
In-vitro and In-situ Measurements:
IMPROVING ACCURACY
From a Commercial Laboratory Perspective
Donald A. Sapienza, PhD

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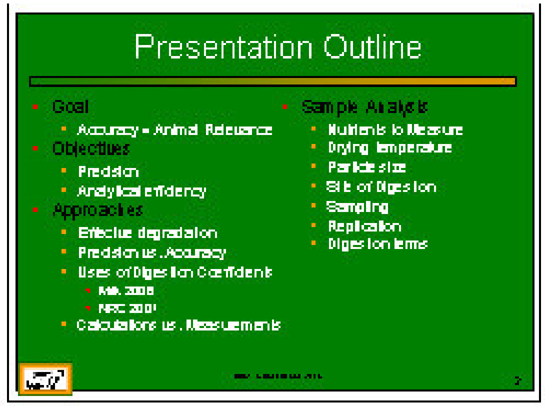
Presentation Outline
Goal
Objectives
Approaches
Sample Analysis

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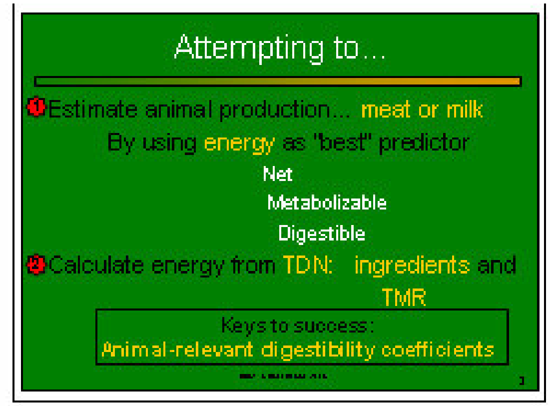
Attempting to…
Estimate animal production… meat or milk
By using energy as "best" predictor
Net
Metabolizable
Digestible
Calculate energy from TDN: ingredients and TMR
Keys to success:
Animal-relevant digestibility coefficients

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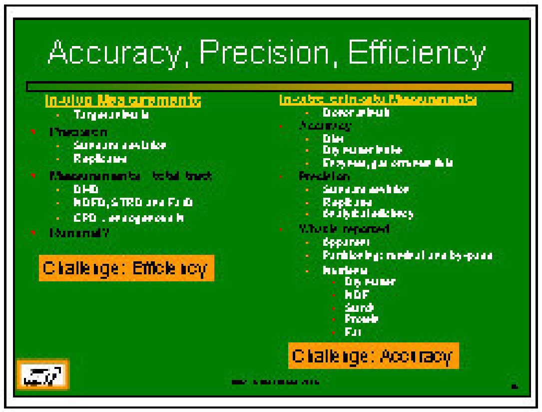
Accuracy, Precision, Efficiency
Challenge: Efficiency
Challenge: Accuracy

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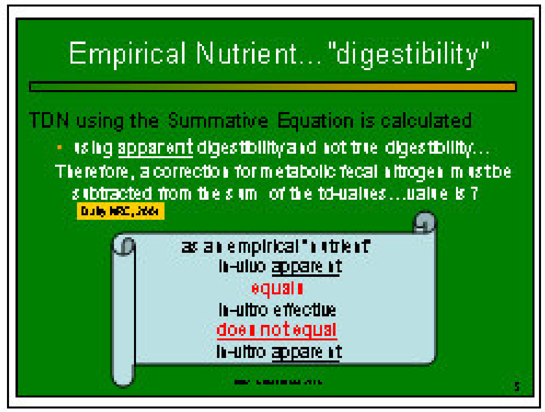
Empirical Nutrient… "digestibility"
TDN using the Summative Equation is calculated
in-vitro effective

