A Blind Alfalfa Study of 39 NFTA-Certified Laboratories: The NDF Issue

Introduction

There have been two reported blind studies of NFTA-certified NIR laboratories. The first was by the National Forage Testing Association (NFTA), the National Hay Association, and the University of Wisconsin (2007-2008). The second was done by the University of Nebraska (2008). The samples sent to the laboratories in both studies did not resemble typical alfalfa samples. One important issue to be learned from these studies is there are serious issues with the determination of NDF.

Goals

There are two goals for this study. First, to prepare blind samples with uniform composition that are visibly indistinguishable from routine alfalfa samples. Second, to help improve the determination of Relative Feed Values (RFV).

Sample Preparation

A large set of alfalfa cores from the face of a single alfalfa bale were separated into stems, leaves, and fines using Tyler screens and a homemade air separator. Fines were defined as the material which passed a 2 mm screen, "leaves" (material on the 2 mm screen), and stems. The bulk stems, leaves, and fines were split into halves, quarters, and eighths by conventional methods to afford 24 subsets. Eight samples were assembled from the 24 subsets using a balance (0.01 gram) so that each sample had the same component percentages. The assembled samples were indistinguishable from regular samples. They were dried, ground, and analyzed by NIR. The results for moisture, protein, ADF, and NDF were letter grades of A or B with relative standard deviations (RSD) of 3.6, 3.0, 3.1, and 2.6%, respectively. Based on these experimental results, forty samples were prepared using more accurate (0.001 to 0.005 grams) masses. This afforded sample variations of <0.03%. Starting with about 1,500 grams (3.3 pounds) of alfalfa cores, five sets of eight samples were prepared with each sample containing stems (37.25%), leaves (7.59%), and fines (55.16%). These samples were sent to 40 NFTA-certified laboratories in 2013 for NIR and/or chemical analysis. Based on a basic exploratory data analysis (EDA) and a multivariate analysis of variance of the results, the five sets of 8 were combined into 1 set of 40.

Data Analysis

The averages for the four analytes were determined using the NFTA protocol and NFTA letter grade ranges were calculated, **Table 1.** The data is presented in **Table 2** and the RFV Distribution in **Figure 1**.

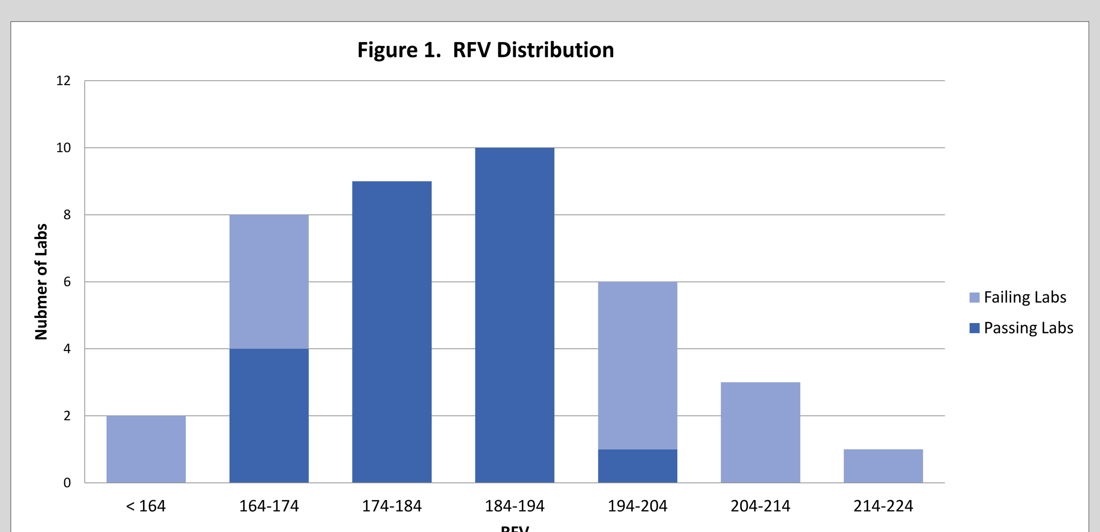
Table 1. Analyte Averages and Letter Grade Ranges.

