

# Variation in Analytical Results Sources and Solutions

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# Factors Affecting Nutritive Value

- Animal performance = Feed Access X Nutrient Content X Intake X Digestibility X Metabolism
  - Intake = 50-75% of the difference
  - Digestibility = 25-50%
  - Metabolism = 5-15%
- Need to estimate the intake, digestibility and metabolism of the feed

# Objectives

- Describe the measurement of NDF and NDFD
- Discuss sources of variation in each associated with sampling and laboratory reproducibility
- Describe how to use NDF and NDFD to determine For:Conc ratios in dairy TMR
- Informal – will follow the lead of audience questions

# Principles of Analytical Variation

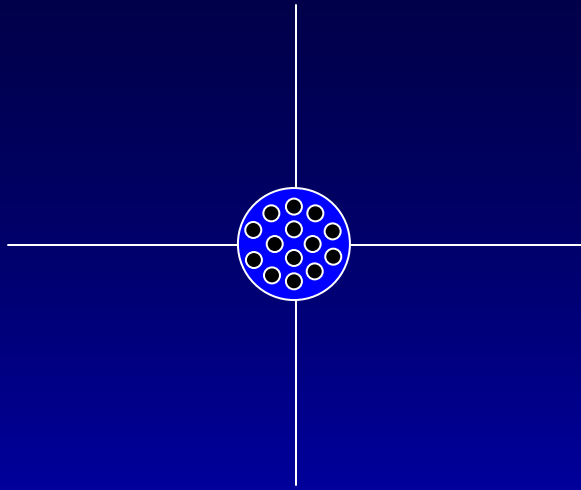
- All analytical results are estimates of the feed actually fed,
- Variation is natural and unavoidable in any measurement,
- Variation can be partitioned into accuracy and precision, and
- Replication and statistical inference are needed to detect differences and provide confidence intervals for analytical results.

# All Analytical Results are Estimates of the True Value

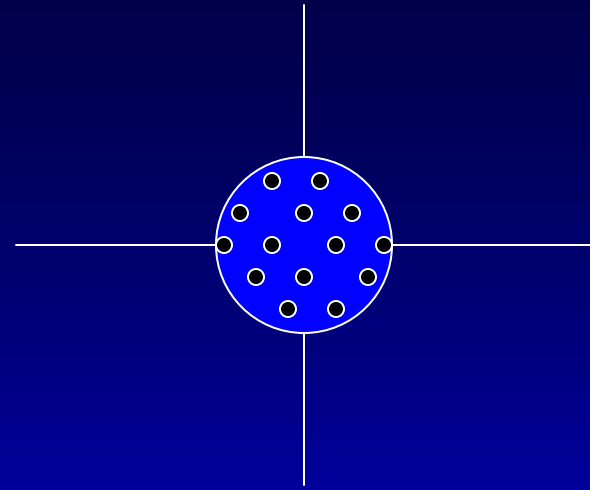
- The only way to determine the CP of a 200 ton stack of hay is to analyze every pound of it
- Taking 20 cores of .5 oz give a sample of 10 oz to represent 200 ton
  - 1/640,000<sup>th</sup> of the stack
- Lab analyzes 1 gm of the sample
  - 1/181,600,000<sup>th</sup> of the stack
- Any wonder that two samples won't agree exactly – each is an estimate of the total

# Difference Between Precision and Accuracy

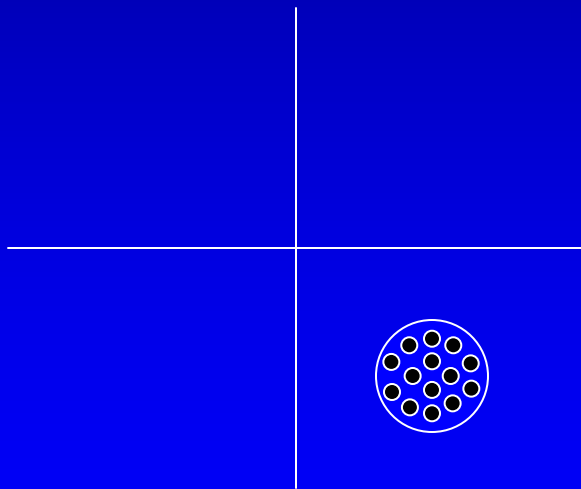
- Accuracy and precision are two different measures of closeness of results
  - Accuracy measures how close the measured result is to the true value
  - Precision measure how closely one measured result agrees with another



