Variation When Determining dNDF and NDFD and Its Prediction by NIRS

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Introduction

- Nutritional importance of NDFD
  - 2001 Dairy NRC suggests it can be used to determine dNDF for estimating TDN1X
  - Oba and Allen () indicates it is related to intake of lactating cows
- Difference between NDFD and dNDF
  - NDF Digestibility = NDFD (% of NDF) = digestion coefficient of NDF
  - digestible NDF = dNDF (% of DM) = proportion of DM that is digestible NDF
  - dNDF = NDF*NDFD/100
    - 24% dNDF = 40% NDF*60%NDFD/100
    - dNDF is always less than NDF

Introduction

- Methods of determining NDFD
  - In vivo – using total collection or markers
    - Lactating cows fed mixed diets
    - Sheep at maintenance fed forage only
  - In situ – using porous bags
  - In vitro
    - Using flasks or tubes
    - Using filter bags – Ankom Daisy system
  - Estimated using chemical composition
    - Related to lignin and silica

In Vivo Digestibility

- Is a biological evaluation of a feed that is not a constant, but varies with
  - Species
  - Size
  - Production level
  - Intake
  - Selection and sorting
  - Methodology

Digestibility as a Measure of Animal Performance

- In vivo production digestibility protocol
  - Specific for the performance status of animals
  - Production level of intake (1-5X Mnt)
  - Ad libitum (free choice) intake with refusals = selection
  - Measures digestibility during production
  - Much greater variability = difficult to measure inputs and outputs

Digestibility as a Measure of Feed Nutritive Value

- Standardized in vivo digestibility protocol
  - Designed to assign a value to a feed by minimizing animal performance differences
  - Mature animals
  - Maintenance level of intake (1X Mnt)
  - No selection or refusals
  - Measures maximum digestibility
  - Weigh feed, refusals and feces for 5-7 days

In Situ / In Sacco Digestibility

- Feed is sealed in a porous bag and suspended in the rumen of fistulated cows
- Assume in situ = in vivo
  - But only measures fermentative digestion
- Apparent value is in mimicking ruminal digestion for production levels and diets
- More difficult to standardize, especially among labs when used for feed evaluation
  - Bag dimensions and pore sizes
  - Washing of bags and removal of fines
  - Cyclic and variable ruminal conditions
  - Variability among animals

In Vitro Digestibility

- Single-stage IVDMD
  - Incubate ruminal fluid with feed in buffer
  - Dry residues and weigh
- Two-stage Tilley & Terry IVDMD
  - Incubate ruminal fluid with feed in buffer
  - Incubate undigested residue in acid pepsin
  - Dry residues and weigh

In Vitro Digestibility

Two-stage Van Soest IVDMTD

- Incubate ruminal fluid with feed in buffer
- Extract undigested residue in neutral detergent
- Dry NDF residues and weigh

- In vitro methods measure different things
  - Single and two-stage T&T IV measure apparent DM digestibility
  - Two-stage Van Soest IV measures true DM digestibility
  - T&T IVDMD will always be lower than VS IVDMTD

Digestibility is a Variable

- NDFD and dNDF are a function of the feed and system in which it is measured
  - Not simply a feed characteristic
  - In vivo digestibility is affected by the animal, its level of intake and the diet in which the feed is fed
  - In situ and in vitro digestibility are affected methodology

Objectives

- Discuss the factors that affect the in vitro and in situ measurement of NDFD
- Indicate the magnitude of variation in NDFD
- Discuss approaches to minimize variation in NDFD within and among laboratories

NDFD Determination
Basic Steps

- Material preparation
- Test sample selection
- Inoculum preparation
- Buffer
- Media supplementation
- Fermentation
- Residue collection

Factors Affecting IV Digestibility

- Test sample preparation
  - Drying – less than 60C to minimize heat damaged protein and artifact lignin
  - Grinding recommendations vary
    - 8-mm screen Wiley cutter mill
      - Maximizes detection of physical effects
    - 2-mm screen Wiley cutter mill
      - Used for porous bag methods to minimize particle loss
      - Concentrates (1.5 to 2.5 mm), forages (1.5 to 5 mm)
    - 1-mm screen Wiley cutter mill
      - Most commonly used to detect digestibility differences
    - 1-mm screen, cyclone mill
      - Rarely, if ever, used for in vitro

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

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

Mertens and Ferreira (2000)

Material Grind Size

- McLeod and Minson (1969) – Grasses Christy mill
  - 0.40 mm-screen = 54.3% T&T IVDMD 48h
  - 1.00 mm-screen = 52.4% T&T IVDMD 48h
  - 1.96 mm-screen = 49.7% T&T IVDMD 48h
- Alexander (1969) Christy mill
  - 0.60 mm = 53.8% (±.35) T&T IVDMD 48h
  - 1.60 mm = 50.3% (±.70) T&T IVDMD 48h
  - 2.45 mm = 50.1% (±.71) T&T IVDMD 48h

Material Grind Size

- Saldivar et al. (1982)
  - 0.5 UD = 52.5% T&T IVOMD 48h
  - 0.5 W = 52.3% T&T IVOMD 48h
  - 1.0UD = 50.3% T&T IVOMD 48h
  - 1.0 W = 47.1% T&T IVOMD 48h