Analyte	Average	Grade A	Grade B	Grade C
Moisture	8.0%	±0.508	±1.02	±1.52
Protein	23.9%	±0.593	±1.19	±1.78
ADF	28.0%	±0.677	±1.32	±2.03
NDF	34.0%	±0.800	±1.60	±2.40

Table 2. Blind Study Full Data Set

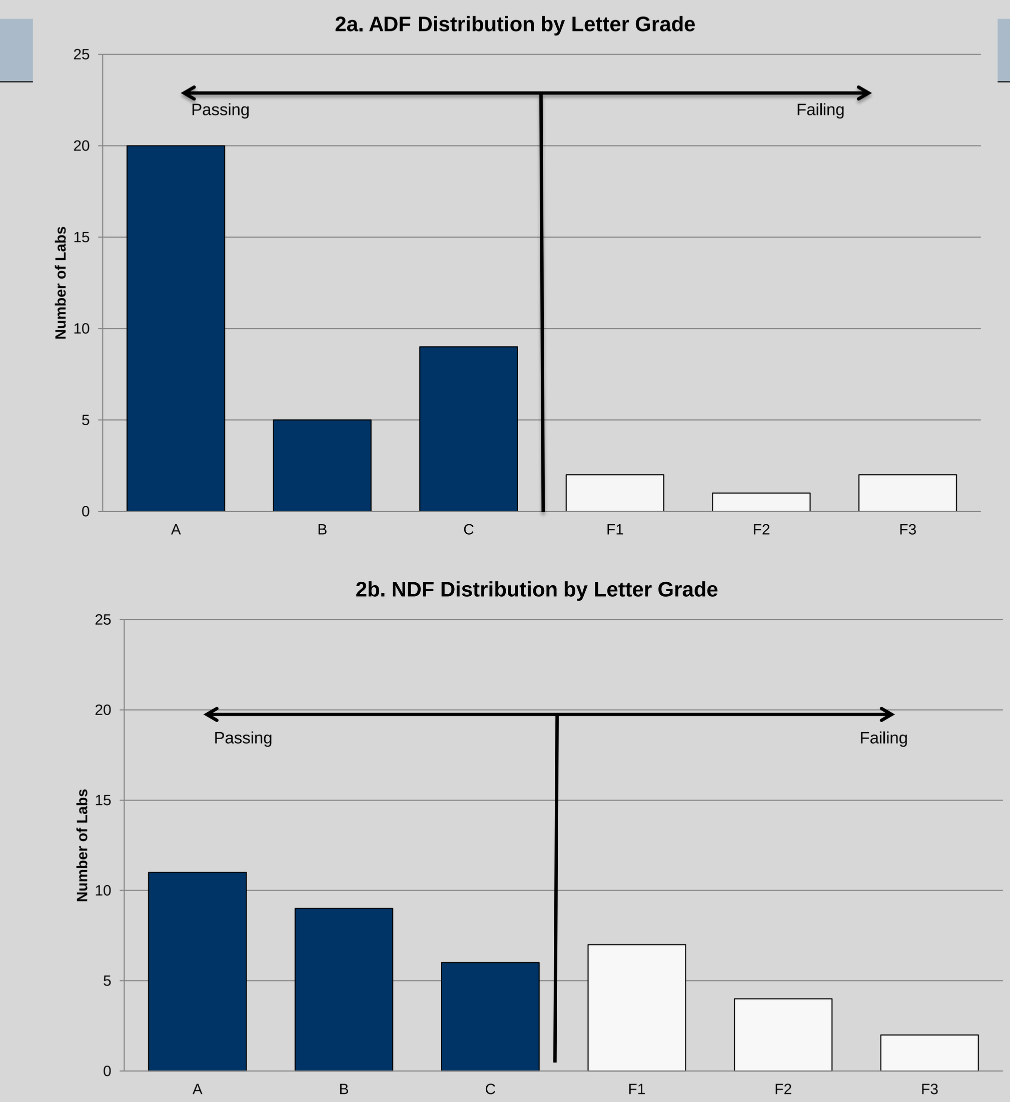
Labs Passing Protein, ADF, and NDF							Labs Failing One or More Analytes					
ID	%M	%Prot	% ADF	%NDF	RFV		ID	%M*	%Prot	%ADF	% NDF	RFV
2N	6.7	23.0	29.9	35.8	171		**1N**	**8.6**	23.7	27.5	**36.8**	171
4N	6.6	22.5	28.3	33.6	185		**3N**	**10.3**	23.4	**31.6**	36.7	163
5N	7.0	23.6	27.9	34.3	182		**6N**	**5.9**	24.4	**25.6**	32.0	200
8N	8.2	25.1	27.7	33.0	190		**7N**	7.6	24.8	28	**31.4**	199
12N	5.9	23.2	28.0	33.3	187		**9C**	9.4	23.2	26.3	**31.6**	202
15N	11.6	25.4	29.4	35.8	171		**10N**	7.7	23.5	27.0	**30.5**	207
16N	6.5	22.6	28.3	35.4	176		**11N**	9.5	23.6	28.3	**37.2**	167
17N	8.2	24.4	27.5	35.8	175		**13N**	8.5	22.5	26.3	**30.6**	208
