

# Improvement of the Rumen Fluid Priming Method for Measuring *in vitro* NDF Digestibility

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## Introduction

We recently developed an alternative *in vitro* NDF digestibility (ivNDFD) technique based on rumen fluid inoculum priming that partially removed inter-assay error, but yielded lower ivNDFD estimates

## Objective

Compare intra-assay and inter-assay error of ivNDFD estimates of novel rumen fluid priming ivNDFD method, an unprimed method, and a modified Goering and Van Soest (1970) *in vitro* assay

## Methodology

•Dried, ground (1mm) alfalfa silage or wheat straw weighed into Ankom F57 forage fiber bags and placed in 125 ml Erlenmeyer flasks with continuous CO<sub>2</sub> flow

•Goering and Van Soest (1970) *in vitro* media and proportions

### Three Inoculum Preparation Methods

Goering and Van Soest Method, Experiment A (GV)

•Strained inoculum used to immediately inoculate samples for modified Goering and Van Soest (1970) technique

Combs-Goeser NDF Digestibility<sup>®</sup> (CG), Experiment A and B

•Strained inoculum combined with buffer, reducing solution, and 0.125 mg ground primer per ml rumen fluid in multiple 1000ml Erlenmeyer Flasks

•The primer consisted of a mix of carbohydrates and NPN

•Solution, in 1000ml Erlenmeyer side-arm flasks, allowed to produce 0.12 ml gas per ml rumen fluid prior to inoculation

Unprimed Method, Experiment A (UN)

•CG without primer

### Experiment A

•Rumen fluid inoculum from two lactating cows

•5 time points per technique per replicate, 3 subsamples per time point, 5 replicates completed

### Experiment B

•Related CG to NIRS spectral data for 54 forage samples (local calibration)

•Set later expanded to 122 forage samples (universal calibration)

Table 1: Forage sample composition in Experiment A

Item	NDF, % of DM
Forage Sample	
Alfalfa silage	44.94
Wheat straw	73.58

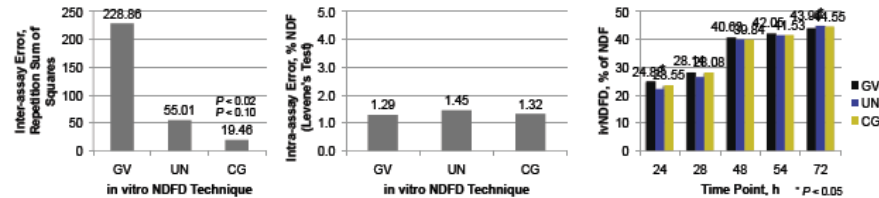


Table 2. NDF composition and *in vitro* digestion estimate statistics for the means of 122 forages in experiment B

Parameter	N	Mean	Minimum	Maximum	Range	SD
NDF, % of DM	122	41.20	22.17	76.37	54.19	10.89
24 h ivNDFD, % of NDF	122	28.85	7.54	56.35	48.82	7.42
30 h ivNDFD, % of NDF	122	35.87	10.67	73.11	62.44	8.35
48 h ivNDFD, % of NDF	122	47.17	24.49	84.59	60.11	8.76

Table 3. Calibration statistics for NIRS prediction of NDF and *in vitro* NDF digestibility for 54 feeds using the Combs-Goeser *in vitro* NDF Digestibility Assay (CGNDFD) - Local Calibration

Parameter	N	Mean	Calibration Statistics					
			Est. Min	Est. Max	SEC	R <sup>2</sup>	SECV	1-VR
NDF, % of DM	53	45.43	10.95	79.90	1.86	0.97	2.44	0.95
24 h ivNDFD, % of NDF	53	23.12	0.00	51.78	2.49	0.93	3.72	0.85
30 h ivNDFD, % of NDF	53	30.25	0.00	62.79	2.82	0.93	4.40	0.83
48 h ivNDFD, % of NDF	53	44.93	11.86	77.99	3.57	0.89	5.01	0.79

Table 4. Calibration statistics for NIRS prediction of NDF and *in vitro* NDF digestibility for 122 feeds using the CGNDFD in experiment B - Universal Calibration

Parameter	N	Mean	Calibration Statistics					
			Est. Min	Est. Max	SEC	R <sup>2</sup>	SECV	1-VR
NDF, % of DM	115	40.32	10.47	70.17	0.88	0.99	1.28	0.98
24 h ivNDFD, % of NDF	119	29.11	7.18	51.04	2.00	0.93	3.66	0.75
30 h ivNDFD, % of NDF	116	35.86	15.55	57.18	1.76	0.94	4.29	0.64
48 h ivNDFD, % of NDF	115	47.25	21.74	72.77	2.31	0.93	3.68	0.81
24 h dNDF, % of DM	118	28.97	0.78	57.15	1.07	0.99	1.96	0.96
30 h dNDF, % of DM	118	26.09	0.00	52.65	0.98	0.99	1.95	0.95
48 h dNDF, % of DM	113	21.06	0.45	41.67	1.16	0.97	1.55	0.95