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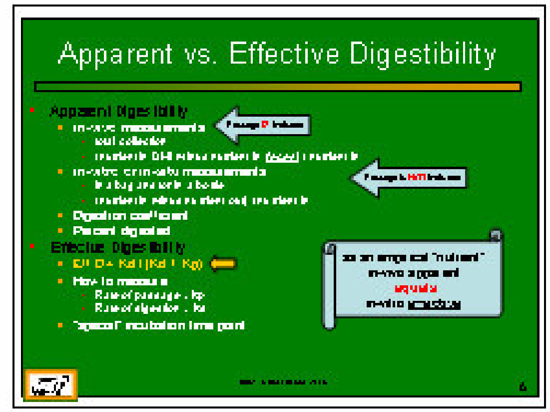
Apparent vs. Effective Digestibility

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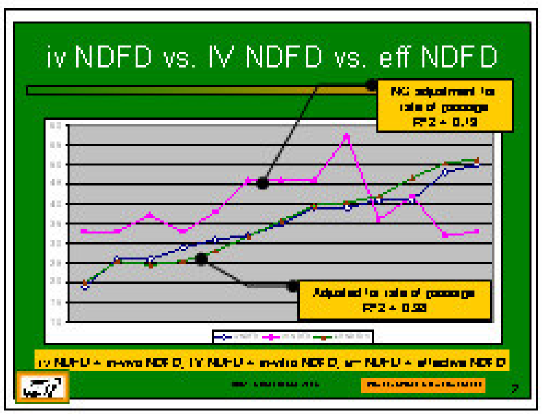
iv NDFD vs. IV NDFD vs. eff NDFD

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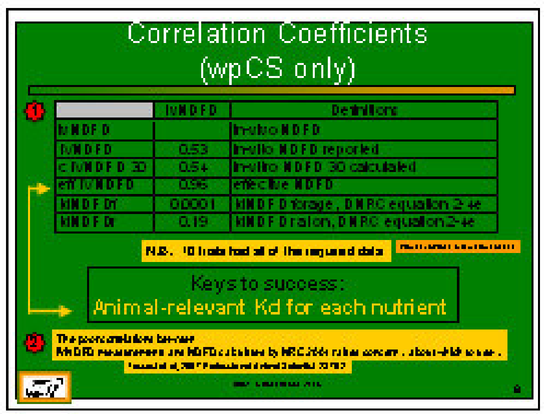
Correlation Coefficients
(wpCS only)
IVNDFD
Definitions
ivNDFD
In-vitro NDFD
IVNDFD
0.53
In-vitro NDFD reported
cIVNDFD 30
0.54
In-vitro NDFD 30 calculated
eff IVNDFD
0.96
effective NDFD
Keys to success:
Animal-relevant Kd for each nutrient

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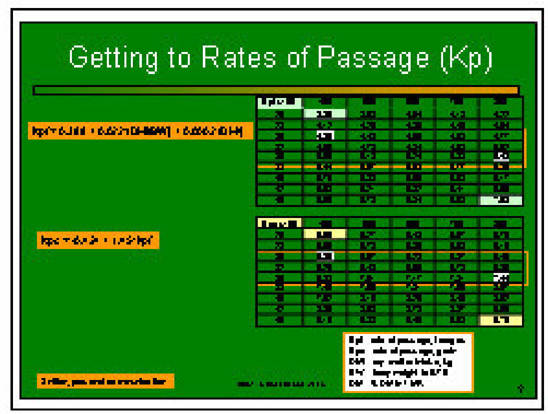
Getting to Rates of Passage (Kp)

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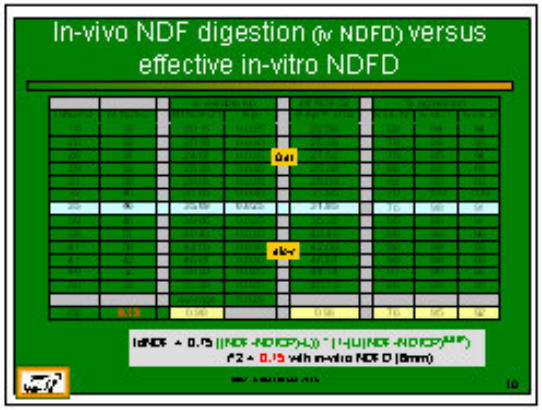