18N	7.9	25.2	28.5	35.0	177		**14N**	**9.9**	23.0	28.4	**36.8**	169
20C	8.1	25.3	27.0	32.1	197		**19N**	**12.6**	23.3	**25.5**	32.4	198
23N	8.2	25.4	27.6	34.2	183		**21N**	7.3	25.0	27.7	**31.3**	200
24N	7.1	23.4	27.3	34.6	182		**22C**	**8.8**	25.6	**33.1**	**42.6**	138
25N	9.2	24.8	27.7	33.4	187		**26C**	8.3	**22.0**	26.0	**30.0**	210
28C	8.1	24.1	28.1	33.2	188		**30N**	**6.4**	25.0	26.9	**29.4**	217
29N	7.7	23.1	27.1	33.3	189		**33N**	**10.2**	**22.0**	29.9	35.9	170
31N	6.4	24.0	28.1	33.1	188		**35N**	**8.9**	24.7	25.2	**37.6**	171
32N	7.7	23.4	29.5	35.3	174							
34N	7.1	23.4	29.5	34.5	178		* Results exceeding three Horwitz Standard Deviations in bold.					
36C	7.1	24.1	27.8	35.6	176							
37N	8.7	23.5	29.4	35.0	176							
38N	8.4	24.6	28.0	33.5	186							
39N	7.6	23.7	28.9	33.2	186							
40C	7.3	24.7	28.2	33.2	188							

