

Transformational Changes In Feed Analysis – How did we get to where we are?©

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History of Nutritional Fiber

- Pre-1860s
 - Maceration in water to obtain “woody” or “fibrous” matter in feeds
 - Thought the residue would be indigestible, but discovered that some woody fiber disappeared in animals
 - Feeds were compared to a standard feed (barley or starch values) based on animal performance
- 1860s-1970s 1st Transformational Change
 - Henneberg and Stohmann (1860,1864) Weende or Proximate System
 - Feeds evaluated based on chemical composition

Definition

- **Transformational Change** is a fundamental shift in concept or process (method) that alters our understanding (knowledge) in such a way that performance or application is dramatically improved.

History of Nutritional Fiber

- 1860s -1970s 1st Transformational Change
 - Henneberg and Stohmann (1860,1864) Weende or Proximate System
 - Ash
 - Crude Protein
 - Crude Fat (EE)
 - **Crude Fiber** – extracted by weak acid and base, ash-free
 - Assumed to be indigestible or poorly digested
 - $NFE = DM - Ash - CP - EE - CF$: calculated by difference
 - Supposed to be the readily carbohydrates

Objectives

- History of fiber chemical analysis
 - Identify transformational changes in concepts and methods that altered ruminant nutrition
- Role of fiber in digestibility
 - Fiber digestion kinetics
- Fiber physical analysis and rumen function
 - Fiber passage kinetics
- Fiber particle size and intake and rumen health (peNDF)

History of Nutritional Fiber

- 1860s -1970s 1st Transformational Change
 - There were problems with chemical methods
 - 1887 (Richardson) AOAC Committee on Cattle Foods “*The crude fiber*”. Doubtless all who have taken part in the work of testing methods of analysis of feeding stuffs, expected wide divergence in results in the fiber, and certainly these expectations are not disappointed.”
 - 1897 First AOAC “Official Method” for CF

History of Nutritional Fiber

- Nutritionists had problems with CF
 - Quickly discovered that CF was digested, but
 - Total **Digested** Nutrients could be determined and adjusted for the extra energy in fats using the proximate analysis system
 - $TDN = dCP + 2.25 * dEE + dCF + dNFE$ (all entities are OM – ash-free)
 - But the relationship between TDN and animal production (net energy) **varied among feed type**

History of Nutritional Fiber

- 1930s – 1950s **Next Transformational Change?**
 - Nutritionists began looking for something with more consistent digestibility than CF
 - Lignin = Indigestible fiber?
 - 72% H₂SO₄ Methods
 - Norman and Jenkins (1934a,b)
 - Crampton and Maynard (1938) as modified by Lancaster (1943)
 - Ellis et al. (1946) as modified by Thacker (1954)
 - **But most of these “lignins” were digestible**

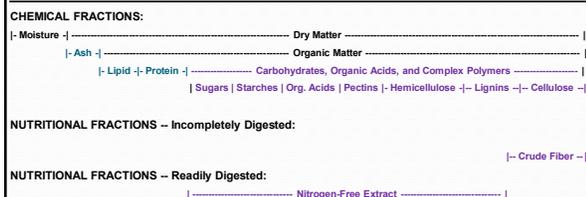
History of Nutritional Fiber

- Nutritionists had problems with CF
 - Richardson and Reid (1953) summarized the problems with CF and NFE
 - the “readily digestible” NFE contains lignin and the indigestible CF was digested to a considerable extent
 - in many feeds, CF is more digestible than NFE
 - CF and NFE do not represent precise (accurate) chemical constituents
 - their composition varies with plant species, maturity and conditions of determination.

History of Nutritional Fiber

- 1930s – 1950s **Next Transformational Change?**
 - Search for chemically consistent carbohydrates in the hope that their nutritive value would be consistent
 - Fibrous carbohydrates
 - Cellulose
 - Crampton and Maynard (1938), Matrone et al., (1946)
 - Hemicelluloses (pentosans)
 - Weihe and Phillips (1942), AOAC (1950)
 - Pectins
 - Carre and Haynes (1922), Nanji and Norman (1928)