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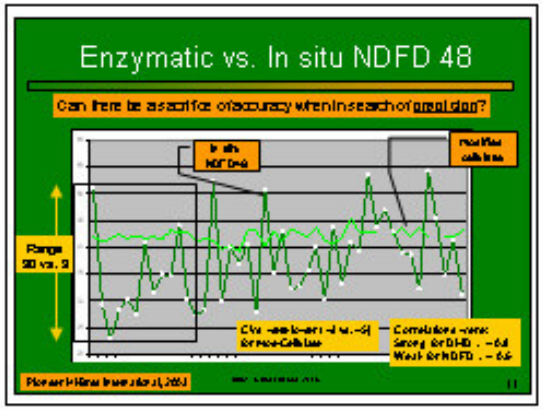

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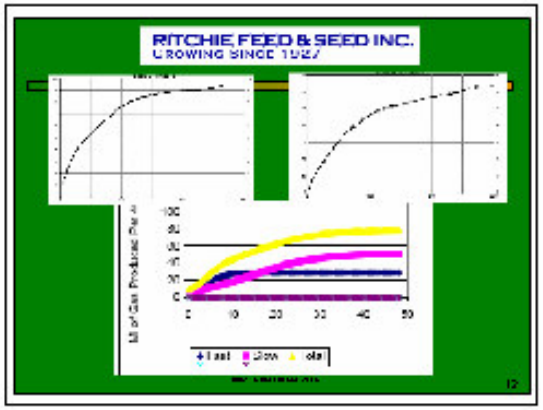

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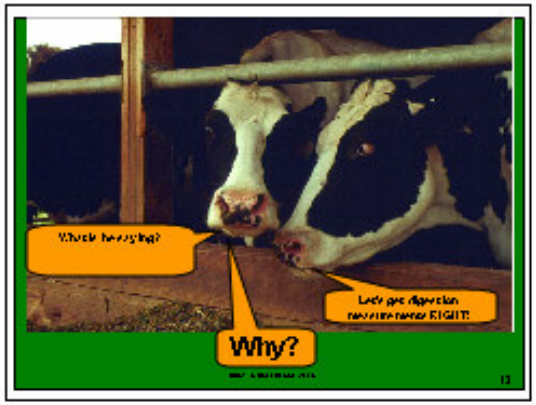

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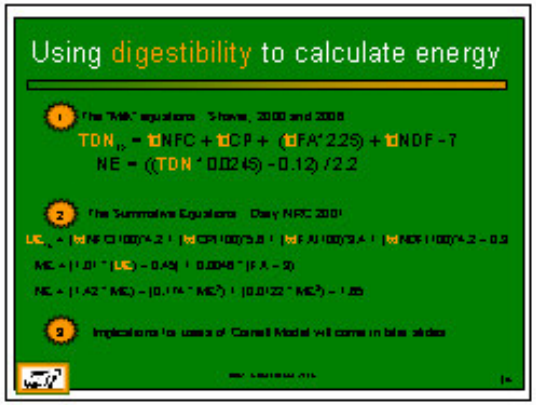

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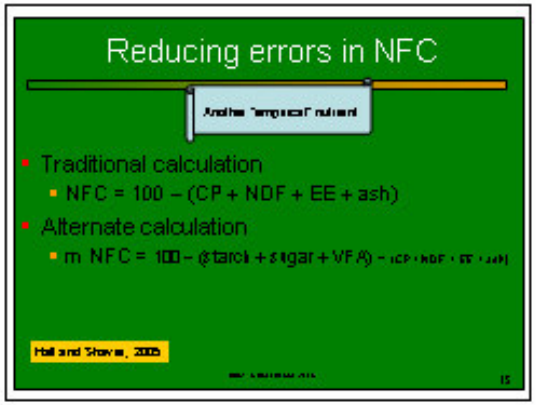