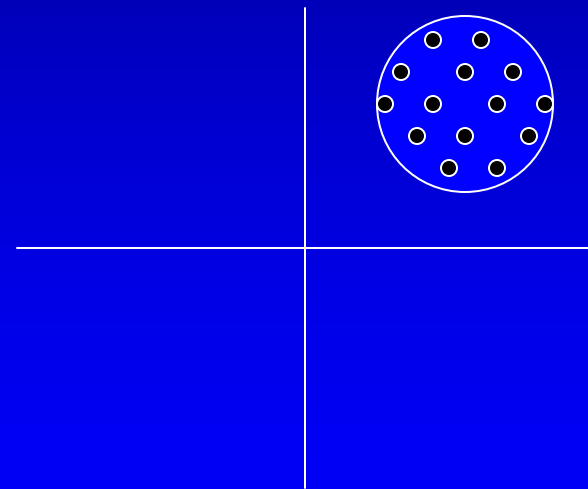
**a. Accurate & Precise**



**b. Accurate, but Imprecise**



**c. Inaccurate, but Precise**



**d. Inaccurate & Imprecise**

# Difference Between Precision and Accuracy

- Just because a lab can repeat results does not mean it is accurate, but only that it is precise
  - Precision tells nothing about accuracy
  - Two close results can still be inaccurate or wrong!
- Accuracy is more important than precision
  - Imprecision can be overcome by more replication, but inaccuracy cannot
- Importance of lab certification by NFTA – comparison of results to a reference value
  - It measures accuracy

# Horwitz Equation for Expected Analytical Variation

- Horwitz summarized data from a broad spectrum of AOAC Official Method
- Observed that coefficient of variation and standard deviation of reproducibility among results was proportional to the mean
  - $RSD(R) = 2^{(1-.5*Conc*.01)}$
  - Probably occurs because methods and equipment are more sensitive when the concentration of the analyte is small
  - May not hold if concentration varies from 0 to 100% for a gravimetric method (based on weighing)

# Expected Variation by Horwitz Single Analyses Among Labs

%Conc.	%CV	SD	Typical Method
10	2.83	0.28	Residual H <sub>2</sub> O, Ash
20	2.55	0.51	CP forages
30	2.40	0.72	ADF forages
40	2.30	0.92	NDF legumes
50	2.22	1.11	NDF legumes
60	2.16	1.30	NDF grasses
70	2.11	1.48	NDF grasses
80	2.07	1.66	

**Always compare analyses  
on an as-is or as-received basis  
DM-adjusted results contain variation in  
both nutrient and DM determinations**

<b>Nutrient</b>	<b>Lab A&amp;B</b>	<b>Lab A</b>	<b>Lab B</b>	<b>Lab diff</b>
	<b>As-Is %</b>	<b>88%DM</b>	<b>92%DM</b>	
		<b>DM basis</b>	<b>DM basis</b>	<b>DM basis</b>
<b>CP</b>	<b>20</b>	<b>22.7</b>	<b>21.7</b>	<b>1.0</b>
<b>NDF</b>	<b>50</b>	<b>56.8</b>	<b>54.3</b>	<b>2.5</b>

# Measuring NDF

- Most variation in NDF among labs is differences in method
- There are several methods for NDF that differ in values
  - NDF (Van Soest and Wine, 1967)
    - With sulfite and without amylase
  - NDR (Robertson and Van Soest, 1981)
    - Without sulfite and with amylase
  - aNDF (Mertens, 2002)
    - With sulfite and amylase

# Variation due to Differences in NDF Methods

Feed description	NDF	NDR	aNDF	aNDF/NDR
Wheat straw	83.9	86.0	82.8	96.3
Timothy	67.2	68.0	65.1	95.7
Corn silage	55.9	55.0	52.6	95.6
Alfalfa hay	47.2	50.4	46.3	91.9
Alfalfa silage		43.6	42.2	96.8
Citrus pulp		21.3	20.2	94.8
Corn grain		11.4	10.1	88.6
Brewer's grains		52.3	40.9	78.2
Distiller's grains		38.6	27.9	72.3
Soybean meal		18.5	12.4	67.0