Figure 1. RFV Distribution



The Major Problem

There are on 23 labs that had passing grades for protein, ADF, and NDF while 16 labs failed one or more analytes. RFV is calculated from the ADF and NDF and the distribution of fiber results are shown in **Figure 2.**



Goal 1: Do These Samples Have Uniform Composition?

The standard deviations and RSD are presented in **Table 3**. One would expect samples with uniform composition to have low standard deviations approaching those for NFTA check samples. This is the case.

Table 3. Standard Deviations and RSD Ranges

ID	Protein SD	RSD%		ADF SD	ADF RSD%		NDF SD	NDF RSD%
NFTA*	0.35-0.46	0.38-2.07		0.69-0.76	2.03-2.36		0.72-1.01	1.91-2.68
WL**	0.64	2.68		0.65	2.31		1.30	3.81

***Five alfalfa samples – 2015. **This Study.**

In **Table 4** the distributions of letter grades are presented. There are 39 samples (5 Rows X 8 subsamples) and 19 sets of twins. Twins are samples that were constructed from the same alfalfa quarter, as such; these twins are the most similar samples in the study.

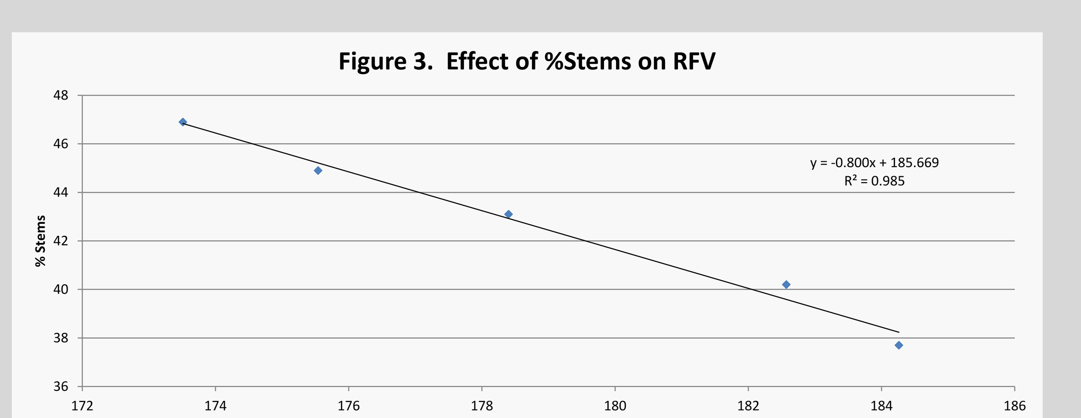
Table 4. ADF Distributions of 39 Letter Grades by Twins

	1	2		3	4		5	6		7	8
1	A	C		F	A		A	F		A	A
2	C	B		A	A		C	A		C	A
3	A	A		F	B		A	F		A	A
4	A	C		X*	A		B	B		A	C
5	C	C		F	A		C	A		B	A

There are 5 F's in the set and their twins are four A's and a B (in yellow). This strongly supports the position that the F's resulted from poor laboratory analyses and not sample variation. Lab 28 received an A and its twin (lab 27) did not report.

There is one additional piece of information that supports the claim that these samples have uniform composition. As expected, there is a linear relationship between the stem percent composition and the RFV ($R^2 = 0.985$), **Figure 3**. There is a linear relationship over the range investigated and to lower the RFV approximately 10 RFV points would require increasing the percent stems by about 10 percentage points. Since fines, leaves, and stems are accurate to better than 0.03%, the samples are not the cause of the large variation.

Figure 3. Effect of %Stems on RFV

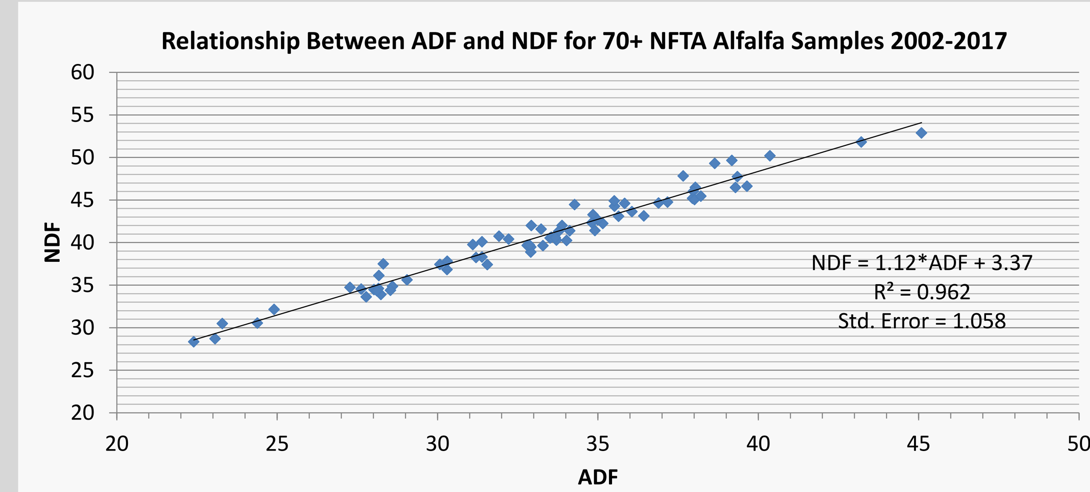


The samples have uniform composition according to three lines of evidence above.

Goal 2: Can these results improve the determination of RFV?