Conceptual Partitioning of Feeds



History of Nutritional Fiber

- 1930s – 1950s **Next Transformational Change?**
 - Fibrous carbohydrates
 - Holocellulose by acid chlorite treatment
 - Cellulose + hemicellulose (similar digestibilities)
 - Ely and Moore (1954, 1955, 1956), Wise et al. (1946)
 - Method would not work on feces; therefore digestibility could not be measured
 - Total CHO = CF + NFE – Lignin
 - Pectin, starch, and sugars were combined with cellulose and hemicellulose – **not a good idea**

History of Nutritional Fiber

- 1960s Acid Detergent Lignin
- 2nd Transformational Change
 - 1957 - Lane Moore hired P. J. Van Soest at Beltsville, USDA-ARS (Waldo, Thomas, Flatt, Bryant)
 - Van Soest and Moore (1959) proposed a comprehensive system of analysis along the lines of work by Ely and Moore (1955)
 - Van Soest switched to detergents for extraction of fiber and published his earliest work on ADF and lignin as abstracts in JDS (Van Soest, 1961).

History of Nutritional Fiber

- 1960s Neutral detergent fiber
- 3rd Transformational Change
 - Van Soest's earliest work on NDF was published as abstracts in JDS (Van Soest and Marcus, 1964).
 - Total insoluble fiber - NDF (Van Soest and Wine, 1967)
 - NDF is not Plant cell-wall constituents (CWC)
 - NDF contains lignin, cellulose, hemicellulose, but not pectin
 - Easily extractable pectin is almost completely digested by ruminants
 - Sulfite used to improve protein extraction
 - Use of detergent fibers was accepted quickly, which confirmed the discontent with previous methods

History of Nutritional Fiber

- 1960s Acid Detergent Lignin
- 2nd Transformational Change
 - Search for indigestible fiber (lignin) Van Soest (1963a,b, 1965)
 - ADF and Lignin
 - Prepared fiber with low N and hemicellulose (ADF is a preparatory step for lignin)
 - 72% H₂SO₄ lignin from ADF (AOAC Official Method, 1973)
 - Permanganate lignin (Van Soest and Wine, 1968)

History of Nutritional Fiber

- 3rd Transformational Change
- 1970 - 2002 Refining NDF analysis
 - ND Residue – NDR (Van Soest, et al., 1991)
 - Removed sulfite – concerns about lignin removal
 - Added heat-stable amylase to extract starch
 - Amylase-treated NDF – aNDF (Mertens, 2002)
 - AOAC Official Method
 - Uses sulfite and amylase
 - Sulfite needed to removed heated proteins
 - aNDF organic matter – aNDFOM (Mertens, 2002)
 - Ash-free aNDF adopted around 2015

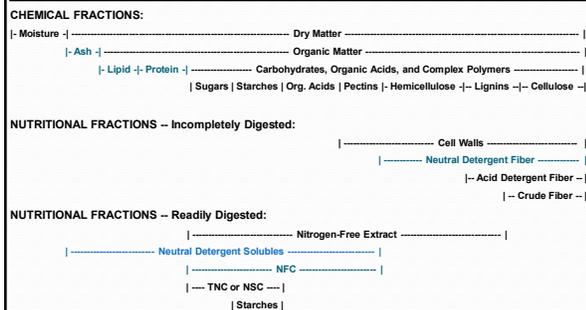
History of Nutritional Fiber

- 1960s Acid Detergent Lignin
- 2nd Transformational I Change
 - Search for indigestible fiber (lignin) Van Soest (1963a,b, 1965)
 - Discovered that **artifact lignin** was due to N contamination (Maillard reaction) when feeds are dried at high temperatures (<60°C)
 - Symposium papers in J. Anim. Sci. (1964, 1965,1967)
 - Lignin only affects fiber and not all OM

History of Nutritional Fiber

- 3rd Transformational Change
- 1980s Extending NDF analysis
 - Non-fibrous carbohydrates (NFC) can be calculated by difference
 - Needed fractions that summed to 100
 - NFC = 100 – ash – CP – EE – NDF
 - Contains sugars, starch, soluble fiber that are rapidly fermented
 - Analogous to NFE
 - NDF – ADF = hemicellulose, **crude and problematic**

