PENNSTATE Dairy and Animal Science

Understanding in-vitro and in-situ digestibility: From the lab to nutritional application

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Learning Objectives

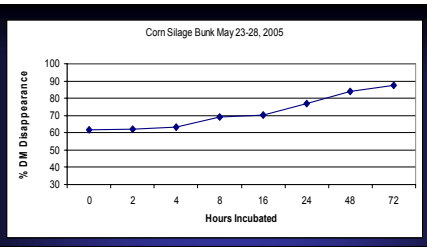
- Appreciate the challenges for determining NDFD using in-vitro or in-situ procedures.
- Understand the multi-faceted dynamics of the rumen and why NDFD values should be interpreted cautiously.
- Realize the limitations of applying NDFD in the field for ration formulation and evaluation.

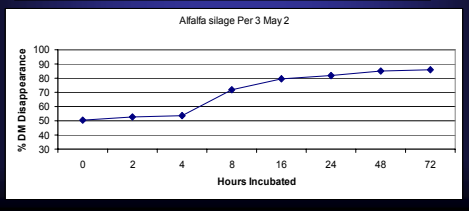
Tilley and Terry
In vitro procedure

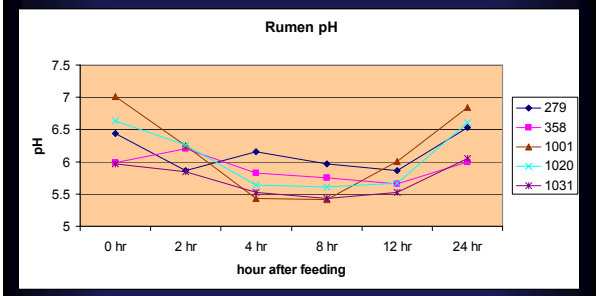
ANKOM DAISY II IN VITRO SYSTEM

In vitro analysis

- Artificial digestion system
  - Not designed to provide info about digestion of the highly soluble components of feedstuffs
  - A highly buffered system and not subject to pH fluctuations found in the rumen of a lactating cow.
  - The test feed is isolated from effects of starch or fat found in lactating cow diets.







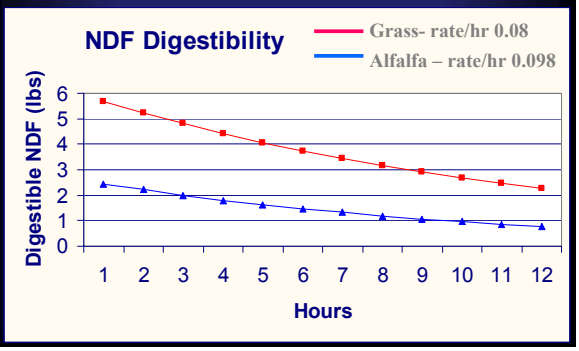
Strained Ruminal Fluid pH

- McLeod and Minson (1969) - Grasses
  - pH 6.1 = 58.8% T&T IVDMD 48h
  - pH 6.7 = 59.2% T&T IVDMD 48h
  - pH 7.2 = 62.5% T&T IVDMD 48h

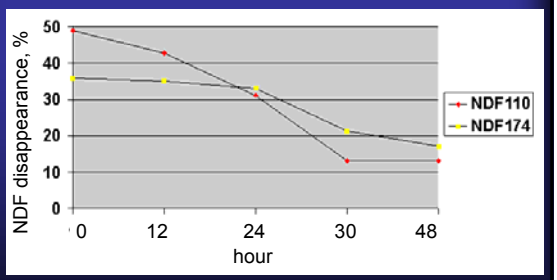
In-vitro fiber digestibility (iVNDF)

- Can rank forages by quality
- Cannot compare in vitro fiber digestibility between grasses and legumes
  - iVNDFD > for grasses than legumes
  - However filling effects for legumes < than grasses
  - Legumes result in > DMI and milk yield than grasses even at similar iVNDFD
- NDFd should be used to rank feeds rather than calculate specific energy values

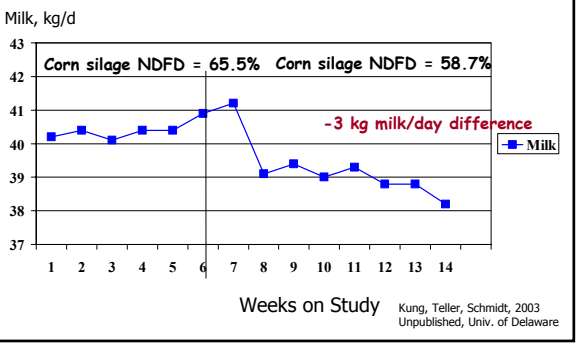
Comparison of Alfalfa Hay at bud stage and Grass Hay at early bloom



Comparison of 2 RFV Forages



NDF-D of Corn Silage on Milk Production



Kung, Teller, Schmidt, 2003
Unpublished, Univ. of Delaware

In-vitro analysis

- Artificial digestion system
  - Ruminal fluid used – type of diet, stage of lactation of cow supplying ruminal fluid
  - Using finely ground samples
    - Labs may vary with type of grind
    - Affects digestibility results

TABLE 3. Mean in vitro DM digestibility (percentage) using either a hay diet or a TMR for the source of rumen inoculum for ten different feeds.