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

Test Sample Amount

- McLeod and Minson (1969) - Grasses
  - 0.5g = 58.0% T&T IVDMD 48h (±1.1)
  - 0.6g = 57.2% T&T IVDMD 48h
  - 0.7g = 56.4% T&T IVDMD 48h
  - 0.8g = 56.1% T&T IVDMD 48h
  - 0.9g = 55.3% T&T IVDMD 48h
  - 1.0g = 55.0% T&T IVDMD 48h (±0.5)

Factors Affecting In Vitro Digestibility

- Fermentation Vessel
  - Flasks versus tubes
    - Changes surface area of submerged material
    - Changes side-wall contact
  - Bag characteristics
    - Size and area
      - 5X5 cm = 50 cm^2
    - Type
      - Filter bag (F57)
      - Dacron bags
    - Pore size
      - 50 µm (range from 20 to 60 µm)

Fermentation Vessel

- Sayre and Van Soest (1972)
  - Erlenmeyer flasks = 75.6% IVDMTD
  - Centrifuge tubes = 72.3% IVDMTD
  - Screwcap vials = 73.3% IVDMTD
- Robertson et al. (per. comm.)
  - 25 mm tubes = 52.3% IVNDFD
  - 32 mm tubes = 54.4% IVNDFD
  - Erlenmeyer flasks = 56.8% IVNDFD
- Grant and Mertens (1992)
  - 50 mL tubes = 66.3% IVNDFD
  - 125mL flasks = 67.8% IVNDFD

Factors Affecting In Vitro Digestibility

- Buffer used to maintain pH during fermentation
  - McDougall's artificial saliva
  - Ohio buffer
  - Kansas buffer
  - Van Soest buffer

Factors Affecting In Vitro Digestibility

- Supplementation of media
  - Trace minerals
  - Ammonia and amino acids
  - Branched-chain fatty acids
- Reduction and anaerobicity
  - Use of sulfide and cysteine
    - Reduced lag time (Grant and Mertens, 1992)
  - Use of indicator (resazurin)
  - CO_2 saturation of media and purging of vessels

Flushing Vessels with CO_2

- Minson and McLeod (1972)
  - Flushing gave no benefit for T&T IVDMD
    - 57.1% with versus 57.5% without
- Alexander (1969)
  - CO_2 buffer+CO_2 flush = 61.0% IVOMD 48h
  - CO_2 buffer+No flush = 59.4% IVOMD 48h
  - No buffer+No flush = 57.8% IVOMD 48h

Flushing Vessels with CO_2

- Robertson et al. (per. comm.)
  - Cont. manifold = 56.5% IVNDFD 48h
  - Bunsen valves = 52.4% IVNDFD 48h
- Grant and Mertens (1992)
  - Cont. manifold = 69.6% IVNDFD 48h
  - Purge + Bunsen = 58.4% IVNDFD 48h

Factors Affecting In Vitro Digestibility

- Sample wetting/submerging
  - Floating material is a problem
    - Related to trapped gas and hydrophobicity
    - May interaction with vessel type
  - Solutions
    - Wet with a small amount of buffer
    - Submerge by evacuation
    - Swirling/mixing of vessels during fermentation
- Clumping a material in bags

Test Sample Wetting

- Minson and McLeod (1972) used evacuation to submerge particles
  - IVDMD = 53.2% without versus 55.2% with

Factors Affecting In Vitro Digestibility

- Inoculum Preparation
  - Donor
    - Single versus composite donors
    - Diet – Intake level
    - Feed restriction prior to obtaining contents
      - Fasting beyond 16 hr is detrimental (Ayers, 1991)
  - Characteristics
    - pH
    - Optical density

Inoculum Preparation

- Ayres (1991)
  - Sheep W19 52.6% IVOMD
  - Sheep W34 51.2% IVOMD
  - Sheep W26 46.6% IVOMD
  - Sheep W31 45.1% IVOMD
  - Composite 51.6% IVOMD
- Mertens, Weimer & Waghorn (unpubl)
  - Composite performed better than individual donors

Strained Ruminal Fluid pH/OD

- 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
- Mertens and Ferriera (unpubl)
  - IVNDFD reduced below an OD threshold

Factors Affecting In Vitro Digestibility

- Inoculum Preparation
  - Strained rumen fluid versus solids extraction
    - Particle associated microbes
  - Time from collection to inoculation
  - Amount of inoculum

Inoculum Preparation

- Craig et al. (1984)
  - Particle-associated microbes collected by washing strained ruminal solid (+PM)
  - Solids were blended with ruminal fluid (B)
  - SRF = 46.3% IVNDFD 48h
  - SRF+PM = 48.6% IVNDFD 48h
  - SRF(B) = 46.1% IVNDFD 48h
  - SRF+PM4°C = 45.8% IVNDFD 48h

Inoculum Preparation Delay

- Alexander (1969)
  - Normal (15min) 68.4% T&T IVDMD 48h
  - 1h delay 38.5 °C 62.3% T&T IVDMD 48h
  - 1h delay cooled 58.3% T&T IVDMD 48h
- Mertens (1973)
  - Delay beyond 20 min (cow to inoculation) increased lag time