NDF = with sulfite and without amylase

NDR = without sulfite and with amylase

aNDF = with sulfite and amylase

# Measuring In Vivo Digestibility

- Maintenance level of intake (1X)
  - Reduces the effects of intake and selection on in vivo measurements of digestibility
  - Most consistent assessment of the “intrinsic” digestibility of feeds because animal effects are minimized
  - Excellent starting value for determining net energy
    - Digestible dry matter (DDM 1X), digestible organic matter (DOM 1X) or total digestible nutrients (TDN 1X)
    - Use models of digestion to adjust digestibility to reflect production levels of intake and dietary interactions

# Measuring In Vivo Digestibility

- Production levels of intake (3-5X maintenance)
  - Digestibility is reduced by decreased retention times
  - Selection can affect results – what the cow ate is not the feed that was sampled and analyzed
  - Crucial reference standard
    - Validation of routine laboratory methods
    - Validation of digestion models
    - Generation of relationships for converting laboratory estimates of digestibility to animal responses

# Laboratory Estimation of Digestibility

- In vivo digestibility cannot be measured in the laboratory
- DMD or NDFD on feed analysis reports are estimates based on a laboratory in vitro or chemical measurement
- Their value depends on their accuracy (closeness to the in vivo reference digestibility) and precision (repeatability and reproducibility among labs)

# Laboratory Estimation of Digestibility

- Ruminant microorganisms are affected by all factors (known and unknown) that limit fiber digestibility
- Most fiber digestion (70-100%) occurs in the rumen
- Two major ruminal techniques for measuring in vitro digestibility in the laboratory
  - Tilley & Terry 48h DM Disappearance
  - Van Soest 48h DM true digestibility

# In Vitro Measurements

- **Tilley and Terry DM disappearance**
  - 48-h fermentation with ruminal inoculum
  - 48-h acid pepsin digestion
  - Traditional in vitro method
  - Highly correlated with in vivo total tract digestibility

