

# In-vitro and In-situ Measurements: IMPROVING ACCURACY

## From a Commercial Laboratory Perspective

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# Presentation Outline

- Goal
  - Accuracy = Animal Relevance
- Objectives
  - Precision
  - Analytical efficiency
- Approaches
  - Effective degradation
  - Precision vs. Accuracy
  - Uses of Digestion Coefficient
    - May 2008
    - Nov 2008
  - Calculations vs. Measurements
- Sample Analysis
  - Nutrients to Measure
  - Drying temperature
  - Particle size
  - Site of Digestion
  - Sampling
  - Replication
  - Digestion time

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# Attempting to...

- 1 Estimate animal production... **meat or milk**  
By using **energy** as "best" predictor  
Net  
Metabolizable  
Digestible
- 2 Calculate energy from TDN: **ingredients and TMR**

Keys to success:  
**Animal-relevant digestibility coefficients**

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# Accuracy, Precision, Efficiency

### In-vitro Measurements

- Tissue/substrate
- Incubation
  - Substrate available
  - Replicates
- Measurements: total tract
  - DM
  - NDFD, STRD, etc. F & D
  - CPD, amylase/alpha
- Humidity

**Challenge: Efficiency**

### In-vitro/In-situ Measurements

- Donor animal
- Nutrition
  - Diet
  - Diets/substrate
  - Dryness, fat content, etc.
- Precision
  - Substrate available
  - Replicates
  - Co-incident efficiency
- What's measured
  - Apparent
  - Particle size: neutral area to pass
- Systems
  - Dry Matter
  - NDF
  - Starch
  - Protein
  - Fat

**Challenge: Accuracy**

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# Empirical Nutrient... "digestibility"

TDN using the Summative Equation

- Is big apparent digestibility and not the digestibility...

Therefore, a correction for metabolic fecal nitrogen must be subtracted from the sum of the TD-values... value is 7

**Goal NRC, 2004**

as an empirical "in-vitro" digestibility

**in-vitro apparent**

**equal**

**in-vitro effective**

**does not equal**

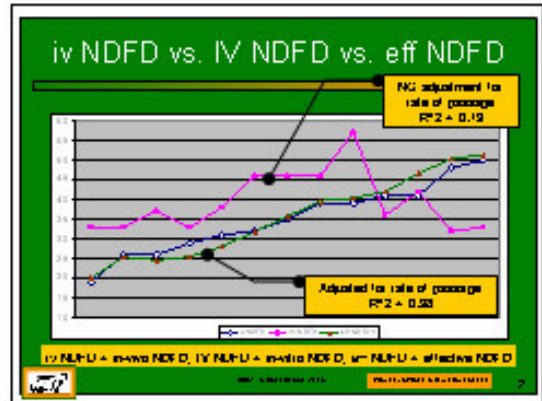
**in-vitro apparent**

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# Apparent vs. Effective Digestibility

- Apparent Digestibility
  - In-vitro measurements
  - Soil collector
  - Incubation: DM, NDF, etc. in fecal material
  - In-vitro/In-situ measurements
  - In-vitro/In-situ measurements
  - Incubation: DM, NDF, etc. in fecal material
  - Digestion coefficient
  - Percent digested
- Effective Digestibility
  - $DM\ D_e = (K_d)(K_d + K_p)$
  - How to measure
    - Rate of passage -  $K_p$
    - Rate of degradation -  $K_d$
  - "apparent" incubation time point

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# Correlation Coefficients (wpCS only)

|              | iv NDFD | IV NDFD | Definitions                       |
|--------------|---------|---------|-----------------------------------|
| iv NDFD      |         |         | In-vitro NDFD                     |
| IV NDFD      | 0.53    |         | In-situ NDFD reported             |
| C iv NDFD 30 | 0.54    |         | In-vitro NDFD 30 calculated       |
| eff NDFD     | 0.56    |         | effective NDFD                    |
| NDFD in      | 0.0001  |         | NDFD in fecal, DMRC equation 2-4e |
| NDFD e       | 0.15    |         | NDFD in fecal, DMRC equation 2-4e |

**N.B. - 10 trials had all of the required data**

Keys to success:  
**Animal-relevant K<sub>d</sub> for each nutrient**

The correlation between  
NDFD measurements and NDFD calculated by NRC 2006 rather than a "best-fit" curve.