Feed	Hay diet	TMR	*P* value
Alfalfa hay	56.13	62.35	*
Grass pasture	60.32	65.10	**
Grass hay	48.37	53.45	*
Mixed haylage	44.11	51.78	0.12
Corn silage	59.74	67.14	0.14
TMR	65.73	72.39	**
Grain mixture	80.13	83.38	0.19
HMSC[1]	81.47	85.56	0.12
Steam flaked corn	81.95	85.94	*
Dry ground corn	82.74	87.28	*

[1]High moisture shelled corn.
*$P < 0.10$.
**$P < 0.05$.

Ranking consistent

Effect of Wiley Grind Size on Corn Silage 24h IV Digestion

Size	IVDMTD	SD	IVNDFD	SD
1-mm screen	77.4	3.96	48.7	5.33
4-mm screen	76.7	3.79	44.9	5.50
Whole	73.2	5.69	37.6	9.27

Mertens and Ferreira (2000)

In-vitro analysis

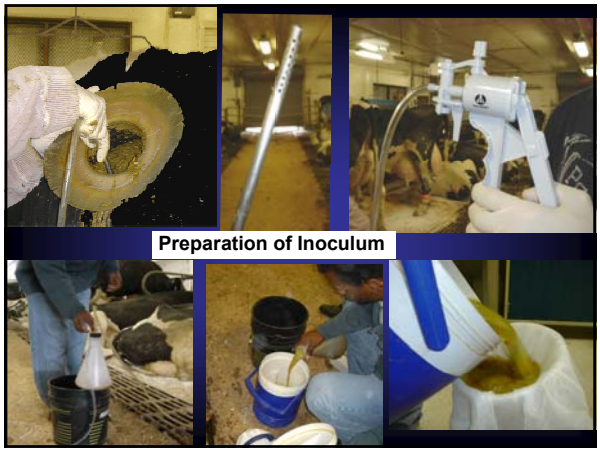
- Artificial digestion system
  - Very small quantity of feed required
    - 0.5 gram
  - High level of precision possible

Factors Affecting In-Vitro Digestibility

- Sample amount
  - Smaller amounts typically increase variation
  - Flask/tube method
    - Ratio of sample amount to buffer and inoculum
    - Typically .5 g per 40 ml buffer & 10 ml inoculum
  - Bag method
    - Ratio of sample amount to buffer and inoculum
    - Ratio of sample amount to bag surface area
    - Typically recommend 10 to 20 mg/cm^2

In-vitro analysis procedure

- **Preparation of Filter Bags and Sample:**



Preparation of Inoculum

ANKOM DAISY II IN VITRO SYSTEM

TABLE 4. Mean in vitro DM digestibility (percentage) for three methods of analyses: traditional method (TM), DAISY[II] with same feeds in jar (DS), or DAISY[II] with different feeds in jar (DD) for ten different feeds.

Feed	TM	DS	DD	*P* value
Alfalfa hay	58.84	58.91	59.89	0.87
Grass pasture	61.88	62.89	63.27	0.62
Grass hay	49.78	51.73	51.22	0.56
Mixed haylage	47.40	48.92	47.52	0.90
Corn silage	63.92	62.85	63.54	0.96
TMR	69.89	68.53	68.75	0.53
Grain mixture	79.53	83.95	81.78	0.31
HMSC[2]	79.83	85.59	86.02	0.11
Steam flaked corn	81.58	84.14	85.11	0.17
Dry ground corn	79.93	86.89	88.21	0.14

[1]ANKOM Technology Corporation, Fairport, NY.

[2]High moisture shelled corn.

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In vitro procedure

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Questions on In-Vitro Procedure?