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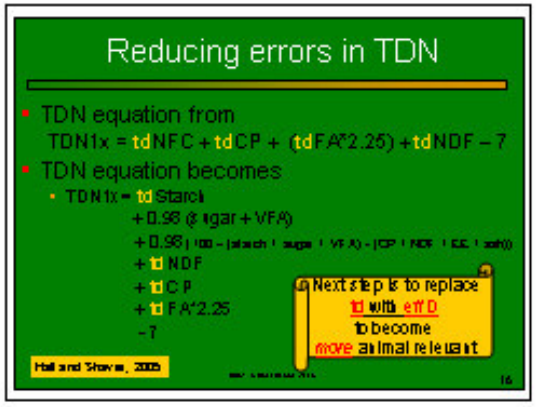

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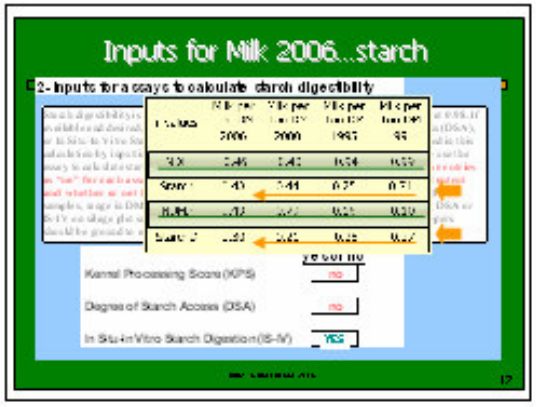

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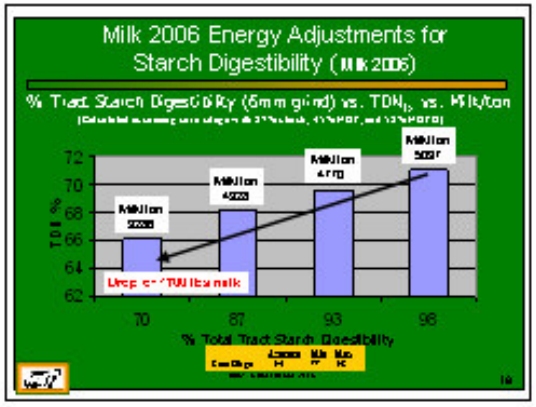

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Why measure, just calculate

- Calculated versus Measured inputs (Shauer, 2002)
- NEL calculated from ADF
- Summative calculations versus Measured inputs

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NEL calculated from ADF

- NE_L = 0.596 - (0.0126 x ADF).....New Hampshire equation
- NE_L = 0.94 - (0.008 x ADF)..... .New York equation
- NE_L = 1.044 - (0.0124 x ADF). ....Pennsylvania equation
- NE_L = 0.92 - (0.0078 x ADF) .....MSU SPARTAN Dairy Ration Evaluation/Balance equation

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Dairy NRC 8th Edition

- TDN using the Summative Equation is calculated
  - using apparent digestibility and not true digestibility.
  - Therefore, a correction for metabolic fecal nitrogen must be subtracted from the sum of the fractions...value is ?
- Dairy NRC 2001 (page 2-14) expected true digestibility of NFC
  - TDNfx...fd NFC = 0.98 times PAF times remainder of items
  - TDNfx...fd NFC = 0.90 times PAF times remainder of items
- Processing activity factor (PAF)
  - PAF is only used in the fdNFC term because
    - "about applying PAF the calculation "will be an over estimation of energy values in some feeds when fed at maintenance,
    - but NEL values when fed at 3X times maintenance should be correct" (Dairy NRC 2001, page 2-15).

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NRC estimates of TDN

- Calculated versus Measured inputs
- Summative calculations versus Measured inputs

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Range in Ruminal Starch Digestibility at 12H

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"lignin" calculations

Values that can be calculated from % Lignin.