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# Getting to Rates of Passage (K<sub>p</sub>)

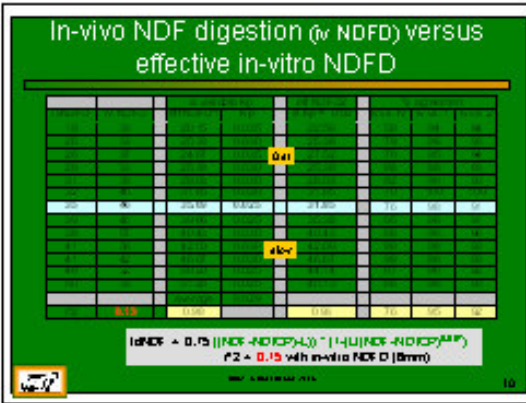
$K_p = 0.284 + 0.0021(DM - 660) + 0.0002(DM)$

$K_p = 0.28 + 1.25K_d$

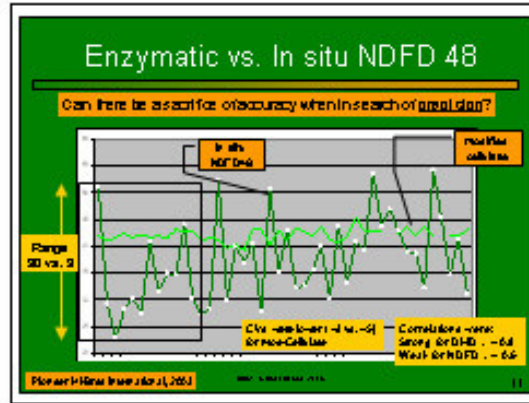
| Rate | DM   | DM   | DM   | DM   | DM   |
|------|------|------|------|------|------|
| 1    | 37.7 | 37.7 | 37.7 | 37.7 | 37.7 |
| 2    | 37.7 | 37.7 | 37.7 | 37.7 | 37.7 |
| 3    | 37.7 | 37.7 | 37.7 | 37.7 | 37.7 |
| 4    | 37.7 | 37.7 | 37.7 | 37.7 | 37.7 |
| 5    | 37.7 | 37.7 | 37.7 | 37.7 | 37.7 |
| 6    | 37.7 | 37.7 | 37.7 | 37.7 | 37.7 |
| 7    | 37.7 | 37.7 | 37.7 | 37.7 | 37.7 |
| 8    | 37.7 | 37.7 | 37.7 | 37.7 | 37.7 |
| 9    | 37.7 | 37.7 | 37.7 | 37.7 | 37.7 |
| 10   | 37.7 | 37.7 | 37.7 | 37.7 | 37.7 |

**Key:** rate of passage, 1 day on DM; rate of passage, 2 day on DM; rate of passage, 3 day on DM; rate of passage, 4 day on DM; rate of passage, 5 day on DM; rate of passage, 6 day on DM; rate of passage, 7 day on DM; rate of passage, 8 day on DM; rate of passage, 9 day on DM; rate of passage, 10 day on DM.

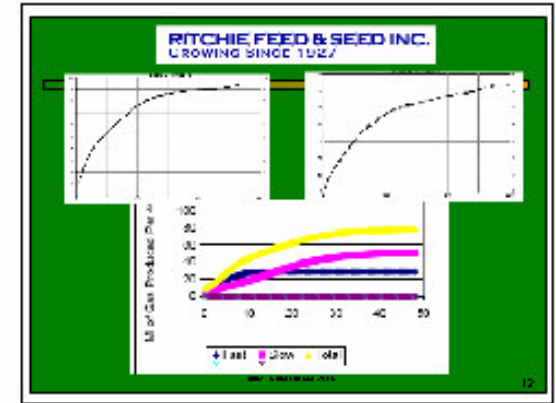
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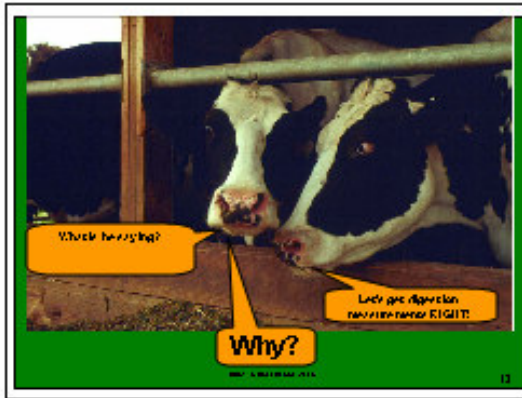
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### Using digestibility to calculate energy