In-situ analysis

- Test feed is weighed in a cloth (dacron) bag with a defined porosity
  - Minimize feed dry matter loss
  - Allows adequate bacterial entry to produce normal digestive activity
- Dacron bag is sealed and incubated in rumen of cannulated animal.
- Test feed can be ground or as-fed

In-situ analysis

- Extent of digestion can be measured using a single incubation point
  - Equal to the expected ruminal retention time of the feedstuff
- Multiple incubation times can be used
  - Develop rates of digestion

In-situ analysis

- Closely simulates the reticulo-rumen, which is being evaluated.
- Can evaluate the digestibility closer to the form in which the feed is fed.
- Feedstuff is exposed to fluctuations in ruminal pH and associative effects from protein and CHO supplementation.

In-situ procedure

- Eight grams of sample are weighed into duplicate pre-tared nylon bags (~54 micron pore size).
- Bags are tied with nylon fishing line and then tied to a 100 cm fishing line attached to a bolt.
- After soaking in 39 C distilled water for 15 minutes bags are placed in the rumen of a cannulated cow consuming the control (untreated) diet.
- All bags are placed into the rumen of the cow at once and then removed over time to represent 0, 2, 4, 8, 16, 24, 48, and 72 hours of incubation in the rumen.
- An alfalfa hay standard is included in the run at 8, 16, 24 and 72 hours.
- Machine wash (5X at 1min/rinse)

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Questions on In-Situ Procedure?

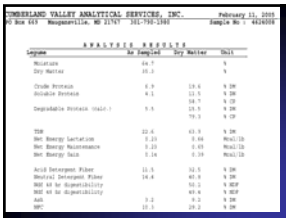
Applying NDFD to Ration Formulation and Evaluation

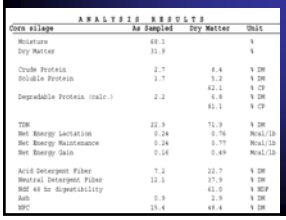
Alfalfa and Grass Silage Based Rations Using Fine/Coarse Ground Shelled Corn

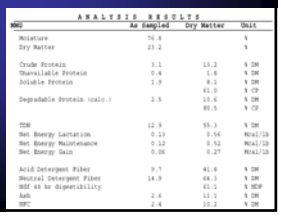
- 50:50 Haycrop Forage:Corn Silage
- 60 cows each trial

Alfalfa based diet – left side of free-stall barn

Grass based diet – right side of free-stall barn

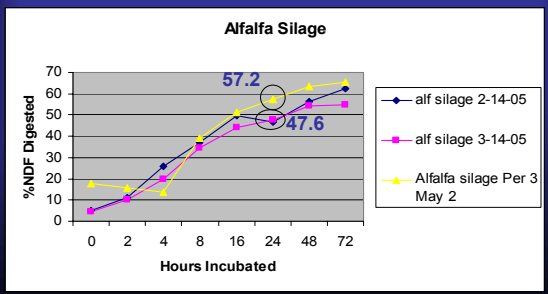


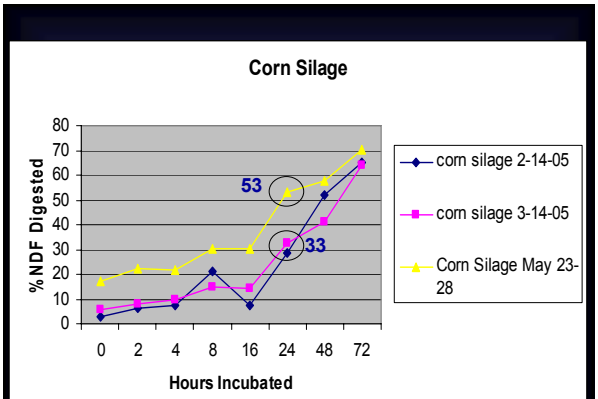


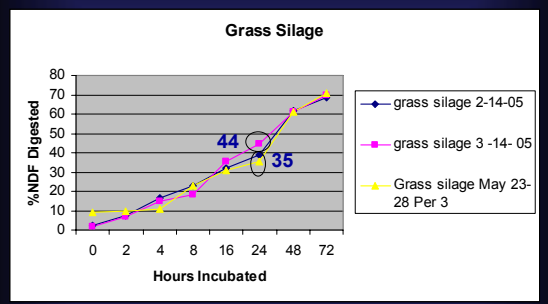


48 hr. NDFD was requested because the 2001 NRC was being used to formulate /evaluate diets.