This blind study (**Figure 2**) and the two previous blind studies have demonstrated that NDF is the primary cause for variation in RFV. It is well known that there is a general correlation between ADF and NDF. To examine this relationship we took 16 years of NFTA alfalfa samples (70+); at a minimum this has 6000 individual chemical determinations of both ADF and NDF. The linear regression exhibits a very good fit ($R^2 = 0.962$) indicating 96.2% of the variation in NDF can be explained by ADF, **Equation 1**. A full cross-validation analysis was run and the RFV was calculated within ± 4 RFV points from the ADF.

Eq. 1 $\quad \text{NDF} = 1.12 \cdot \text{ADF} + 3.37 \quad (R^2 = 0.962)$

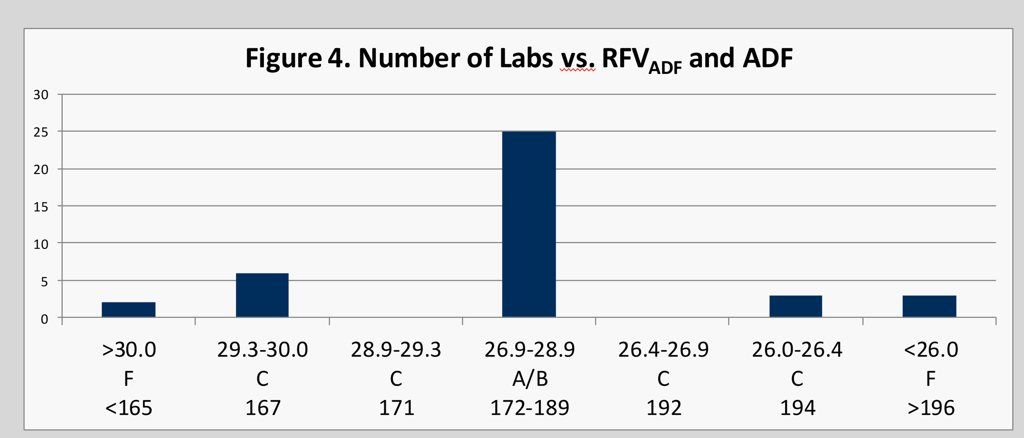


To improve the determination of RFV you need to get more accurate ADF and/or NDF results. To accomplish this, two simple changes are required. **First, find and use laboratories that routinely report ADFs at the B or better level.** If you delete the five labs failing ADF, 74% of the remaining labs meet this performance standard. There are 9 (26%) labs at the C level and their results are within about ± 0.6 points from the B level. NDF presents a different problem; 19 (49%) of the 39 labs NDF results were C's or F's. **Second, replace RFV with the calculated RFV_{ADF}, (Table 5).**

Table 5. Calculated NDF Values

Labs with ADFs of ≥ "B"					C level Labs		
ID	% ADF	$RFV\text{-}_{ADF}$	RFV		ID	%ADF	RFV_{ADF}
1N	27.5	184	171		**2N**	29.9	165
4N	28.3	177	185		**15N**	29.4	168
5N	27.9	180	182		**32N**	29.5	168
7N	28	180	199		**33N**	29.9	164
8N	27.7	182	189		**34N**	29.5	168
10N	27	188	207		**37N**	29.4	169
11N	28.3	177	167				
12N	28	180	187		Avg.	29.6	167
14N	28.4	177	169		SD	0.24	2.0
16N	28.3	177	176				
17N	27.5	184	175		**9C**	26.3	193
18N	28.5	176	197		**13N**	26.3	193
20C	27	188	188		**26C**	26.0	196
21N	27.7	182	200				
23N	27.6	183	183		Avg.	26.15	194
24N	27.3	185	182		SD	0.17	1.73
25N	27.6	182	179				
28C	28.1	179	188				
29N	27.1	187	189				
30N	26.9	189	211				
31N	28.1	179	188				
36C	27.8	181	176				
38N	28	180	187				
39N	28.9	173	187				
40C	28.2	178	187				
Average	27.8	181	185				
Stdev.	0.51	4.1	10.7				

Figure 4. Number of Labs vs. RFV_{ADF} and ADF



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Based on 34 labs, twenty labs reported A's (59%) for ADF (28.0 ± 0.7) and 25 labs (74%) already meet the B or better criterion. For a detailed summary see **Table 7**.