- The NRC equations - Shows, 2000 and 2006  
 $TDN_{1x} = tdNFC + tdCP + (tdFA \cdot 2.25) + tdNDF - 7$   
 $NE = (TDN \cdot 0.0245) - 0.12 / 2.2$
- The Summative Equations - Daily NFC 2001  
 $LC = (0.06 \cdot 100)^{0.42} + (0.03 \cdot 100)^{0.58} + (0.04 \cdot 100)^{0.4} + (0.06 \cdot 100)^{0.42} - 0.3$   
 $ME = (1.01 \cdot LC) - 0.49 + 0.004 \cdot (FA - 3)$   
 $ME = (1.42 \cdot ME) - (0.17 \cdot ME^2) + (0.022 \cdot ME^3) - 1.26$
- Implications for users of Cornell Model will come in the slides

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### Reducing errors in NFC

Another "empirical" nutrient

- Traditional calculation
  - $NFC = 100 - (CP + NDF + EE + ash)$
- Alternate calculation
  - $m\ NFC = 100 - (\$tarcl + \$tgar + VF\ A) - (CP + NDF + EE + ash)$

Hall and Shows, 2006

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### Reducing errors in TDN

- TDN equation from
- TDN equation becomes
- TDN 1x = td Starch + 0.96 (\$tgar + VF A) + 0.96 (100 - (starch + \$tgar + VF A) - (CP + NDF + EE + ash)) + td NDF + td CP + td FA \* 2.25 - 7

Next step is to replace **td** with **eff D** to become **more** animal relevant

Hall and Shows, 2006

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### Inputs for Milk 2006...starch

2-Inputs for a easy to calculate chroli digestibility

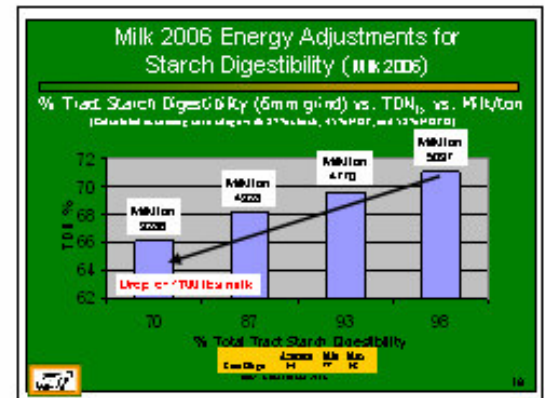
| Starch Digestibility (6mm grind) | TDN  | CP   | EE   | ash |
|----------------------------------|------|------|------|-----|
| 100%                             | 2006 | 2000 | 1995 | 95  |
| 92%                              | 245  | 241  | 154  | 155 |
| 85%                              | 45   | 44   | 27   | 27  |
| 80%                              | 37   | 27   | 22   | 22  |
| 75%                              | 27   | 24   | 22   | 22  |

Kernel Processing Score (KPS)  NO

Degree of Starch Access (DSA)  NO

In Situ In-Vitro Starch Digestion (IS-V)  YES

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### Why measure, just calculate

- Calculated versus Measured inputs (Hauer, 2002)

|                            | ADP | ADP |
|----------------------------|-----|-----|
| ADP from separate analysis | 2.7 | 2.7 |
| ADP calculated from NEL    | 2.7 | 2.7 |
| ADP from separate analysis | 0.5 | 0.5 |
| ADP calculated from NEL    | 0.5 | 0.5 |

- NEL calculated from ADP
- Summative calculations versus Measured inputs

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### NEL calculated from ADF

- $NE_L = 0.566 - (0.0126 \times ADF)$  ... New Hampshire equation
- $NE_L = 0.54 - (0.008 \times ADF)$  ... New York equation
- $NE_L = 1.044 - (0.012 \times ADF)$  ... Pennsylvania equation
- $NE_L = 0.52 - (0.0078 \times ADF)$  ... NASSU SPARTAN Dairy Ration Evaluation/Balance equation

Example: 100 lb Corn Silage, 100 lb Soybean Meal, 100 lb Barley, 100 lb Alfalfa Hay, 100 lb Cottonseed Meal, 100 lb Molasses, 100 lb Vitamin/Mineral Supplement

| Ingredient                 | Weight (lb) | ADF (%)   | NE <sub>L</sub> (Mcal/lb DM) |
|----------------------------|-------------|-----------|------------------------------|
| Corn Silage                | 100         | 48        | 0.48                         |
| Soybean Meal               | 100         | 12        | 0.45                         |
| Barley                     | 100         | 35        | 0.35                         |
| Alfalfa Hay                | 100         | 55        | 0.31                         |
| Cottonseed Meal            | 100         | 10        | 0.46                         |
| Molasses                   | 100         | 5         | 0.49                         |
| Vitamin/Mineral Supplement | 100         | 1         | 0.51                         |
| <b>Total</b>               | <b>700</b>  | <b>35</b> | <b>0.45</b>                  |