Conceptual Partitioning of Feeds



History of Fiber's Definition NDF vs NDS

- 3rd Transformational Change
- Greatest contribution in developing NDF was the hypothesis that uniform nutritional availability, rather than chemical purity, was the most important criteria for measuring total insoluble fiber (Van Soest, 1967; Van Soest and Moore, 1965)
 - NDF is the fraction with variable digestibility
 - ND solubles (NDS = 100 – NDF) have a high and relatively constant true digestibility (uniform nutritional availability) across feeds
 - Variability in NDF among feeds is trivial compared to the differences between NDF and NDS within feeds

History of Fiber's Definition

- 3rd Transformational Change
- After 200 y, we are still using empirical methods (where the resulting measurement is solely a function of the method used to generate it) to measure fiber
- This conundrum is not a failure of understanding or technique, but the result of attempting to measure a nutritional concept, "insoluble fiber," using chemical solubility methods

History of Detailed Chemical Measures of Fiber

- Chemical composition of NDF and ADF varies considerably
 - Detailed analysis of the monomers in fibrous CHO (Wedig et al., 1989; Canale et al., 1991, Miron, et al. 2002, Jung et al., 2011) and lignin (Reeves, 1987; Wedig et al., 1989; Canale et al., 1991)
 - **Nutritional relevance of monomer analysis is unknown**
 - May generate understanding about indigestible NDF

History of Fiber's Definition

- 3rd Transformational Change
- Originally, fiber was the fraction that could not be digested
- Nutritional fiber for ruminants is insoluble fiber that is indigestible or slowly digested and occupies space in the gastrointestinal tract (Mertens, 2003)
- Soluble fiber is relatively unimportant for ruminants because it is rapidly fermented in the rumen like NDS

History of Fiber's Definition NDF vs NDS

- 3rd Transformational Change
- "Simple" summative equation of Van Soest
 - $dDM = dNDF + dNDS$
 - $dDM = \text{digested DM (g dDM/100gDM)}$
 - $dNDF = NDFD * NDF$
 - $dNDF = \text{digested NDF (g dNDF/100gDM)}$
 - $NDFD = \text{NDF Digestibility Coefficient (fraction of NDF)}$
 - $dNDS = 0.98 * NDS - 12.9$
 - $dNDS = \text{digested ND Solubles (g dNDS/100gDM)}$
 - $0.98 = \text{true Digestibility Coefficient of NDS}$
 - $12.9 = \text{Endogenous losses}$

History of Fiber's Definition NDF & NDFD

- 3rd Transformational Change
- "Simple" summative equation of Van Soest
 - $dDM = dNDF + dNDS$
 - $dDM = NDFD * NDF + 0.98 * NDS - 12.9$
 - Because $NDS = (100 - NDF)$
 - $dDM = NDFD * NDF + 0.98 * (100 - NDF) - 12.9$
 - Simplifies to
 - $dDM = 85.1 - (0.98 - NDFD) * NDF$
 - Conclusion: **digested DM is a function of only NDF and NDFD**, assuming all NDS has a constant digestibility
 - Starch digestion can be a potential problem

History of Fiber Digestibility

- Because NDFD is variable, development of a routine method for it is crucial to estimating feed evaluation
- Initially, animal, or in vivo (IVV) methods were standardized and used to evaluate feeds
 - This digestibility data was the starting point for calculating Net Energy Lactation after an adjustment for intake

NDF and NDFD are the Keys to Digestion

- 3rd Transformational Change
- "Simple" summative equation of Van Soest
 - $dDM = 85.1 - (0.98 - NDFD) * NDF$
 - Coefficient for NDF, $(0.98 - NDFD)$, is not constant, which precludes regression analysis of NDF to predict dDM
 - Constant digestibility of NDS works well for forages, but starch in NDS is an exception that has variable digestibility
 - NRC (2001) expanded the NDS portion of the equation into $dNDS = tdCP + 2.25 * tdFA + tdNFC * PAF - EndogLoss (EL)$
 - Shaver (2006) recommended removing starch from NFC
 - $dNDS = tdCP + 2.25 * tdFA + tdNFNSC + tdSt * PAF - EndogLoss$

History of Fiber Digestibility

- In vivo (IVV)
 - Total collection trials for digestibility began in the late 1800s
 - Digestibility by markers began in the 1940s
 - Fiber analysis problems in digesta and feces – **we may still have these problems**
 - **IVV Digestibility is not a constant for a feed**, but can vary considerably based on the situation
 - Variable intakes and rate of passage (particle size)
 - Diet and feeding conditions