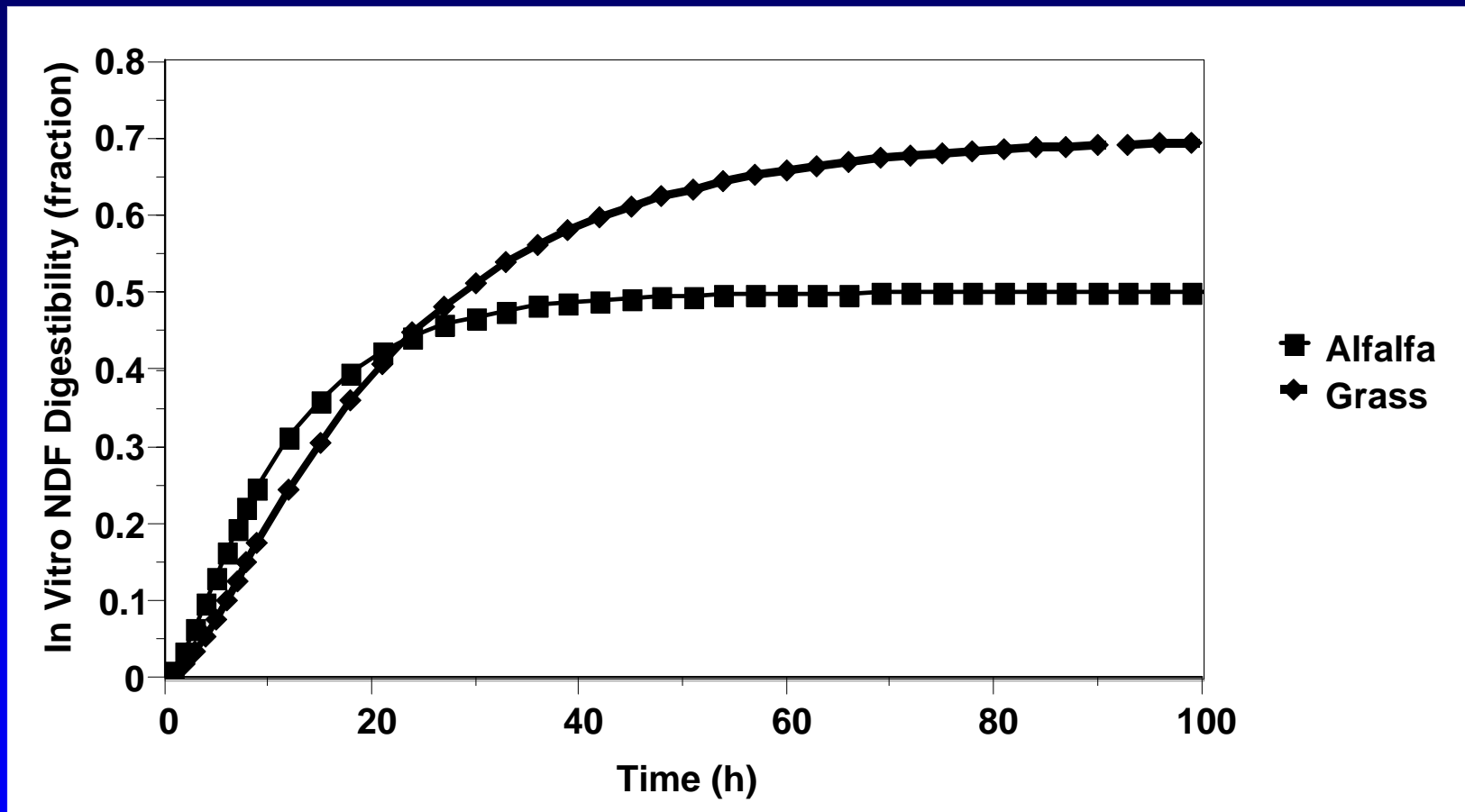
# In Vitro Measurements

- Van Soest True Digestibility
  - 48-h fermentation with ruminal inoculum
  - Extraction in neutral detergent (ND)
  - ND extraction removes microbial and DM contamination so the true digestion of the feed is measured (endogenous matter is removed)
  - 2 days shorter and has lower error of prediction
  - Can simultaneously measure NDF digestibility

# Measuring NDFD

- VS DMTD will always be 8-10%-units higher than T&T DMD because of microbial debris
- Fermentation time is a crucial factor in determining digestibility using laboratory in vitro methods (24, 30 or 48h ??)
- Retention time (reciprocal of passage rate) in vivo is not the same as fermentation time in batch in vitro

# NDF Digestion for Typical Forages



# Measuring IVNDFD

- Routinely measuring in vitro digestibility is difficult
  - Time consuming
  - Fistulated animals are required
  - Methods are not standardized
  - Biological systems are inherently variable
  - Relationship to in vivo digestion has not been established (relative ranking, but perhaps not the absolute value)

# IVNDFD Variability

- IVNDFD will always be twice as variable as DMD
  - Statistical considerations
    - NDFD requires the measurement of two variables NDF and in vitro NDF residue, each of which has variability
  - Mathematical artifact due to scale
    - Because NDF is a fraction of DM, dividing IV disappearance by this fraction will increase the standard deviation
  - Solution = more replication of measurements

# Change in Std Dev with Scale

Observation	Result A	A/.40
1	20.0	50.0
2	21.0	52.5
3	22.0	55.0
4	23.0	57.5
5	24.0	60.0
6	25.0	62.5
7	26.0	65.0
8	27.0	67.5
9	28.0	70.0
10	29.0	72.5
11	30.0	75.0
Mean	25.0	62.5
Std. Dev.	3.16	7.91

# Variability in NDFD

Variable and technique	n=1	n=2	n=4	n=8
In vivo DMD 1X	3-4		1.8	
In vivo NDFD 1X	4-6			1.8
In vivo NDFD 3X	8-10			3.2
In vitro NDFD 48h, within lab between runs	4	2.8	2	
In vitro NDFD 48h, among labs	10	7.5	5	
In vitro NDFD 30h, within lab between runs	5	3.5	2.5	
In vitro NDFD 30h, among labs	12	8.4	6	

# Putting Analytical Variation into Perspective

- Sampling variation is almost always greater than analytical variation
  - Too often only a single sample is split to detect analytical variation –
    - Sample splitting and analytical variation are confounded
  - Splitting multiple samples would evaluate both sampling and analytical variation
    - Can separate splitting and analytical sources of variation
  - Multiple samples should be the rule rather than the exception

# Factors Affecting Forage Testing Results

- Sampling
  - Results are no better than the sample that was submitted and analyzed
  - Lots of feed should be samples separately
  - Truly representative samples require multiple samples (>12) must be taken and combined or composited
  - Many times sampling during harvest is the best way of obtaining a representative sample