RFV_{ADF} Summary for 34 labs Based on ADF: A & B Letter Grades

20 A labs average RFV 181 Range 177-184 (181 ± 4) (59%)
25 A & B average RFV 181 Range 173 – 188 (181 ± 8) (74%)

A change of 0.6 ADF points on the 9 "C" labs would result in all A or B!

Table 7. Detailed ADF and RFV_{ADF} Summary for 39 Labs

No. of Labs	ADF Range	RFV_{ADF} Range	ADF Letter Grade	
5	26.9-27.3	185-189	1 A & 4 B's	Supreme
11	27.5-28.0	180-184	11 A	Premium
8	28.1-28.9	177-179	8 A	Premium
1	28.9	173	B	Premium
At the B or better level, these labs would not pass.				
3	26.0-26.3	193-196	3 C	Supreme
6	29.4-29.9	165-169	6 C	Good
5 failing laboratories exceeded 3 HSD for ADF				
3	< 25.9	199-203	F	Supreme
2	> 30.0	145-154	F	Good/Fair

The USDA guidelines are presented in **Table 8** in violet. The results in yellow are the results calculated from **Eq. 1** using the ADF values from 25.0 to 31.0.

Table 8. Correlation of ADF and NDF to RFV

Supreme				Premium				Good		
ADF	NDF	RFV		ADF	NDF	RFV		ADF	NDF	RFV
<27	<34	>185		27-29	34-36	170-185		29-32	36-40	150-170
25.0	31.5	205		**27.5**	34.3	183		**29.5**	36.6	168
25.5	32.1	200		**28.0**	34.9	179		**30.0**	37.1	164
26.0	32.6	196		**28.5**	35.4	175		**30.5**	37.7	161
26.5	33.2	191		**29.0**	36.0	171		**31.0**	38.8	154
27.0	33.7	187								

The NDF Issue

There have been two blind studies with NIR labs, one blind study with both NIR and chemistry labs, and one ringtest for chemistry labs (Hristov et al. 2010). NDF variation was a major problem in all four studies and a recent article (Severe, Young 2017) also agrees.

Conclusions

Recommendation 1. The NFTA determines the correct ADF answer using the ~30 NFTA-certified wet chemistry labs running the ADF reference method. If you don't like your results have the lab send the ground sample to a different lab that uses the reference method for determining fibers. This should provide a good ADF result to compare results with. This is the best approach we have found to deal with the "NDF Issue." With a little effort you will find a chemistry lab or an NIR lab whose results agree with those labs running the reference method.

Recommendation 2. Use ADF or RFV_{ADF} to compare labs instead of RFV. Half of the labs are reporting C's or F's on NDF. This results in unacceptable variation in RFV. Using results based on ADF and a calculated NDF (RFV_{ADF}) helps to eliminate this issue.

Conclusion. The NDF issue has been addressed before and the following quote (Hristov et al. 2010) sums up the issue very well: "However, a range of 34.2 to 41.3% aNDF for alfalfa hay or 45.9 to 52.0 aNDF for corn silage is not acceptable for feed evaluation or ration formulation." One possible solution is to require satisfactory laboratory performance on blind samples in addition to the routine check samples for certification.

Notes

1. **About 1/3 of labs fail NDF on blind samples across multiple studies. Given the critical role NDF and its derivatives play in dairy models, it is crucial for consumers to recognize the variability in this component.**
2. **This is the first blind study to publish the laboratory data and include both NIR and wet chemistry labs.**
3. **This is the largest blind study, containing 39 labs (7 of which were wet chemistry).**
4. **This is the first blind study to define the composition (leaves, stems, and fines) of the samples and how they were prepared.**
5. **This study is the first to use samples that are visually indistinguishable from routine alfalfa.**
6. **Weld Labs was included in the first two blind studies and the study from Hristov et al. but is not one of the 39 labs in this study.**
7. **One study at one sample per laboratory does not properly assess laboratory performance. Within-lab variance is missing and would improve this study.**
8. **The next generation of blind samples is under development.**