## Statistical Techniques

•Complete data set for Experiment A analyzed as randomized complete block with sampling using SAS Proc Mixed

•Means compared using least squared means statement

•Data set subset by *in vitro* technique and analyzed using Proc GLM to obtain sums of squares

•Repetition sums of squares compared between *in vitro* techniques with an *F*-test to estimate inter-assay error (4,4 numerator & denominator df)

•Analysis of variance performed on absolute deviance of each observation from the median of triplicates (intra-assay error), Levene's Test (1960)

•Digestion curves were compared with a single compartment exponential model and non-linear regression techniques in R

•Parameter comparisons made by comparing parameters with linear regression

## Results and Summary

•The Combs-Goeser *in vitro* NDF Digestibility technique significantly reduced inter-assay error compared with a traditional laboratory technique and yielded similar ivNDFD estimates

•The improvement in precision resulted in successful NIRS calibration using the CGNDFD as a reference method

•The calibration statistics for 24, 30, and 48 h NDF digestion measurements suggest greater *in vitro* precision and accuracy than achieved previously

## Conclusion

•Priming rumen fluid with a low level of carbohydrates and nitrogen prior to sample inoculation significantly reduced inter-assay error without depressing ivNDFD estimates

•Improved ivNDFD procedure precision resulted in NIRS calibrations for ivNDFD approaching the precision of an NDF calibration

## References

Goering, H. K. and P. J. Van Soest. 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, D.C.  
Levene, H. 1960. Pages 278-282 in Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling. I. Olkin, S. G. Ghurye, W. Hoeffding, W. G. Madow, and H. B. Mann, ed. Stanford University Press, Stanford, CA.

# Amount of Sample NDF Affects Estimates of *in vitro* NDF Digestibility

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## Introduction

-The sample size (g, DM) used with different ruminal *in vitro* digestion assays ranges from approximately 200 to 1000 mg DM content

-Sample size (g, DM) has been shown to affect estimates of *in vitro* dry matter and NDF digestibility (Damiran et al., 2008)

-Amount of NDF incubated within the *in vitro* digestion vessel subsequently may range from less than 60 to 800 mg

-Even with a target sample weight (DM) of 500 mg, the amount of NDF incubated in the *in vitro* digestion vessel may range from 110 to 380 mg

## Objective

Determine if altering the amount of NDF in an *in vitro* digestion vessel affects *in vitro* NDF Digestibility (ivNDFD) estimates

## Methodology

*Combs-Goeser NDF Digestibility<sup>®</sup> - UW Madison Laboratory (CG)*

-Goering and Van Soest (1970) *in vitro* media and proportions

-Dried, ground (1mm) forage sample weighed into Ankom F57 forage fiber bags and placed in 125 ml Erlenmeyer flasks with continuous CO<sub>2</sub> flow

-Strained inoculum combined with buffer, reducing solution, and 0.125 mg ground primer per ml rumen fluid in multiple 1000ml Erlenmeyer Flasks

-The primer consisted of a mix of carbohydrates and NPN

-Solution, in 1000ml Erlenmeyer side-arm flasks, allowed to produce 0.12 ml gas per ml rumen fluid prior to inoculation (standardization)

## Statistical Techniques

-Data were analyzed as a randomized complete block design with a factorial arrangement of forage and NDF level treatments using SAS Proc Mixed

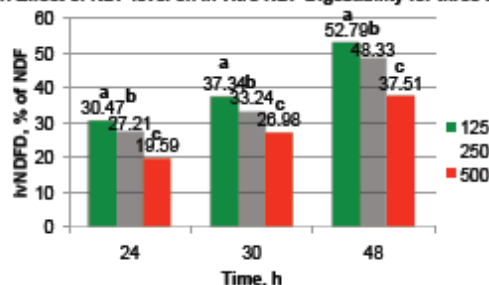
$$Y_{ijm} = \mu + \text{Time (T)}_i + \text{NDF level (N)}_j + \text{Feed (F)}_k + \text{TN}_{ij} + \text{TF}_{ik} + \text{NF}_{jk} + \text{TNF}_{ijk} + e_{ijm}$$

-Data set analyzed separately for each time point after observing a significant three-way interaction between time, NDF content, and forage