# Factors Affecting Forage Testing Results

- Laboratory proficiency
  - Accurate analyses are crucial to both nutritional and financial decisions
  - Most forage testing laboratories attempt to provide reliable and repeatable results
  - National Forage Testing Association (NFTA) manages a proficiency testing program
    - 150 participating laboratories
    - Receive 6 samples per year
    - Evaluate DM, CP, ADF and NDF
    - Certification is based on a cumulative grade for all samples and methods during a year

# Putting Analytical Variation into Perspective

## Comparison of Sources of Variation

Analytical	SE	Hay	SE	Silage	SE
Avg 'A' Lab single analysis	0.60	5 cores	2.15	5 loads	2.46
Avg 'A' Lab duplicate analysis	0.54	10 cores	1.52	10 loads	1.74
Two avg 'A' Labs single analysis	0.48	15 cores	1.24	15 loads	1.42
Avg 'B' Lab single analysis	1.50	20 cores	1.07	20 loads	1.23
Avg 'B' Lab duplicate analysis	1.35	5 grab	3.00	5 grab	2.37
Two avg 'B' Labs single analysis	1.21	10 grab	2.12	10 grab	1.68
Avg 'C' Lab single analysis	2.50	15 grab	1.73	15 grab	1.37
Avg 'C' Lab duplicate analysis	2.27	20 grab	1.50	20 grab	1.19
Two avg 'C' Labs single analysis	2.00				

# Putting Analytical Variation into Perspective

- True values (minimum bias and variation) require multiple samples submitted to multiple laboratories
- Laboratory imprecision can be compensated for by replicated analyses, but accuracy (lack of systematic bias) has no substitute
- Minimum analytical variation cannot compensate for a poorly collected and mixed sample
- Analytical variation is small relative to the relationships between nutrient content and animal responses
- But labs can differ in analytical results – select a lab and use results judiciously

# Relationship of Digestibility to Fiber

adapted from Giger-Reverdin, 1995  
(average parameters and SD of 15 expts)