History of Fiber Digestibility

- "Simple" summative equation of Van Soest
 - NDFD
 - Measured in vivo, in vitro, in situ
 - Predicted from lignin ratio in fiber (ADF or NDF)
 - Relationship between lignin and NDFD may differ between legumes and grasses
 - NDFD increase of 1%-unit in forages related to increases in DMI (0.37, 0.31, & 0.21) and 4%FCM (0.55, 0.26, 0.31) for Oba and Allen, (1999); Jung et al.,(2004); and Mertens, (2006), respectively

History of Fiber Digestibility

- 1930-60s digestibility was measured in animals (in vivo) under carefully controlled conditions
 - Digested proportions were measured as the difference between the total amount eaten and the total amount excreted (total collections) over a fixed period (5–10 d)
 - Consistent intakes were crucial to success
 - Labor intensive and expensive
 - Animals were used as "biological test tubes" to determine differences in feeds, especially forages

History of Fiber Digestibility

- 1930-60s digestibility was measured in animals (in vivo) under carefully controlled conditions
 - Mature animals, not growing, pregnant or lactating
 - “Maintenance” level of intake (~2% BW/d)
 - The rate of passage of these animals was relatively constant
 - Differences among animals were minimized
 - Routine measurements for feed evaluation are impossible due to cost, labor and amount of feed
- Meta-analyses indicate that IVV total tract NDFD varies widely

History of Fiber Digestibility

- In Situ (IS)
 - Early studies measured changes of fiber in the rumen
 - Indigestible bags first used in 1960s
 - Is all degraded (lost from the bag) OM fermented?
- In Vitro (IV)
 - Initial development in the 1950s
 - Results were reported as digestibility (the fraction that disappeared)
 - The IV **residue is a measure of undigested fiber**

Variability in IVV Total Tract NDFD

Reference	Type	N	Avg	Range
Whiting et al., 2017	All	337	0.504	0.195 to 0.840
Weld & Armentano, 2017	Fat	98	0.494	0.288 to 0.668
Farraretto & Shaver, 2015	C. Sil.	81	0.438	0.242 to 0.625
Stergiadis et al., 2015	Grass	464	0.803	0.623 to 0.899
Ferraretto et al., 2013	All	414		0.35 to 0.57
Ferraretto et al., 2013	In vivo Ruminant NDFD			0.28 to 0.52

Shouldn't IVV ruminal and IVV total tract NDFD be nearly equal?
If fiber digestion occurs primarily by microbial fermentation, it would appear that there is little NDF digestion in the lower gut.

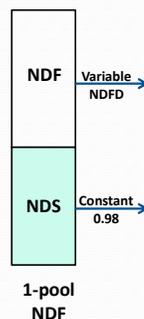
History of Fiber Digestibility

- 4th Transformational Change
- Two IV methods became “standard” methods
 - Both were two-step methods
 - Step 1. Fermentation with ruminal inoculum for 48 h
 - Step 2. Extraction of undigested residues
 - Tilley and Terry (1963) – measured **apparent digestibility**
 - Step 2 was acid pepsin incubation for 48 h
 - Van Soest et al. (1966) or Goering and Van Soest (1970) – measured **true digestibility**
 - Step 2 was ND extraction

History of Fiber Digestibility

- 1950-60s Development of routine in vitro “artificial rumen” digestibilities 4th Transformational Change
- In situ was also developed at this same time, but was adopted more for protein and starch than for fiber rumen degradabilities

Models of NDF Digestion



History of Fiber Digestion Kinetics

- IV methods led to a fundamental change in our concept of fiber **5th Transformational Change**
 - Wilkins (1969) used a 6-day IV to measure “potentially digestible” cellulose
 - Waldo (1969) made the conceptual breakthrough
 - Cellulose not attacked by long-term fermentation should be excluded from a model of cellulose digestion because it is **indigestible cellulose**
 - **Potentially digestible cellulose** might follow first-order kinetics
 - Suggested inability of chemical methods to measure this distinction in cellulose