**Table 1. Main effect of forage type on *in vitro* NDF digestibility for three digestion time point**

Forage	Time point		
	24	30	48
	ivNDFD, % of NDF		
Normal Corn	17.19 <sup>a</sup>	22.08 <sup>a</sup>	37.79 <sup>a</sup>
Normal Corn Stover	25.27 <sup>a</sup>	35.77 <sup>b</sup>	48.53 <sup>b</sup>
BMR Corn	23.43 <sup>ad</sup>	29.96 <sup>ad</sup>	48.48 <sup>b</sup>
BMR Corn Stover	38.59 <sup>a</sup>	46.81 <sup>a</sup>	63.34 <sup>a</sup>
Alfalfa Silage	29.48 <sup>b</sup>	32.98 <sup>bc</sup>	41.68 <sup>c</sup>
Wheat Straw	20.58 <sup>d</sup>	27.55 <sup>d</sup>	37.54 <sup>c</sup>
SEM	1.12	1.03	2.24

**Figure 1. Effect of NDF level on *in vitro* NDF Digestibility for three time points**



**Table 2. Forage type and NDF level means for 24, 30, and 48 h *in vitro* NDFD**

Sample	Time point, h									
	24			30			48			
	Sample Size, mg of NDF									
	ivNDFD, % of NDF									
Normal Corn	39.79	23.43	14.86	13.27	26.82	23.81	15.60	50.65	40.13	22.58
Normal Corn Stover	63.92	20.09	28.89	26.82	34.42	36.81	36.07	49.55	50.58	45.46
BMR Corn	41.90	31.16	23.94	15.19	41.32	31.56	17.00	61.84	54.84	28.45
BMR Corn Stover	62.20	49.23	42.58	23.97	53.83	45.76	40.83	69.46	64.45	56.09
Alfalfa Silage	42.10	32.57	32.05	23.82	38.70	33.06	27.18	44.44	42.14	38.47
Wheat Straw	71.63	26.35	20.92	14.47	28.96	28.47	25.22	40.81	37.82	33.96

## Statistical Techniques Continued

-Least square means were compared with the SAS lsmeans statement, and significance was declared at  $P < 0.05$  and tendencies discussed at  $P < 0.10$ .

-Appropriate *F*-test denominator degrees of freedom were calculated with the Kenward-Rodger approximation

## Results

-Confounding effect of forage type on NDF content removed by assessing three levels of NDF content with each of six forage types

-Average NDF contents incubated *in vitro* were 128.5, 251.8 and 509.9 mg NDF for the 125, 250 and 500 mg NDF treatments, respectively

-Time, NDF content and forage type all significantly affected *in vitro* NDF digestibility

-Significant interactions for all parameters, means presented in Table 2

-Increasing NDF content within the *in vitro* digestion vessel significantly depressed ivNDFD estimates across all time points using this system

-Results suggest the amount of NDF may complicate conclusions regarding ivNDFD for samples that widely differ in NDF content

-Difficult interpretation for high NDF samples that happen to have corresponding lesser ivNDFD estimates over time or low NDF samples with greater ivNDFD estimates

-Future *in vitro* NDF digestibility research may opt to take the effect of NDF content on ivNDFD into account

## Conclusion

-For ivNDFD comparisons across forages, sample DM weight may need to be adjusted to provide similar amounts of NDF *in vitro*

## References

Damiran, D., T. Del Curto, D. W. Bohner, and S. L. Findholt. 2008. Comparison of techniques and grinding size to estimate digestibility of forage based ruminant diets. *Anim. Feed Sci. Tech.* 141:15-35

# Comparison of Means and Run to Run Variation of *in vitro* NDFD Between Two Labs Using Different *in vitro* NDFD Techniques

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## Introduction

We recently developed an *in vitro* NDF digestibility (ivNDFD) assay that reduced inter-assay error, significantly improving assay precision

## Objective

Compare intra-assay and inter-assay error of ivNDFD estimates for Combs-Goeser NDFD and an ivNDFD technique used in a commercial laboratory

## Methodology

• 9 forage samples coded so that neither laboratory could identify the samples

• Also coded differently for each *in vitro* repetition

• Three *in vitro* repetitions completed by each lab over a three week period

• 24, 30, and 48 h ivNDFD measured for in duplicate within each rep

Modified Goering and Van Soest Method (COM) – UW Marshfield Soils and Forage Laboratory

• Strained rumen fluid inoculum used to immediately inoculate samples using modified Goering and Van Soest (1970) technique

Combs-Goeser NDF Digestibility\* – UW Madison Laboratory (CG)

• Goering and Van Soest (1970) *in vitro* media and proportions

• Dried, ground (1mm) forage sample weighed into Ankom F57 forage fiber bags and placed in 125 ml Erlenmeyer flasks with continuous CO<sub>2</sub> flow

• Strained inoculum combined with buffer, reducing solution, and 0.125 mg ground primer per ml rumen fluid in multiple 1000ml Erlenmeyer Flasks

• The primer consisted of a mix of carbohydrates and NPN

• Solution, in 1000ml Erlenmeyer side-arm flasks, allowed to produce 0.12 ml gas per ml rumen fluid prior to inoculation (standardization)