Values that are routinely measured at SALLIC.

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Presentation Outline

- Goal
  - Accuracy
- Objectives
  - Precision
  - Analytical efficiency
- Approaches
  - Enzyme degradation
  - Prediction vs. Accuracy
  - Uses of Digestion Coefficients
    - Milk 2000
    - NRC 2001
  - Calculation vs. Measurement
- Sample Analysis
  - Nutrients to Measure
  - Drying Temperature
  - Particle size
  - Site of Digestion
  - Sampling
  - Replication
  - Digestion terms

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Where the differences are

Important considerations for the Cornell Model.

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Protein Influence, HMC

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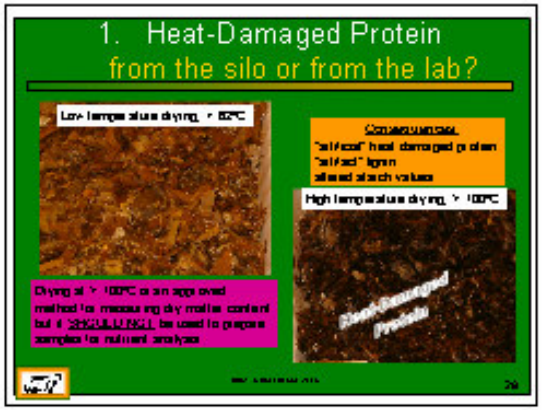

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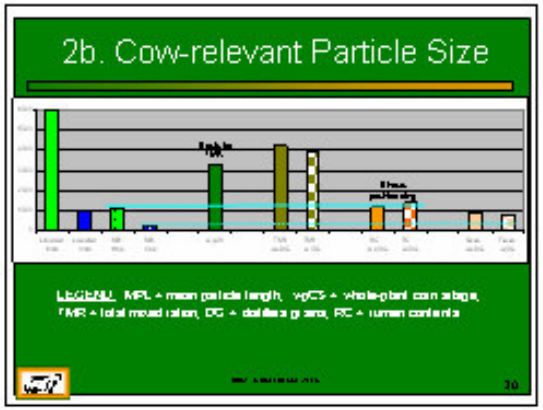

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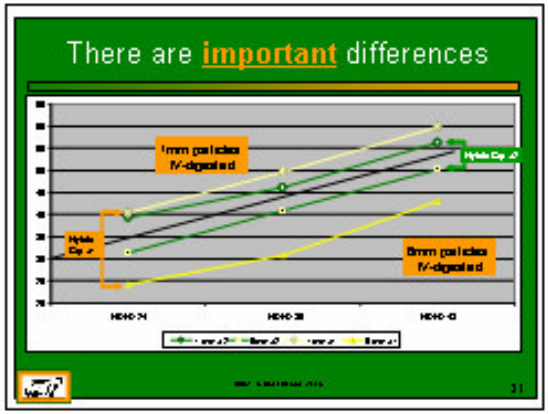

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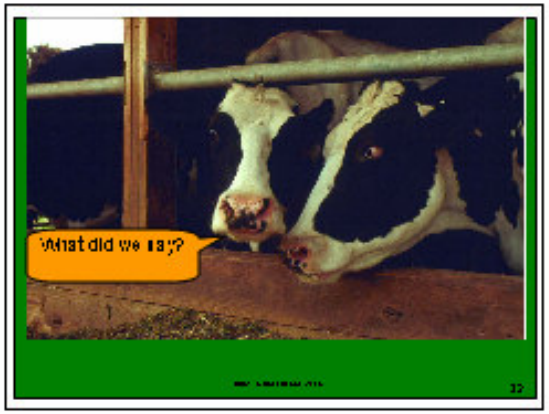