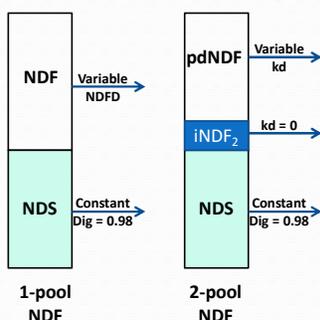
History of Fiber Digestion Kinetics

- Measuring the iNDF concept
 - Smith et al. (1971) reported NDF kd ranging from 0.057 to 0.270/h
 - iNDF can be estimated when pdNDF digestion is >99% complete

kd of pdNDF	Time to 99% digested
0.270/h	17.1 h
0.150/h	30.7 h
0.090/h	51.2 h
0.060/h	76.8 h
0.050/h	92.1 h

uNDF₇₂ appears to be an adequate estimate of iNDF₂ in a 2-pool model

Models of NDF Digestion



History of Fiber Digestion Kinetics

- Measuring the iNDF concept and parameter
 - Mertens (1973, 1977) observed that if iNDF was estimated by uNDF_{144h}, the pdNDF generated curvilinear semilog plots
 - Either pdNDF pool was not first-order or
 - There is more than one pool of pdNDF
 - Lippke (1986) used 6, 7, and 8d fermentations to estimate iNDF
 - Using IS bags with small pores, Huhtanen and coworkers used fermentations of 10d or more

History of Fiber Digestion Kinetics

- **5th Transformational Change**
- 2-pool NDF is a novel concept
 - Important variation in NDF is not related chemical composition, but rather nutritional availability
 - Crucial to estimate the iNDF that has a uniform kd = 0
 - Undigested NDF (uNDF_{xxh}) is measured to estimate iNDF (model parameter)
 - Only the pdNDF has a kd and not total NDF
 - Changes the model for NDFD, but also changes the model of ruminal disappearance (digestion and passage)

History of Fiber Digestion Kinetics

- Measuring the iNDF concept and parameter
 - For slowly digesting pdNDF the time for uNDF to estimate iNDF with minimal contamination increases
 - Krizsan and Huhtanen (2013) also observed that IS uNDF₁₄₄ was > uNDF₂₈₈, but uNDF₂₁₆ was not

kd of pdNDF	Time to 99% digested
0.040/h	115.1 h
0.030/h	153.5 h
0.020/h	230.3 h
0.010/h	460.5 h

Variation in measuring uNDF > 120h, makes it impossible to detect the differences in uNDF between long fermentation times

History of Fiber Digestion Kinetics

- Measuring the iNDF concept
 - In addition to fermentation time, IV or IS technique affect measurement of uNDF>120h
 - Grind size of test samples affects uNDF recovery
 - Porous bags lose small uNDF particles, small pore bags can inhibit digestion
 - Diet of the animal may be important (Van Milgen et al., 1992)
 - Complete collection of IV residues requires filters with small pore ~1 micron (also crucial for lignin determination)
 - Van Soest et al. (2005) argued that (2.4*lignin), which was derived from 60d bio-digester residues, could be used to estimate iNDF in the CNCPS

History of Fiber (Rumen Fill) and Intake

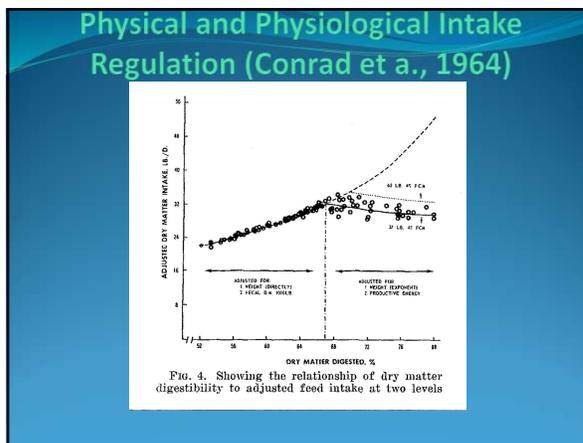
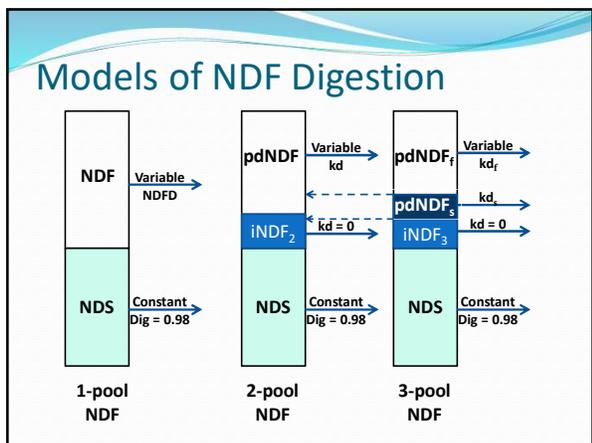
- 6th Transformational Change
 - In his first symposium paper, Van Soest (1965) proposed a relationship between fiber and intake
 - “. . . The only consistent effect that can be observed for all forages is that . . . As this fraction (NDF) increases, voluntary intake declines with an increasingly negative slope.”
 - The concept of “fill” or “ballast” was old, but relating it to fiber was new
 - Part of the confusion about fiber or fill and intake regulation may be related to the observation that two distinct mechanisms regulate intake

