Van Amburgh (2010). In some cases, non-forage feeds do not show the partition of NDF into a fast and slow degrading pool, therefore two different equations were used to describe degradation parameters. Equation 1 was used for non-forage feeds having one pdNDF (potentially digestible NDF) pool degrading with one rate:

$$(1) \quad \text{NDF}_t = \text{pdNDF}^{[-K(t-L)]} + \text{uNDF}$$

where NDF_t is the residue at time t ; L is the lag time; K is the rate of digestion of pdNDF; and uNDF is the undigestible NDF, as percentage of NDF.

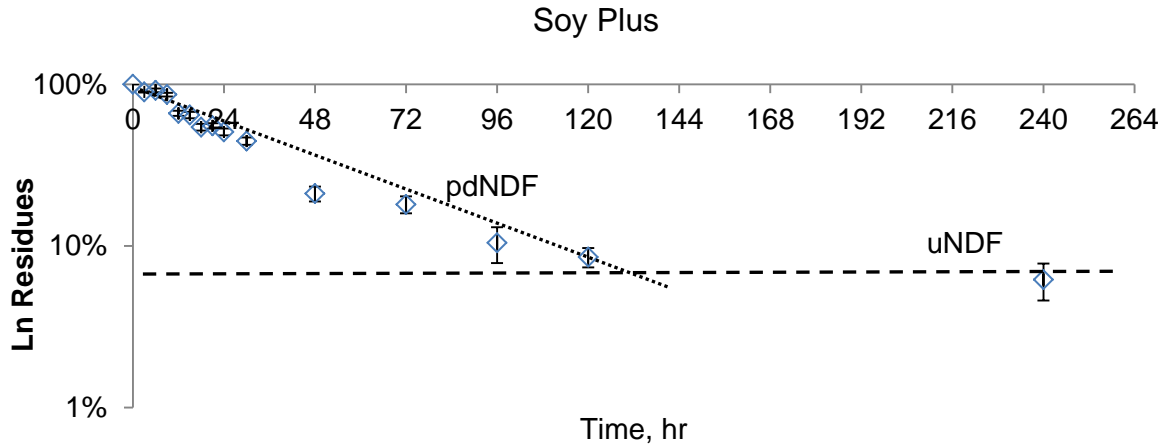


Figure 1. The semi-log plot showing partition of Soy Plus into potentially digestible NDF and undigested pool. The potentially digestible NDF pool was exhausted by 120 h of fermentation.

Equation 2 was used for non-forage feeds demonstrating two pdNDF pool behavior, a fast and slow degrading pool.

$$(2) \quad \text{NDF}_t = \text{pdNDF1}^{[-K1(t-L)]} + \text{pdNDF2}^{[-K2(t-L)]} + \text{uNDF}$$

where NDF_t is the residue at time t ; L is the lag time; $K1$ is the rate of digestion of pdNDF1 ; $K2$ is the rate of digestion of pdNDF2 ; and uNDF is the undigestible NDF, as percentage of NDF.

Furthermore a dynamic model can be used as a tool to validate the biology of NDF in feeds (Raffrenato and Van Amburgh, 2011). For example, if a feed shows a multiple pool behavior on a semi-log plot, than the dynamic model can better predict its degradation using Equation 2; or in the case a feed shows a single pool behavior on a semi-log plot, using Equation 1. As described in Figures 3 and 4, the NDF digestion characteristics of wheat midds are more accurately described using Equation 2, indeed R^2 is higher (0.94 vs 0.88) and the slope of the line is closer to 1 (0.97 vs 1.1) in Figure 4.

In addition, a dynamic model (Raffrenato and Van Amburgh, 2010) was used to select four time-points throughout the 120 h of fermentation that allowed for the most accurate estimation of the degradation decay and would allow for the prediction of digestion rates. The dynamic model allows for the use of fewer data points, compared to statistical models, to estimate the desired parameters (Raffrenato, 2010). Considering that two points are needed to draw a line, a selection of 4 time points; two for each digestible pool was believed to be necessary to characterize NDF pools, and subsequently rates of degradation. Then, the dynamic model was utilized for selection of the best combination of time-points to use when analyzing non-forage feeds for commercial laboratories application.