Strained Rumen Fluid to Buffer Ratio

- McLeod and Minson (1969) – Grasses
  - 25:25 = 52.9% T&T IVDMD 48h
  - 15:35 = 51.2% T&T IVDMD 48h
  - 10:40 = 48.5% T&T IVDMD 48h
  - 5:45 = 43.9% T&T IVDMD 48h
  - 2.5:47.5 = 43.9% T&T IVDMD 48h
- Weimer (per. comm.)
  - IV digestion reduced below 10 mL SRF

Factors Affecting In Vitro Digestibility

- Incubation temperature
  - Recommended varies from 38-39.5 °C
  - Gas pressure measurements were extremely sensitive (Mertens and Weimer)
    - 10% reduction per 1 °C difference from 39 °C

Incubator Temperature

- Alexander (1969)
  - 35.5 °C = 56.4% IVOMD 48h
  - 38.5 °C = 58.7% IVOMD 48h
  - 42.0 °C = 61.0% IVOMD 48h
- Minson and McLeod (1972)
  - 35.0 °C = 54.4% IVOMD 48h
  - 37.0 °C = 58.4% IVOMD 48h
  - 39.0 °C (min SD) = 58.9% IVOMD 48h
  - 41.0 °C = 59.7% IVOMD 48h
  - 43.0 °C = 58.4% IVOMD 48h

Factors Affecting In Vitro Digestibility

- Adjustment using standards
  - Traditionally used in vitro versus in vivo calibration curves
    - Required 4 to 5 calibration samples per run
    - Variable effectiveness
  - Use standards to normalize or correct individual results
  - Use standards to determine validity of the entire run without correction

Adjustment of IV Digestibility Using Standards

- Adjustment using standards
  - Alexander (1969)
    - Scaling for std mean was ineffectual
    - Correcting using 4 ref std reduced single result SD from 1.27 to 0.89
  - Ayers (1991)
    - No adjustment if standards with 95% confidence level
    - Adjust by mean deviation, if the deviations of 4 standards are consistently different
    - Re-run if standards outside the 95% CI and are inconsistent
  - Mertens
    - Using standards as covariate rarely improves statistical analysis

Factors Affecting In Vitro Digestibility

- Time of fermentation
  - T&T 48h IVDMD consistently related to in vivo digestibility measured at maintenance levels of intake (Feed Evaluation Protocol)
  - Allen et al indicate that producing dairy cows have a fiber retention time of 30 to 36 h
  - Some have suggested that 24h IV fermentations may be a better indication of dairy cow performance

In Vitro Fermentation Time versus In Vivo Retention Time

- In vivo Retention Time DOES NOT equal in vitro fermentation time
  - i.e., digestion at 30 hr retention time DOES NOT equal digestion at 30 hr fermentation time
    - $1/kp$ = retention time ≠ fermentation time
  - In vivo digestion = $kd / (kd + kp)$
  - In vitro digestion = $1 - DM*\exp(-k*t)$

In Vitro Variation

- Alexander (1969)
  - 1-stage rumen fluid
    - Between run SD = 0.99
    - Within run SD = 0.73
  - 2-stage rumen fluid + acid pepsin (T&T)
    - Between run SD = 0.63
    - Within run SD = 0.38

In Vitro Variation

Reference	Within run	Among run
Alexander (1969)	.39	.66
Tilley & Terry (1963)	.61	1.90
Dent (1963)		1.50
Minson and McLeod (1972)	.94	2.24
Martin and Barnes (1969) A	.83	
Martin and Barnes (1969) B	.50	
Barnes (1967) 5-lab average	2.80	2.35
Ayers (1991)		1.18

In Vitro Variation

- IVNDFD is more variable than IVDMTD or IVDMD
  - IV undigested NDF (uNDF) has a variance
  - NDF determination has a variance
  - IVNDFD is the quotient of two variables
    - IVNDFD = 100*(NDF-uNDF)/NDF
  - Mathematical consequence of dividing mean and SD by a fraction
    - Mean = 50 and SD = 5, if all measurements are divided by .5 then Mean =100 and SD = 10

NDFD Variation – Statistics 101

- Summation of errors
  - SD of determining NDFD using in vitro method = ±4.0
  - SD of predicting IVNDFD using NIRS = ±3.0
  - Total SD of estimating NDFD using NIRS
    - = square root ($IV\_SD^2 + NIRS\_SD^2$) = ±5.0
- Outlier population
  - ± 1 SD = 31.7% of estimates outside ± 5
  - ± 2 SD = 4.6% of estimates outside ± 10
  - ± 3 SD = 0.26% of estimates outside ± 15

In Vitro Digestibility – Final Caution

- IVDMD DOES NOT EQUAL in vivo DMD, especially at production levels of performance
- Improvement in IVDMD and IVNDFD of bmr corn does not translate into improved dairy cow digestibility
  - Instead performance is increased due to increased intake
  - Not certain this is a universal response, but should indicate caution in using in vitro data