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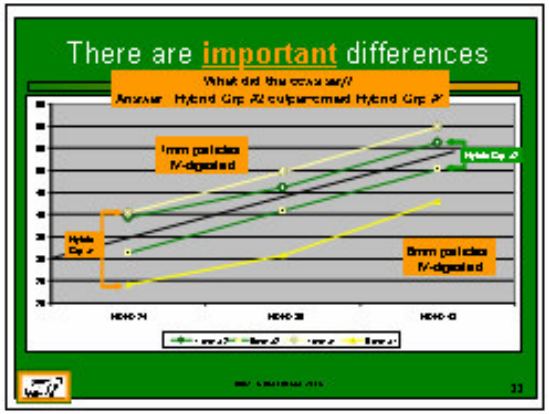

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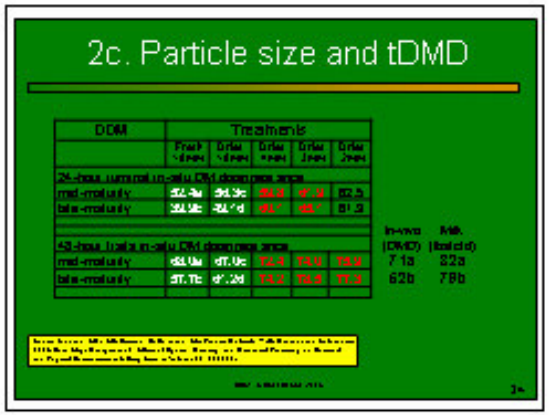

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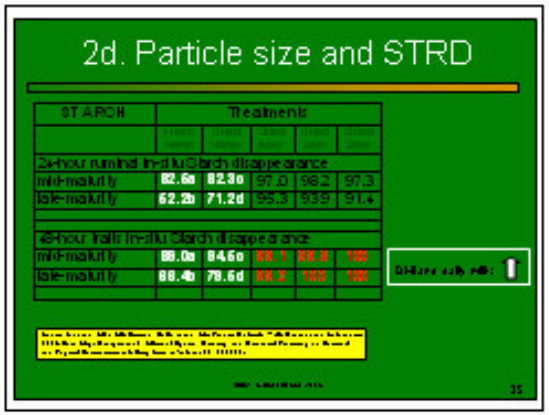

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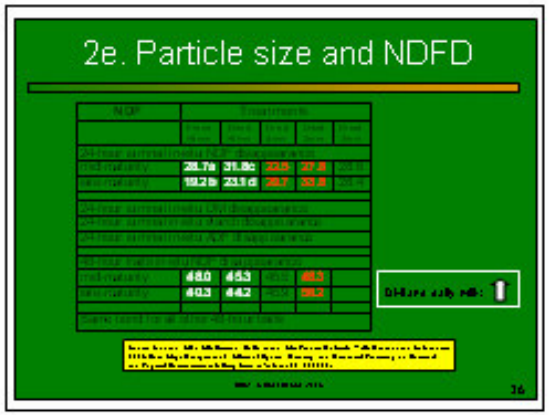

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DSA and IVSTRD

	% starch	Average	Minimum	Maximum
Dry Corn	DCA	96.4	86.9	98.9
	CTRD 12	64.3	48.7	74.3
	IVSTRD	86.2	72.9	96.3
HMC	% starch	Average	Minimum	Maximum
	DCA	94.9	80.1	98.1
	CTRD 12	73.1	58.9	93.4
	IVSTRD	87.9	74.3	98.6
Corn Silage	% starch	Average	Minimum	Maximum
	DCA	93.7	50.1	97.2
	CTRD 12	89.7	75.8	98.8
	IVSTRD	96.0	96.1	99.5

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Whole-plant Corn Silage (particle-size adjustment)