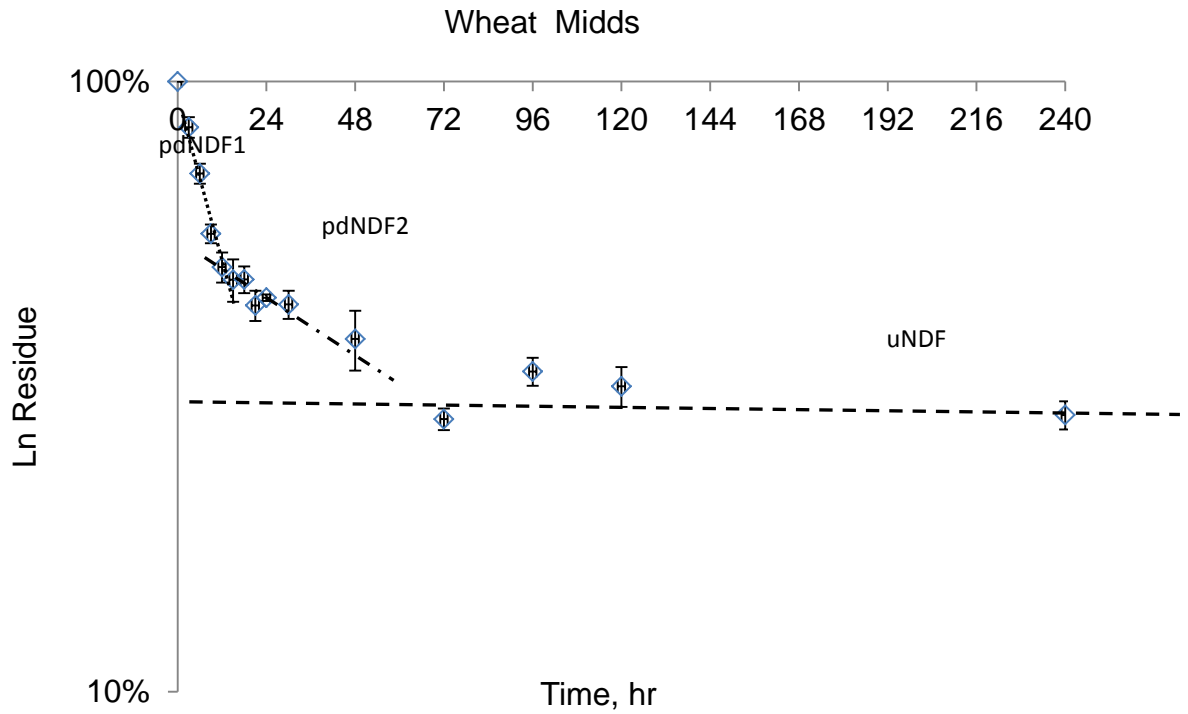


Figure 2. Semi-log plot showing the partition of wheat midds into fast, slow, and undigestible NDF pools. The pdNDF fast pool was exhausted by 18 h of fermentation.

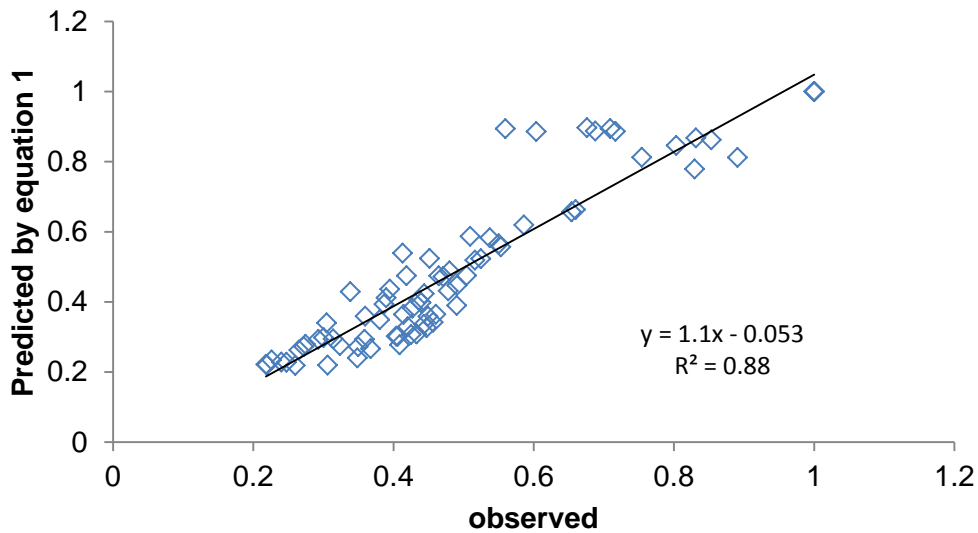


Figure 3. The plot of observed versus predicted NDF digestion values for wheat midds using Equation 1.