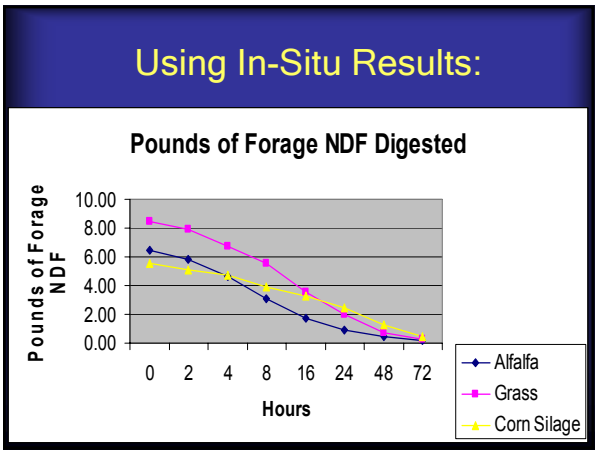
In-Situ Results - Changes Over Time



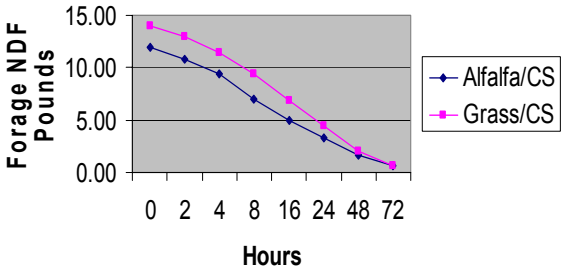




Using In-Situ Results:



Alfalfa vs Grass Based Ration - Forage NDF Digested



Ingredients		Gras ration	Alf ration
Alfalfa silage			37.90
Grass silage		46.00	
Western Hay			
Bunk CS		42.10	42.20
Coarse corn		13.20	9.98
Fine corn		13.20	9.98
Cotton hulls		0.50	5.10
Rst beans		5.33	5.30
Canola		1.40	1.75
Cookie meal		1.50	1.00
Sugar		4.00	4.00
Amino Plus		2.66	1.83
Mineral		2.30	1.87
Bicarb		0.33	0.33
Bent		0.10	0.10

What is the interaction of the other ingredients on NDFD of the ration?

- CHO sources: Sugar, Starch (particle size)
- Protein balance: Adequate substrate for SC bugs?
- Cottonseed hulls (influence of byproduct feeds)

PSU Alfalfa and Grass Silage Based TMRS - In-vitro and In-situ results

NDFD-In Vitro	Alf-F	Alf-C	Gras-F	Gras-C
24 hr	39.0	40.0	50.1	47.4
30 hr	47.8	46.1	54.6	54.4
48 hr	56.4	51.2	58.5	59.7
NDFD-In Situ				
24 hr	41	42	50	50
48 hr	50	57	65	61

Effects of ration particle size

Particle Size Distribution

Suggested
Up 2-8%
Md 30-50%
Lo 30-50%
Bp < 20%

TMRs	Upper	Middle	Lower	Bottom pan
	----------Particles Remaining - % of total----------			
Alfalfa silage				
Fresh	7	45	39	10
Refusals	2	40	47	11
Grass silage				
Fresh	27	29	34	11
Refusals	17	41	35	7

Alfalfa based diet 60 cows housed in freestall barn

	As-fed, lbs	TMR DM%	DMI, lbs	
Period 1	129	0.52	67	Fine
Period 2	136	0.51	69	Coarse
Period 3	124	0.53	66	Fine
Period 4	114	0.54	63	Coarse

Grass based diet 60 cows housed in freestall barn

	As-fed, lbs	TMR DM%	DMI, lbs	
Period 1	115	0.47	54	Coarse
Period 2	119	0.45	53.5	Fine
Period 3	120	0.46	55	Coarse
Period 4	115	0.48	55	Fine

Alfalfa based diet 60 cows housed in freestall barn

Milk, lbs	Fat %	Prot %	3.5%FCM	ECM	DMI-Eff	
93.1	4.00	3.00	100.8	97.7	1.51	Fine
93.7	3.97	3.16	101.0	99.1	1.46	Coarse
95.6	3.83	2.98	100.8	98.1	1.53	Fine
103.2	3.94	2.94	110.7	107.1	1.76	Coarse

Grass based diet 60 cows housed in freestall barn

Milk, lbs	Fat %	Prot %	3.5%FCM	ECM	DMI-Eff	
83.2	4.00	3.00	90.1	87.3	1.66	Coarse
83.1	3.85	3.09	87.9	86.2	1.64	Fine
85.6	3.69	2.91	88.3	85.9	1.61	Coarse
85.4	4.1	3.26	93.8	92.3	1.71	Fine

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How did NDFD help in formulating rations?

Learning Objectives

- Appreciate the challenges for determining NDFD using in-vitro or in-situ procedures.
- Understand the multi-faceted dynamics of the rumen and why NDFD values should be interpreted cautiously.
- Realize the limitations of applying NDFD in the field for ration formulation and evaluation.