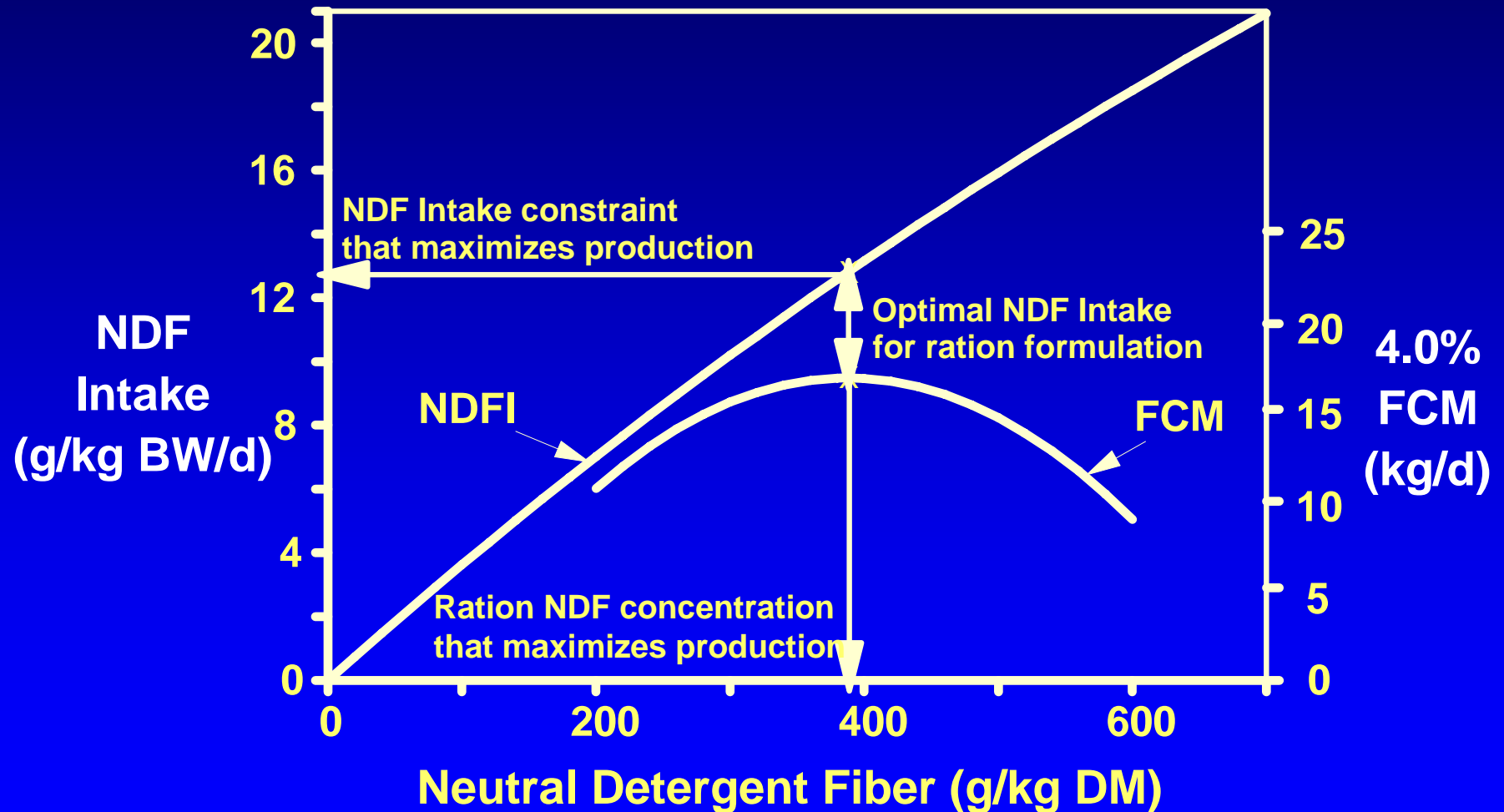
	Intercept	Slope	R <sup>2</sup>	Reg.SE
<b>CF</b>	<b>89.7</b>	<b>-0.772</b>	<b>0.63</b>	<b>5.31</b>
	7.2	0.259	0.16	2.53
<b>NDF</b>	<b>97.7</b>	<b>-0.544</b>	<b>0.67</b>	<b>5.92</b>
	7.8	0.174	0.12	3.28
<b>ADF</b>	<b>95.4</b>	<b>-0.714</b>	<b>0.68</b>	<b>4.95</b>
	9.1	0.429	0.16	2.53
<b>ADL</b>	<b>83.1</b>	<b>-2.975</b>	<b>0.79</b>	<b>4.84</b>
	3.7	0.683	0.08	2.86

# Using NDF to Formulate Rations Containing Grasses

- Mertens (1992) developed a system that maximizes forage in the ration for a target milk production using on NDF
- Based on simple theories of intake regulation
  - Cows limit intake of high-energy diets based on energy demand
  - Cows limit intake of high-fiber diets based on fill limitations

# NDF-Energy Intake System

## Defining the NDF Intake Constraint



# Maximum Forage Rations

Forage is 75% corn silage (40% NDF)  
and 25% Grass (55% NDF)

Target 3.5% fat milk, lb/d	77	88	99	110	121
Opt. NDFI, % of BW/d	1.20	1.20	1.20	1.20	1.20
Forage, % of TMR DM	76.3	67.1	59.1	52.0	45.7
NDF, % of TMR DM	36.2	33.3	30.8	28.5	26.5
DMI, lb/d	47.4	51.5	55.8	60.2	64.7
DMI, % of BW/d	3.31	3.60	3.90	4.21	4.52
NEL3X, Mcal/kg	1.676	1.716	1.752	1.783	1.811
Intake adj. NEL, Mcal/kg	1.644	1.659	1.668	1.672	1.671

# Maximum Forage Rations

Cow producing 99 lb/d of 3.5% fat  
75% corn silage and 25% grass

Grass NDF, % of DM	51	53	55	57	59
Opt. NDFI, % of BW/d	1.20	1.20	1.20	1.20	1.20
Forage, % of TMR DM	61.4	60.2	59.1	58.0	57.0
NDF, % of TMR DM	30.9	30.8	30.8	30.7	30.7
DMI, lb/d	55.6	55.7	55.8	55.9	56.0
DMI, % of BW/d	3.89	3.89	3.90	3.91	3.91
NEL3X, Mcal/kg	1.758	1.755	1.752	1.749	1.746
Intake adj. NEL, Mcal/kg	1.673	1.671	1.668	1.665	1.662