History of Fiber Digestion Kinetics

- The 3-pool model of NDF Transformational Change ?
 - Mertens and Ely (1979, 1982) developed a computer model of digestion and passage in the rumen that used a 3-pool model of NDF digestion
 - Raffrenato and Van Amburgh (2010) suggested that if uNDF₂₄₀ is used to estimate iNDF then a 3-pool model of NDF digestion is appropriate
 - Nutritional value of the 3-pool model needs to be determined and is probably related to the size of the slowly digesting pool

History of Fiber (Rumen Fill) and Intake

- 6th Transformational Change
 - Conrad et al. (1964) proposed two primary mechanisms of intake regulation: physical and physiological and derived empirical equations for each
 - At low digestibility, intake was a function of (body weight, undigested residue/BW/d [Fiber effect], and DMD)
 - At high digestibility, intake was a function of (metabolic body size, production and digestibility)
 - It is not a matter **if** fiber affects intake, but **when**



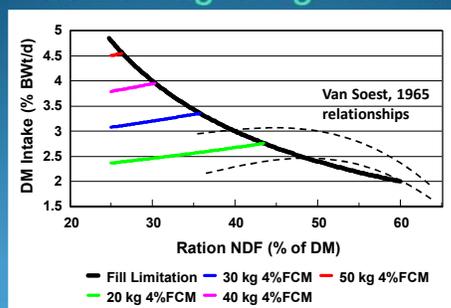
History of Fiber (Rumen Fill) and Intake

- 6th Transformational Change
- Mertens (1985, 1987) used the physical and physiological regulation concepts to derive theoretical relationships based on NDF
 - Physical fill limitation
 - $C = I_f \times F$; C = Fill (NDF) processing constraint (kg/d); I_f = fill limited intake (kg/d) and F = NDF concentration of the diet
 - $I_f = C/F$
 - Fill limited intake is a linear effect of the animal's processing constraint and a reciprocal function of diet NDF

Development of the Fibrousness Concept

- 7th Transformational Change peNDF
- Mertens (1997) clarified these concepts
 - effective NDF (eNDF) = the sum total ability of a feed to replace forage so that milk fat percentage is effectively maintained
 - physically effective NDF (peNDF) = the physical properties of fiber that stimulate chewing activity and a biphasic ruminal environment
 - Neither definition mentions particle size!
 - peNDF concept is being confused by attempts to define it by too many different particle sizing methods

NDF-Energy Intake System Maximizing Forage Intake



History of Fiber Analyses - NIRS

- 8th Transformational Change
- Use of NIRS to estimate fiber concentration and digestibility (undigested NDF) measurements
 - Allows the rapid and economical prediction of NDF and NDFD in its various forms
 - Allows the analysis of millions of samples annually

History of Fiber and Rumen Function

- 7th Transformational Change
- Fiber particle size affects the rumen environment
 - Fiber effectiveness produces two animal responses of interest
 - Two distinct concepts about fiber effectiveness
 - Fibrousness = related to chewing activity (and ruminal function)
 - Effectiveness = related to animal health and performance (milk fat depression in dairy cows)
 - Historically, fibrousness was related to chewing activity and effectiveness was related to milk fat depression