NFTA Dairy 6 Feed # 64

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### Dairy NRC 8<sup>th</sup> Edition

- TDN using the Summative Equation is calculated
  - using apparent digestibility and not true digestibility.
  - Therefore, a correction for metabolic loss of nitrogen must be subtracted from the sum of the digestibilities... value is 7
- Dairy NRC 2001 (page 2-14) expected true digestibility of NRC
  - TD MIX... DMFC = 0.80 times PAF times remainder of feeds
  - TD MIX... DMFC = 0.80 times PAF times remainder of feeds
- Processing adjustment factor (PAF)
  - PAF is only used in the DMFC term because
    - without 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 2 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

| Ingredient   | Weight (lb) | TDN (%)   | TDN (%)   |
|--------------|-------------|-----------|-----------|
| Ingredient 1 | 100         | 65        | 65        |
| Ingredient 2 | 100         | 70        | 70        |
| Ingredient 3 | 100         | 75        | 75        |
| Ingredient 4 | 100         | 80        | 80        |
| Ingredient 5 | 100         | 85        | 85        |
| Ingredient 6 | 100         | 90        | 90        |
| Ingredient 7 | 100         | 95        | 95        |
| Ingredient 8 | 100         | 100       | 100       |
| <b>Total</b> | <b>700</b>  | <b>75</b> | <b>75</b> |

Measured (NDF PD 20)

Formula:  $TDN = NRC - (ADF - NDF) \times (CP + 64H)$

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### Range in Ruminal Starch Digestibility at 12H

Dairyland Laboratories, Inc.

| Sample    | Starch Digestibility (%) |
|-----------|--------------------------|
| Sample 1  | 44                       |
| Sample 2  | 45                       |
| Sample 3  | 46                       |
| Sample 4  | 47                       |
| Sample 5  | 48                       |
| Sample 6  | 49                       |
| Sample 7  | 50                       |
| Sample 8  | 51                       |
| Sample 9  | 52                       |
| Sample 10 | 53                       |
| Sample 11 | 54                       |
| Sample 12 | 55                       |
| Sample 13 | 56                       |
| Sample 14 | 57                       |
| Sample 15 | 58                       |
| Sample 16 | 59                       |
| Sample 17 | 60                       |
| Sample 18 | 61                       |
| Sample 19 | 62                       |
| Sample 20 | 63                       |
| Sample 21 | 64                       |
| Sample 22 | 65                       |
| Sample 23 | 66                       |
| Sample 24 | 67                       |
| Sample 25 | 68                       |
| Sample 26 | 69                       |
| Sample 27 | 70                       |
| Sample 28 | 71                       |
| Sample 29 | 72                       |
| Sample 30 | 73                       |
| Sample 31 | 74                       |
| Sample 32 | 75                       |

Average 22, with Max 27 and Min 44

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### "lignin" calculations

Values that can be calculated from % Lignin.

Values that are routinely measured at SALLC.

| Sample   | WBCP (%) | WBCP (%) | WBCP (%) | WBCP (%) |
|----------|----------|----------|----------|----------|
| Sample 1 | 0.073    | 0.070    | 7.25     | 7.25     |
| Sample 2 | 0.024    | 0.048    | 10.21    | 10.21    |
| Sample 3 | 0.039    | 0.034    | 2.83     | 2.83     |
| Sample 4 | 0.032    | 0.034    | 7.88     | 7.88     |
| Sample 5 | 0.033    | 0.029    | 1.23     | 1.23     |

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### Presentation Outline

- Goal
  - Accuracy
- Objectives
  - Precision
  - Analytical efficiency
- Approaches
  - Effective degradation
  - Precision vs. Accuracy
  - Use of Digestion Coefficient
    - Feb. 2008
    - NRC 2001
  - Calculation vs. Measurement
- Sample Analysis
  - Multiple to Measure
  - Drying temperature
  - Particle size
  - Site of Digestion
  - Sampling
  - Replication
  - Digestion time

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### Where the differences are

Important considerations for the Cornell Model.