# Differences Between High and Low Digestibility Forages

(compiled from 17 published reports)

Variable	High Dig Forage	Low Dig Forage
IVNDFD30h	.544	.437
Forage NDF, % of DM	46.6	47.5
Forage, % of TMR DM	53.4	53.6
Dietary NDF, % of DM	36.0	36.5
NDF from forage, % of DM	68.7	66.5
IVNDFD48wtd	43.6	34.7
DM Intake, kg/d	23.7	21.7
NDF Intake, % of BW/d	1.37	1.32
4% Fat-corrected milk, kg/d	32.7	31.0
Body weight change, kg/d	0.19	-.02
In vivo NDF Digestibility	.502	.437

# Differences Between High and Low Digestibility Forages

- Relationships with IVNDFD48h
  - $\text{DMI} = \text{Trial} + .097(\pm.0242)*\text{NDFD48h} - .312(\pm.125)*\text{DietNDF}; R^2 = .949,$
  - $\text{NDFI} = \text{Trial} + .00485(\pm.00178)*\text{NDFD48h} - .0237(\pm.0092)*\text{DietNDF}; R^2 = .930,$
  - $4\%\text{FCM} = \text{Trial} + .139(\pm.037)*\text{NDFD48h} - .520(\pm.202)*\text{DietNDF}; R^2 = .977$
- NDFD coefficients differed from Oba and Allen (1999)
  - DMI, kg/%NDFD48h (.097 versus .17)
  - 4% FCM, kg/%NDFD48h (.139 versus .25)
- Diet NDF coefficient is 3 times the NDFD48h coefficient
  - for DMI, .312 vs .097; for 4%FCM, .520 vs .139

## Maximum Forage Ratios (Mertens, 1992)

75% corn silage and 25% grass

Increase grass 10 IVNDFD %-units

Target 3.5% fat milk, lb/d	77	88	99	110	121
Opt. NDFI, % of BW/d	1.20	1.20	1.20	1.20	1.20
Forage, % of TMR DM	76.3	67.1	59.1	52.0	45.7
NDF, % of TMR DM	36.2	33.3	30.8	28.5	26.5
DMI, % of BW/d	3.31	3.60	3.90	4.21	4.52
Opt. NDFI, % of BW/d	1.22	1.22	1.22	1.22	1.22
Forage, % of TMR DM	78.6	69.2	61.0	53.7	47.3
NDF, % of TMR DM	37.0	34.0	31.4	29.1	27.0
DMI, % of BW/d	3.30	3.59	3.89	4.20	4.52

# Variation in Analytical Results Sources and Solutions

Questions?

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# Take-Home Message

- All analytical results are estimates of the feed actually fed
- Variation is natural and unavoidable in any measurement
  - Variation cannot be eliminated only minimized or controlled
- Variation can be partitioned into accuracy and precision
  - Being precise does not mean you are accurate

# Take-Home Message

- Replication and statistical inference are needed to detect differences and provide confidence intervals for analytical results
  - Multiple samples need to be split to separate splitting variation from analytical variation
- Putting analytical variation in perspective
  - Sampling variation is almost always greater than analytical variation
  - True value is best determined by multiple samples analyzed by multiple labs
  - Select a lab and use results judiciously

# Summary: Take-Home Message

- Grass NDF can be used to formulate rations that will maximize forage for a target milk production
- Changing the digestibility of NDF can be incorporated into the NDF system for formulating rations

# Variability in the aNDF Method

Feed	n	Fiber	Mean(%)	SDr	SDR	R
Forages	5	aNDF	52.2	0.84	1.10	3.08
Forages	5	aNDFom	50.4	0.93	1.18	3.31
Concentrates <10% fat	3	aNDF	33.5	1.18	1.42	3.98
Concentrates <10% fat	3	aNDFom	32.5	1.16	1.46	4.08
Concentrates >10% fat	3	aNDF	8.7	1.01	1.61	4.50
Concentrates >10% fat	3	aNDFom	8.8	0.58	1.20	3.37
All materials	11	aNDF	38.7	1.05	1.33	3.72
All materials	11	aNDFom	37.7	1.02	1.28	3.58

**SDr = Standard deviation of repeatability within labs**

**SDR = Standard deviation of reproducibility among labs**

**R = Confidence range of 95% of single analyses among labs**