Fiber – Future Transformational Changes Not in Order of Priority

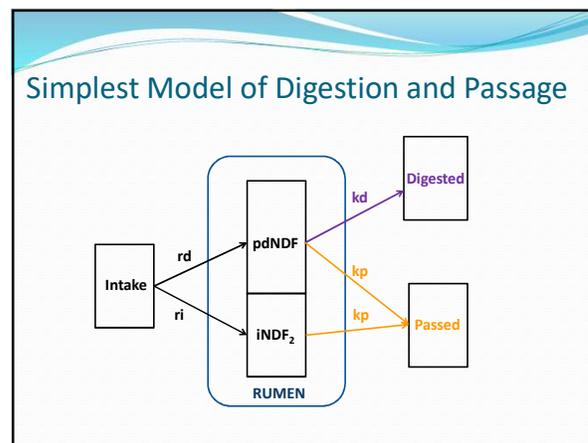
- Improve in vitro methods and applications
- Improvements in NIRS calibration and “big data” applications
- Ability to predict nutrient interactions with fiber (negative impact of starch on fiber digestion)
- Effective and efficient measurement of fiber particle size

Fiber fermentation Future Transformational Changes

Ferraretto et al. (2013) Meta-analysis suggested that starch concentration in the diet was inversely related to ruminal (upper) and total tract NDFD (lower)

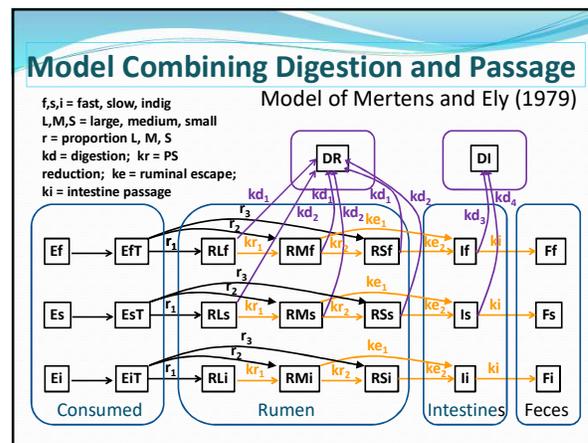
How can we relate changes in total NDFD to kd or iNDF?

Figure 1. Effect of starch concentration of the diet on ruminal and total tract digestibility of dry NDF adjusted for the median effect of total tract digestible area (total NDFD) on ruminal digestibility from the following equation: $y = 14.799x + 1.0287 \times 10^4 - 0.0001x^2$, $r^2 = 0.92$, $n = 70$; total tract digestible area (total NDFD) vs starch concentration: $y = 0.0001x^2 - 0.0241x + 1.0287 \times 10^4$, $r^2 = 0.92$, $n = 70$. Ruminal digestible area (total NDFD) vs starch concentration: $y = 0.0001x^2 - 0.0241x + 1.0287 \times 10^4$, $r^2 = 0.92$, $n = 70$.



Ideal Flow of Fiber in Rumen

Selective retention of large particle and escape of small dense ones



Fiber – Future Transformational Changes

Not in Order of Priority

- Simple models that can relate IV rates to IVV performance where passage occurs
- Simple models of nutrient interactions
- Measurement and use of digestion lag
- Complex models that include both digestion and passage kinetics based on chemical, biological (IV) and particle size analyses

Conclusions

- 1st Transformational change – Proximate analysis and Crude Fiber
 - Useful, but not consistent among feeds
- 2nd Transformational Change – Measurement of ADSL
 - First chemical measurement of a truly indigestible fiber

Conclusions

- **3rd Transformational Change** – Measurement of NDF (total insoluble fiber for ruminants)
 - Feeds are partitioned into NDS with almost complete digestibility and NDF with variable digestibility
- **4th Transformational Change** – Development of routine in vitro “artificial rumen” fiber digestibilities
 - NDFD can be measured routinely to estimate dDM or TDN

Sometimes I think I should be working on Transformational Changes and then



Conclusions

- **5th Transformational Change** – IV methods led to a fundamental change in our concept of fiber
 - Digestion kinetics – NDF consists of two fractions, indigestible and potentially digestible fractions
- **6th Transformational Change** – NDF is related to intake
 - Physical fill (fiber) can limit intake when low energy diets are fed to animals with high energy requirement

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Questions

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Conclusions

- **7th Transformational Change** – peNDF
 - Both chemical NDF and is physical particle size affect rumen function and health
- **8th Transformational Change** – NIRS
 - Rapid and economical analyses are the key to improving ration formulation and animal performance