| Sample   | Range       | Average | St. Dev. | Range         | Average | St. Dev. |
|----------|-------------|---------|----------|---------------|---------|----------|
| Sample 1 | 3.4 - 4.7   | 4       | 0.7      | 11.78 - 13.13 | 12.45   | 0.68     |
| Sample 2 | 3.23 - 7.23 | 5.23    | 2.0      | 3.27 - 8.31   | 4.79    | 2.54     |
| Sample 3 | 4.4 - 8.4   | 6.4     | 2.0      | 4.27 - 8.27   | 6.27    | 2.0      |
| Sample 4 | 2.45 - 4.75 | 3.6     | 1.15     | 1.27 - 4.27   | 2.77    | 1.5      |
| Sample 5 | 3.23 - 5.23 | 4.23    | 1.0      | 3.27 - 4.27   | 3.77    | 0.5      |
| Sample 6 | 3.23 - 5.23 | 4.23    | 1.0      | 3.27 - 4.27   | 3.77    | 0.5      |
| Sample 7 | 3.23 - 5.23 | 4.23    | 1.0      | 3.27 - 4.27   | 3.77    | 0.5      |
| Sample 8 | 3.23 - 5.23 | 4.23    | 1.0      | 3.27 - 4.27   | 3.77    | 0.5      |

Microbial growth... Synthesis of Microbial Protein

Energy Carbon Source Carbon Sinks

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### Protein Influence, HMC

Protein Solubility vs. TFCRSE


Protein Solubility vs. 12H Ruminal STED

| Sample   | Range       | Average | St. Dev. | Range       | Average | St. Dev. |
|----------|-------------|---------|----------|-------------|---------|----------|
| Sample 1 | 3.23 - 5.23 | 4.23    | 1.0      | 3.27 - 4.27 | 3.77    | 0.5      |
| Sample 2 | 3.23 - 5.23 | 4.23    | 1.0      | 3.27 - 4.27 | 3.77    | 0.5      |
| Sample 3 | 3.23 - 5.23 | 4.23    | 1.0      | 3.27 - 4.27 | 3.77    | 0.5      |
| Sample 4 | 3.23 - 5.23 | 4.23    | 1.0      | 3.27 - 4.27 | 3.77    | 0.5      |
| Sample 5 | 3.23 - 5.23 | 4.23    | 1.0      | 3.27 - 4.27 | 3.77    | 0.5      |
| Sample 6 | 3.23 - 5.23 | 4.23    | 1.0      | 3.27 - 4.27 | 3.77    | 0.5      |
| Sample 7 | 3.23 - 5.23 | 4.23    | 1.0      | 3.27 - 4.27 | 3.77    | 0.5      |
| Sample 8 | 3.23 - 5.23 | 4.23    | 1.0      | 3.27 - 4.27 | 3.77    | 0.5      |

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### 1. Heat-Damaged Protein from the silo or from the lab?

Low temperature drying, < 82°C



Conventional silage  
"silage" heat damaged protein  
"silage" lignin  
affected starch values

High temperature drying, > 100°C



Drying at > 100°C is an approved method for measuring dry matter content but if **STARCH**, **NDF**, be used to prepare samples for nutrient analysis

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### 2a. Particle Size

Appropriate for:  
- Reference chemistry  
- NIR



1 mm

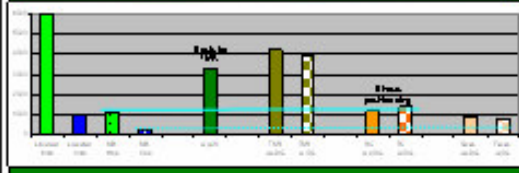
Appropriate for:  
- Diets for ruminants



6 mm

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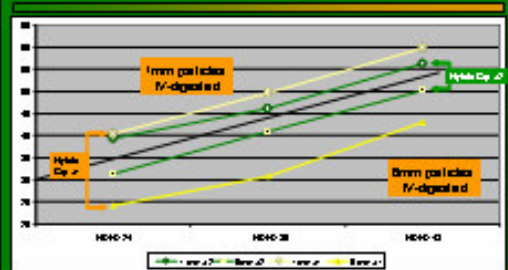
### 2b. Cow-relevant Particle Size




LEGEND: MPL = mean particle length, wCS = whole-grain corn silage, TMR = total mixed ration, DG = double grass, RC = rumen contents

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### There are **important** differences



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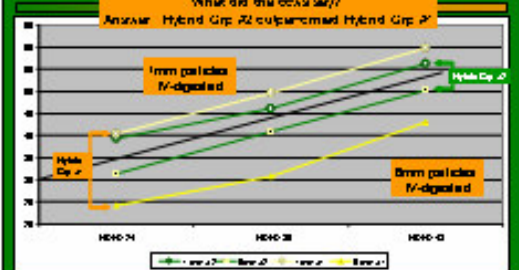
What did we say?