## Statistical Techniques

• Complete data set for Experiment A analyzed as randomized complete block with sampling using SAS Proc Mixed

• Means compared using least squared means statement

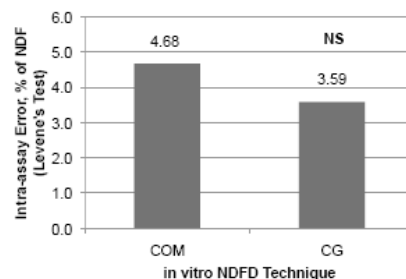
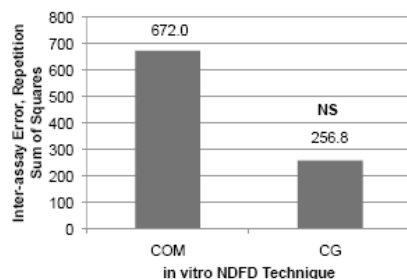
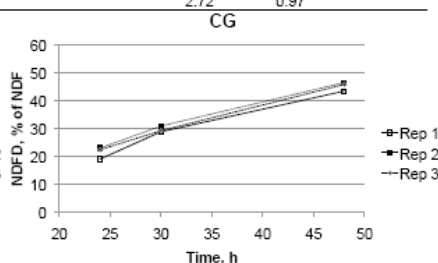
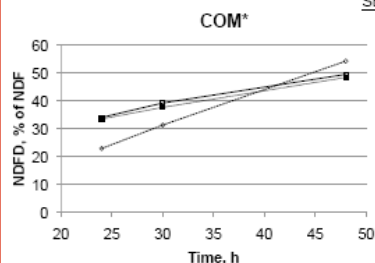


Table 1. NDF content for 9 forages analyzed by two separate laboratories

Forage	NDF, % of DM
Alfalfa Hay	24.90
Alfalfa Silage A	44.76
Alfalfa Silage B	46.61
bm3 Corn Silage	50.60
Corn Silage	36.54
Orchardgrass Silage	50.50
Timothy Hay	63.41
Timothy Silage A	65.43
Timothy Silage B	70.06

Table 2. Mean ivNDFD estimates across 9 forages for two laboratories using different ivNDFD techniques

Item	Laboratory		SEM <sup>1</sup>
	Marshfield	Madison	
Mean ivNDFD, % of NDF	38.84 <sup>a</sup>	32.07 <sup>c</sup>	0.37
Repetition mean ivNDFD, % of NDF			
1	40.77	30.38	
2	39.71	33.41	
3	36.02	32.42	
Time point mean ivNDFD, % of NDF			
24	30.03 <sup>b</sup>	21.45 <sup>e</sup>	
30	35.93 <sup>b</sup>	29.61 <sup>d</sup>	
48	50.55 <sup>a</sup>	45.15 <sup>a</sup>	
SEM <sup>2</sup>	2.72	0.97	



Mean ivNDFD for each repetition by time points for two laboratories using different ivNDFD techniques, <sup>a</sup>significant repetition and time interaction observed for COM

## Statistical Techniques continued

• Data set subset by *in vitro* technique and analyzed using Proc GLM to obtain sums of squares

• Repetition sums of squares compared between *in vitro* techniques with an *F*-test to estimate inter-assay error (2,2 numerator & denominator df)

• Intra-assay error estimated with Levene's Test (1960)

• ANOVA performed on absolute deviance of each observation from the median of its group

## Results

• CG means were significantly lower than COM (Table 2), however both techniques ranked forages in similar order

• Intra-assay error similar between methods

• Inter-assay error:

• CG repetition sums of squares was 2.6 times lower than COM, however not significant

• Limited degrees of freedom with conservative *F*-test (2 and 2 df)

• CG repetition mean ivNDFD numerically closer for three reps compared with COM (Table 2)

• Ranges were 3.03 and 4.75 % of NDF for CG and COM, respectively

• COM did not offer precision necessary to differentiate 24 and 30 h time point mean ivNDFD (Table 2)

• CG standard errors numerically smaller than COM

• Significant repetition and time interaction with COM

• Interaction with COM uncovers poor precision of modified Goering and Van Soest (1970) ivNDFD method

• ivNDFD results obtained in different runs were comparable with the CG method

## Conclusion

The Combs-Goeser *in vitro* NDF Digestibility technique offered improved precision compared with the commercially available method by incorporating a novel rumen fluid inoculum priming procedure

## References

Goering, H. K. and P. J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, D.C.

Levene, H. 1960. Pages 275-282 in Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling. I. Olkin, S. G. Okun, W. Hoerling, W. G. Madow, and H. B. Mann, ed. Stanford University Press, Stanford, CA.