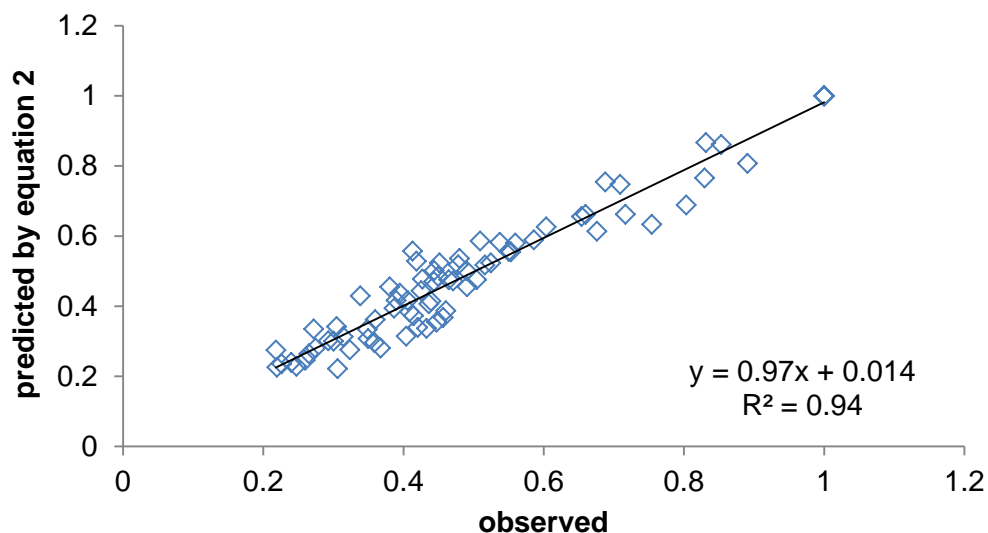


Figure 4. The plot of the observed versus predicted NDF digestion values for wheat midds using Equation 2.

From this analysis, the time points necessary to best describe the digestion curve for routine analysis are 0,15, 48 and 96 h (Table 11) assuming the lab understands the lag period and can account for that in a similar manner as forages. The range in time points for the first observation was 12 h to 18 h to ensure that the NDF digestion observed was in the linear portion of the fast pool digestion.

Table 11. Accuracy of the model among a different combination of time points. Reported are the absolute differences of the slopes from 1 (|1-slope|). The highest accuracy was achieved with 15, 48 and 96 h of in vitro digestion.

| Time points | 0,9,48, 96 | 0,24,48, 96 | 0,15,48, 96 | 0,15,48, 72 | 0,12,48, 72 | 0,12,72, 120 | 0,12,72, 96 |
|-------------|---------------|----------------|----------------|----------------|----------------|-----------------|----------------|
| MEAN | 0.0471 | 0.0393 | <u>0.0383</u> | 0.0495 | 0.0479 | 0.0505 | 0.0451 |
| STD | 0.0164 | 0.0322 | <u>0.0245</u> | 0.0394 | 0.0362 | 0.0248 | 0.0250 |

Finally, parameters of degradation can be defined using the correct equations and the best combination of time points. Wheat midds is used as example for feeds that degrade following a multiple pool behavior and Soy Plus for feeds that degrade following one pool behavior. The model of Raffrenato and Van Amburgh (2010) can be used to fit both approaches, an integrated single pool rate and the multiple pool behavior for incorporating this approach into diet formulation with models like the CNCPS.

Of the feeds analyzed there were similarities between the lignin*2.4 estimation of unavailable NDF and the measured uNDF for corn gluten, soybean hulls, soybean meal and whole cottonseed. For canola, distillers, citrus pulp, the lignin factor greatly over-estimated the indigestible fraction of NDF in these feeds and this observation helps explain why, under certain conditions, more energy is realized from feeding these byproducts compared to current estimates (Table 13).