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### There are **important** differences

What did the cows say?

Answer: Hybrid Grp 22 culprits Hybrid Grp 2



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### 2c. Particle size and tDM

| DDM                                      | Treatments |       |      |      |      |      | tDM  | tMR  |
|--|------------|-------|------|------|------|------|------|------|
|  | Frk        | Grk   | Grk  | Grk  | Grk  | Grk  |      |      |
| 28-hour ruminal in-situ DM disappearance |            |       |      |      |      |      |      |      |
| med-variability                          | 22.3a      | 24.3c | 25.4 | 27.3 | 29.3 | 32.3 |      |      |
| low-variability                          | 24.3a      | 26.3d | 27.3 | 29.3 | 31.3 |      |      |      |
| 42-hour total in-situ DM disappearance   |            |       |      |      |      |      |      |      |
| med-variability                          | 24.0a      | 27.0c | 28.0 | 29.0 | 30.0 | 32.0 | 7.1a | 22a  |
| low-variability                          | 27.0b      | 29.0d | 30.0 | 31.0 | 32.0 |      | 6.2b | 7.9b |

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### 2d. Particle size and STRD

| STARCH                                   | Treatments |       |      |      |      |     |
|--|------------|-------|------|------|------|-----|
|  | Frk        | Grk   | Grk  | Grk  | Grk  | Grk |
| 24-hour ruminal in-situ DM disappearance |            |       |      |      |      |     |
| med-variability                          | 82.6a      | 82.8a | 87.0 | 88.2 | 87.3 |     |
| low-variability                          | 62.2b      | 71.2b | 85.3 | 93.9 | 91.4 |     |
| 24-hour total in-situ DM disappearance   |            |       |      |      |      |     |
| med-variability                          | 88.0a      | 84.6a | 88.1 | 88.8 | 100  |     |
| low-variability                          | 88.4a      | 79.6b | 88.3 | 100  | 100  |     |

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### 2e. Particle size and NDFD

| NDF                                      | Treatments |       |      |      |     |     |
|--|------------|-------|------|------|-----|-----|
|  | Frk        | Grk   | Grk  | Grk  | Grk | Grk |
| 24-hour ruminal in-situ DM disappearance |            |       |      |      |     |     |
| med-variability                          | 28.7a      | 31.8a | 33.5 | 37.8 |     |     |
| low-variability                          | 19.2b      | 23.5b | 28.7 | 33.8 |     |     |
| 42-hour total in-situ DM disappearance   |            |       |      |      |     |     |
| med-variability                          | 48.0       | 45.3  | 48.5 |      |     |     |
| low-variability                          | 40.3       | 44.2  | 48.3 |      |     |     |

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### DSA and IVSTRD

| Dry Corn   |        |         |         |         |
|------------|--------|---------|---------|---------|
|            | % Hard | Average | Minimum | Maximum |
| DCA        | 96.4   | 86.9    | 86.9    | 96.9    |
| STP D 12   | 64.3   | 48.7    | 74.3    |         |
| STP D      | 86.2   | 72.9    | 96.3    |         |
| HMC        |        |         |         |         |
|            | % Hard | Average | Minimum | Maximum |
| DCA        | 94.9   | 89.1    | 86.1    | 96.1    |
| STP D 12   | 73.1   | 59.9    | 93.4    |         |
| STP D      | 87.9   | 74.3    | 98.8    |         |
| Com Silage |        |         |         |         |
|            | % Hard | Average | Minimum | Maximum |
| DCA        | 93.7   | 90.1    | 87.2    | 97.2    |
| STP D 12   | 89.7   | 75.8    | 96.8    |         |
| STP D      | 96.0   | 96.1    | 99.5    |         |

Tzuc et al., 2007 Proceedings for Nut Science 23-25

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### Whole-plant Com Silage (particle-size adjustment)

- 105 samples submitted for analysis
- Table contains regression coefficients

|     | CP     | CP2    | CP3    | CP4    |
|-----|--------|--------|--------|--------|
| CP  | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| CP2 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| CP3 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| CP4 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