- 105 samples submitted for analysis
- Table contains regression coefficients

- Animal implications

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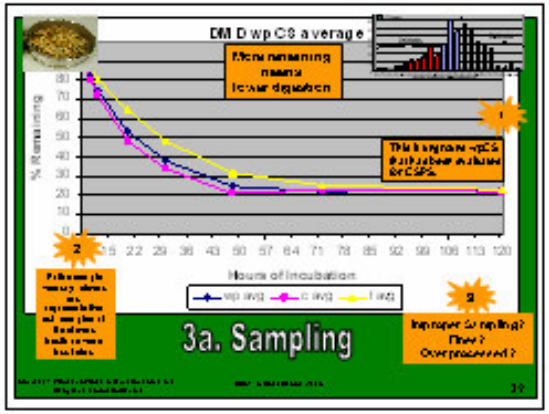
3a. Sampling



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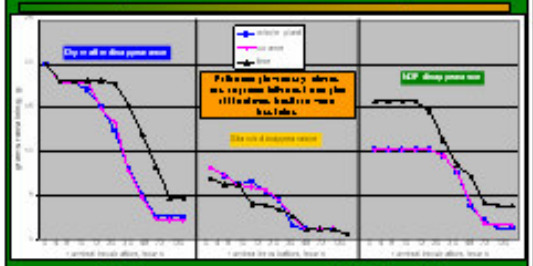
3b. CSPS and Nutrient Disappearance



Note. Fewer grams remaining means higher % digested

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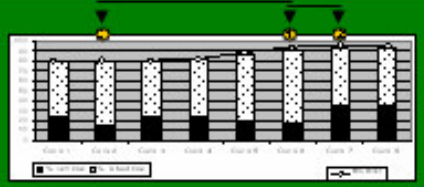
Total Tract and Partitioning



	Average	Min	Max	
Corn Silage	94	77	99	Total Tract Digestibility, % of Starch
Corn Silage	84	45	99	Ruminal Starch Digestibility, % of Starch

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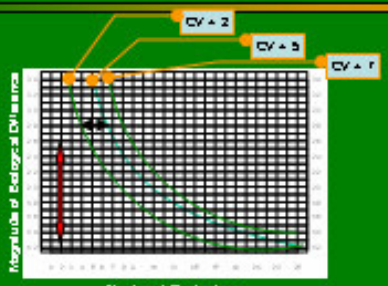
4. Number of Replicates



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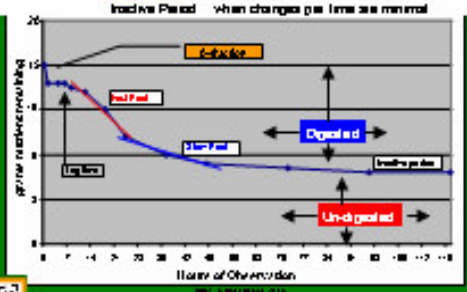
5a. Digestion Terms…by nutrient

Lag When no change is apparent
Fast Period When changes per time are great
Slow Period When changes per time are small
Inactive Period When changes per time are minimal



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5b. The Digestion Fractions

- A-fraction…that plant material that is "instantly" digested…but it must be distinguished from plant material that is of a lesser size than the pores in the recovery filter or bag
- B-fraction…that plant material that is digested at a variable rate
  - measured in the rumen…two pools (fast and slow)
  - measured post-ruminally
- C-fraction…that plant material that is not digested…also called undigested fraction, simply not digested in given time
- Indigestible fraction…that plant material resistant to the digestive process, indigestible or simply un-digested

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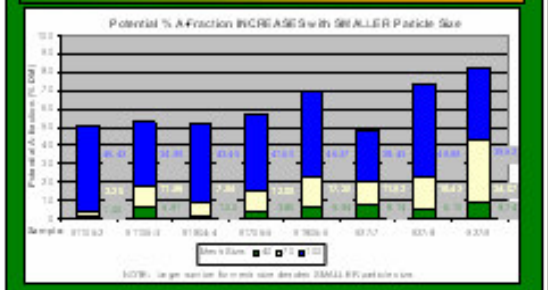
Particle Size Influences Losses from Bags