Table 12. The pdNDF behavior of wheat midds and Soy Plus.

| Feed | Fast Pool NDF (% of NDF) | Slow Pool NDF (% of NDF) | uNDF (% of NDF) | kd 1 (%/h) | kd 2 (%/h) | kd 3 (%/h) | Lag (h) |
|-------------|-----------------------------|-----------------------------|--------------------|---------------|---------------|---------------|------------|
| Wheat midds | 49 | 23 | 28 | 17 | 4 | 0 | 0 |

| Feed | pdNDF (% of DM) | Kd (%/h) | Lag (h) |
|----------|--------------------|-------------|------------|
| Soy Plus | 94 | 4 | 0.7 |

Future Work at Cornell

To fully evaluate the concept of uNDF and rumen fill, the Cornell group will be conducting a study later this winter where we are formulating diets around uNDF. The diets will be formulated to be high or low in forage content, iso-NDF, iso-nitrogenous and iso-starch and be quantitatively different in uNDF which based on the data from the Miner and Bologna studies, should hypothetically result in different DMI due to the amount of the uNDF content of the TMR. Within the structure of the developmental version of the CNCPS, the multiple pool NDF model based on uNDF has been incorporated. The modeling approach allows us to quantitatively predict the difference in rumen NDF content under steady state conditions at the gram level. Incorporating the information on uNDF intake from the Miner and Bologna studies allows us to predict the expected difference in uNDF intake and by back-calculation, the difference in the predicted DMI among diets based on the relative amounts of uNDF consumed, assuming uNDF intake is similar.

SUMMARY AND PERSPECTIVES

The “Informal Fiber Working Group” has been an extremely useful collaborative effort. The ideas and concepts discussed have been actualized into studies and resulting data shared among the groups in an effort to strengthen and develop our understanding of fiber digestion. Furthermore, the data have been generated in a manner that allows us to be quantitative which enhances our ability to develop and incorporate the information into the CNCPS and other models of interest so that nutritionists can learn to possibly implement the approach in the field.

The primary members of the informal Fiber Group who have been involved in this research since the beginning, in addition to the authors, are Dave Mertens, Charles Sniffen, and John Metcalf.

To-date, the combined data sets from Cornell, Bologna, and Miner indicate:

- Daily uNDF intake equals uNDF output in the feces.
- Maximum uNDF mass in the rumen is approximately 0.48 to 0.62 % of BW.

- Maximum NDF intake is approximately 10 kg/d or 1.47% of BW (range of 1.27 to 1.47).
- Maximum uNDF intake is 0.39 % of BW (Miner data) to 0.48% (Bologna data).
- Ratio of rumen uNDF: intake uNDF is 1.60 regardless of diet.

Table 13. The NDF analysis using amylase, sodium sulfite and ash correction, lignin, in vitro digestibility and determination of uNDF of byproduct feeds.

| | Beet pulp | Canola meal | Citrus pulp | Corn gluten | Corn distiller grain | Whole cottonseed | Soy bean meal | Soy hulls | Soy Plus | Wheat midds | Wheat distiller grain | Flaked corn |
|---------------------------------|-----------|-------------|-------------|-------------|----------------------|------------------|---------------|-----------|----------|-------------|-----------------------|-------------|
| aNDFom, % DM | 46 | 25 | 23 | 38 | 42 | 45 | 9 | 74 | 30 | 45 | 38 | 13 |
| Lignin, % DM | 2.10 | 8.39 | 2.63 | 1.94 | 4.50 | 12.88 | 0.50 | 1.94 | 1.13 | 2.7 | 6.20 | 1.77 |
| Lignin*2.4/NDF | 0.11 | 0.81 | 0.27 | 0.12 | 0.26 | 0.69 | 0.13 | 0.06 | 0.09 | 0.14 | 0.39 | 0.33 |
| <u>NDF digestibility, % NDF</u> | | | | | | | | | | | | |
| 15 h | 0.57 | 0.34 | 0.64 | 0.34 | 0.49 | 0.10 | 0.71 | 0.34 | 0.35 | 0.53 | 0.39 | 0.41 |
| 48 h | 0.75 | 0.56 | 0.80 | 0.70 | 0.85 | 0.29 | 0.85 | 0.85 | 0.79 | 0.62 | 0.70 | 0.89 |
| 120 h | 0.77 | 0.58 | 0.84 | 0.86 | 0.86 | 0.29 | 0.89 | 0.92 | 0.91 | 0.68 | 0.75 | 0.93 |
| uNDF (120 h) | 0.23 | 0.42 | 0.17 | 0.14 | 0.14 | 0.71 | 0.11 | 0.08 | 0.09 | 0.32 | 0.25 | 0.07 |

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