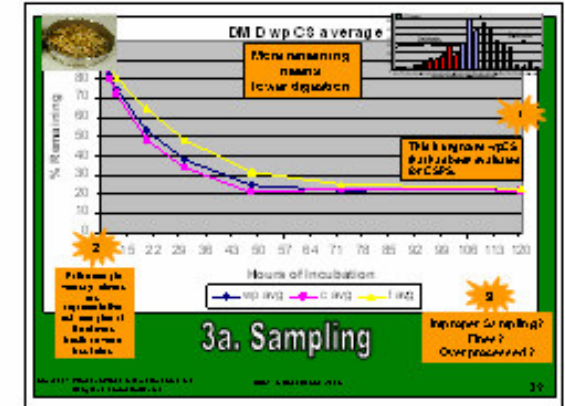
Equation:  $Y = 0.0000 \cdot CP + 0.0000 \cdot CP2 + 0.0000 \cdot CP3 + 0.0000 \cdot CP4 + 0.0000$

Particle Size:  $r^2 = 0.99$   
 Moisture:  $r^2 = 0.76$   
 Dry-matter:  $r^2 = 0.99$

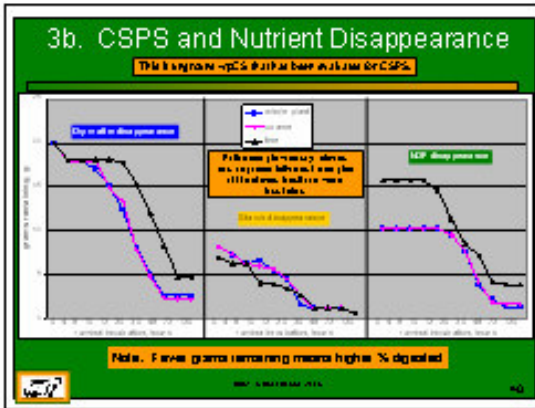
Animal Implications



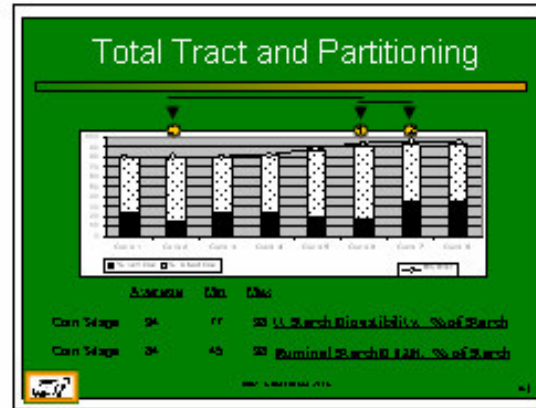
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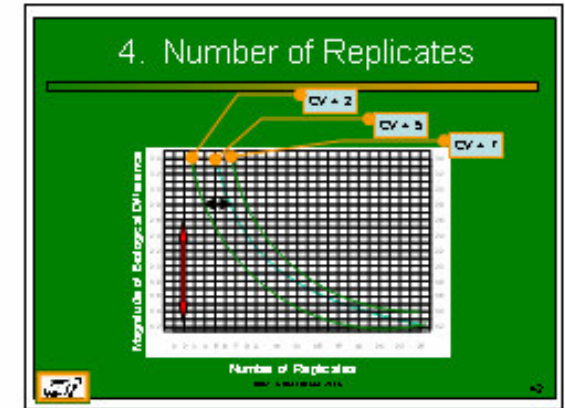
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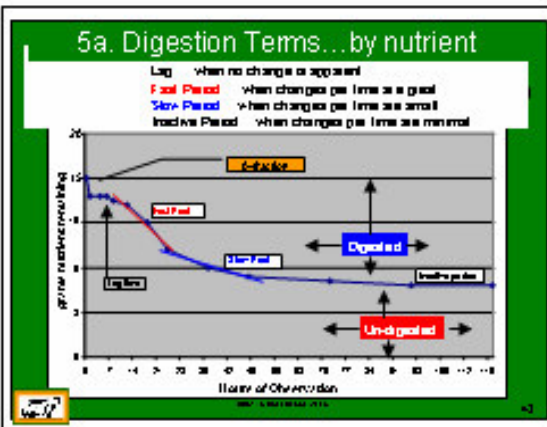
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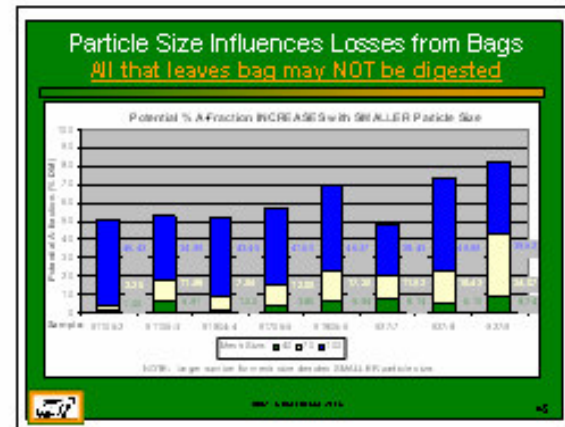