All that leaves bag may NOT be digested



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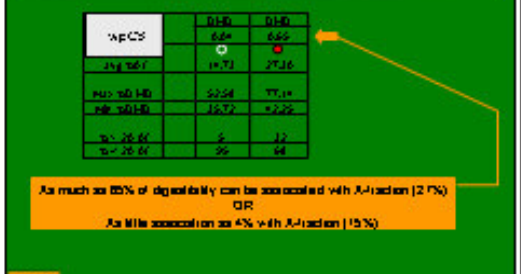
A-fraction effect in set of diverse samples



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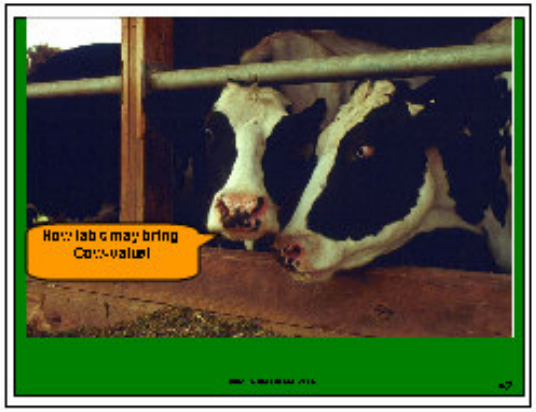

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Lessons Learned at SALLC

- Gravimetric, by-nutrient analysis...not gas analysis
- Sample in bags increases analytical efficiency
  - Ease of blank correction
  - Versatility of digesta for measurement of nutrient content
- 4 to 8 replicates of 6mm particles
- Short time from withdrawal of rumen fluid from animal until beginning of in-vitro incubation...20 minutes
- Van Soest buffer to set pH to that of donor animals
- Maintain part of the fiber mat in the in-vitro vessel
- "Vented" incubation in air-jacketed, anaerobic incubator
- Artificial rumination of bags in the IV vessels discharges gas "trapped" in bags and eliminates "putrefaction"

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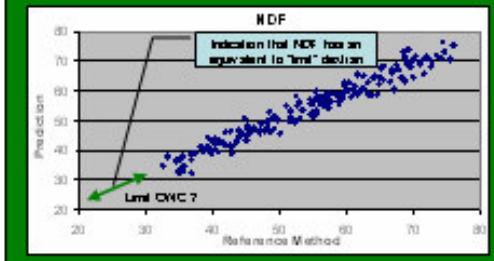
Residual NDF...indigestible NDF



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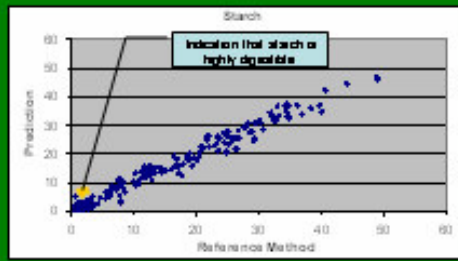
Residual Starch



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Five Items to Consider When Measuring Cow-Value

- Cattle eat heterogeneous particles;
  - Heterogeneous → Suggestions
- Use a cow-relevant particle size when measuring digestion → 6 mm
- Replication is a must → 4 to 8
- Cow-relevant nutrients:
  - STARCH and FIBER,
  - NOT just dry matter or "fat/ash"
- Measured rather than calculated nutrient values are cow-relevant → starch and NDF; eff deg

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Cow-Relevant TDN

- TDN equation becomes
  - TDN = effD Starch (rumINAI) + ID starch
    + 0.98 (sugar + VFA)
    + 0.98 (100 – (starch + sugar + VFA) – (CP + NDF + EE + ash))
    + effD NDF (rumINAI)
    + effD CP (rumINAI) + IDCP
    + ID FA*2.25
    – 7
- reduce the errors associated with nitrogen-free extract (NFE) and crude fiber
- reduce the errors associated with NFC only by difference
- account for partitioning of digestibility between ruminal and post-ruminal
- associate digestion with rate of passage

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