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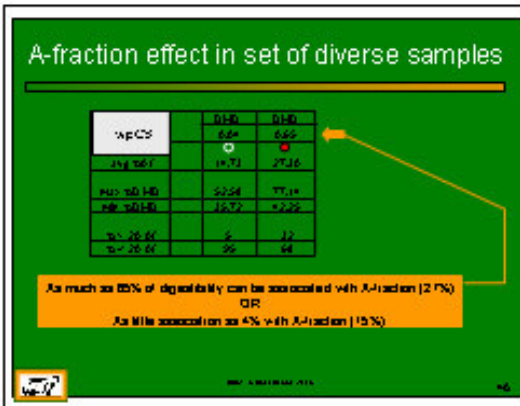
### 5b. The Digestion Fractions

- A-fraction... that plant material that is "instantly" digested... but it must be digested to be removed 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 digested in glass
- Indigestible fraction... that plant material resistant to the digestive process, indigestible or simply not-digested

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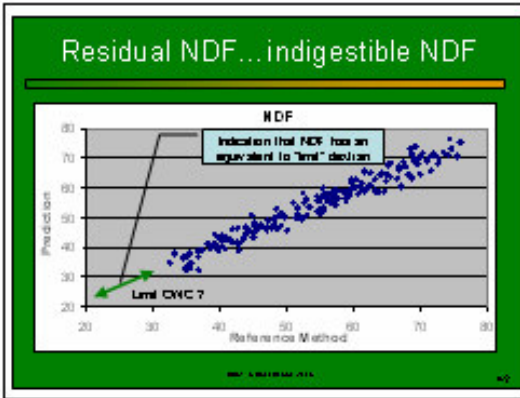
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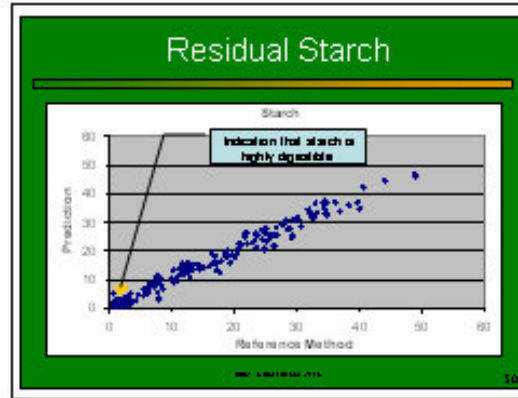
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- ### Lessons Learned at SALLC
- Gravimetric, bio-nutrient analysis, not gas analysis
    - TDN = 4NFC + MCP + 10FA'2.25 + 4NDF-T
    - CP = 10(NFC66) + 2 + 10(CP66) + 2 + 10(F66) + 10(NDF66) + 2 + 6.1
  - Samples in bags increases analytical efficiency
    - Ease of blank correction
    - Versatility of digesta for measurement of nutrients
    - 4 to 6 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 vessel; discharge gas "trapped" in bags and eliminates "putrefaction"

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- ### Five Items to Consider When Measuring Cow-Value
- Cattle eat heterogeneous particles.
    - THE CHOICE:** 6 mm
    - SUGGESTIONS:** 4 to 8
  - Use a cow-relevant particle size when measuring digestion
  - Replication is a must
    - 4 to 8
  - Cow-relevant nutrients:
    - STARCH and FIBER
    - NOT just dry matter or "fat to flow"
  - Measured rather than calculated nutrient values are cow-relevant
    - eff deg

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- ### Cow-Relevant TDN
- TDN equation becomes
    - TDN = eFD Starch (10m/kg) + ID starch
    - + 0.98 (g/gar + VFA)
    - + 0.98 (100 - (starch + sugr + VFA) - CP + NDF + EE + ash)
    - + eFD NDF (10m/kg)
    - + eFD CP (10m/kg) + ID CP
    - + ID FA'2.25
    - T
  - reduce the error associated with nitrogen-free extract (NFC) and crude fiber
  - reduce the error 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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