USDA Agricultural Research Service
U.S. DEPARTMENT OF AGRICULTURE

# Understanding the Digestion of Forage Fiber

U.S. Dairy Forage Research Center

## Why Seek to Understand Forage Fiber Digestion?

Ruminants have the unique ability to utilize fiber derived from plant cell walls, largely by means of microbial action within the anaerobic environment of the rumen. Unfortunately, the ingestion of plant cell walls also can limit the production potential of domesticated livestock because fiber consumption contributes to gut fill, which suppresses intake. Further, fiber is incompletely digested and has structural volume of varying rigidities compared to the associated cell solubles that are (in contrast) highly digestible. The incomplete digestion of forage fiber, as well as the impact of its structural volume on gut fill, serve to restrict the energy available to the animal.

To illustrate the incomplete nature of forage fiber digestion, consider Lucas tests for 19 forages (11 grasses, 8 legumes) as summarized by Van Soest (1967, 1982). For a Lucas test, the digestible amount of the forage component is regressed linearly against the concentration of that same entity in the forage. The resulting slope of the regression line is an estimate of true digestibility, while the negative intercept is a constant representing the metabolic and endogenous matter excreted in the feces. As summarized in Figure 1, the Lucas test for cell solubles yielded a slope of 0.98, which indicates cell solubles are an 'ideal' or 'uniform' fraction that is completely digestible, irrespective of its concentration within the forage. In contrast, the same tests for insoluble residual forage fiber (**NDF**) resulted in regression slopes of 0.46 (grasses) and 0.60 (legumes), thereby indicating non-uniformity or incomplete availability to the ruminant animal. This is especially important because forage fiber, as defined on the basis of insolubility in neutral detergent solution, can (generally) comprise between 20 and 80% of the total forage dry matter (**DM**).

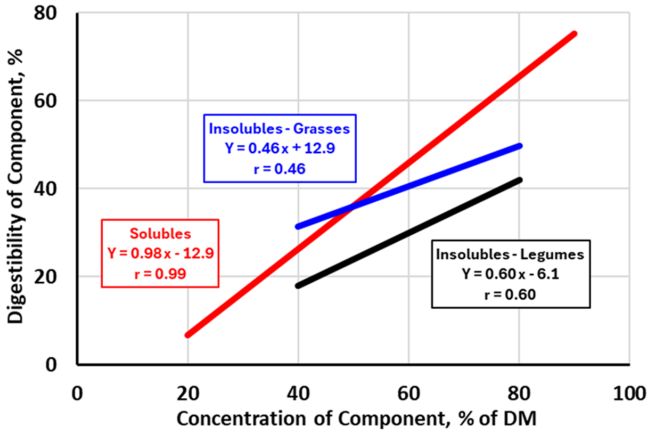


*Figure 1. Lucas tests for neutral detergent solubles (—), neutral detergent insolubles of grasses (—), and neutral detergent insolubles of legumes (—), where insolubles generally represent forage fiber [adapted from Van Soest (1967, 1982)].*

***Note:*** *The terms "cell wall" and "fiber" are sometimes used interchangeably, but this is inappropriate within the context of ruminant digestion. "Cell wall" includes pectin, cellulose, hemicellulose, lignin, some ash, and varying amounts of crude protein (**CP**). Unlike the other components of the cell wall, pectin is uniquely soluble in neutral detergent and completely digestible. For purposes of the ensuing discussions, references to "fiber," "residual fiber," or "neutral detergent fiber (**NDF**)" are nutritionally related. They are specifically equivalent to the residue remaining after extraction in neutral detergent solution. These residues following extraction in neutral detergent include cellulose, hemicellulose, lignin, ash, and some CP, but not pectin. The varying amounts of CP retained within residual NDF residues can be profoundly affected by the inclusion of sodium sulfite within neutral detergent extraction procedures.*

The structural volume of forage fiber also may affect the energy available to the animal consuming the forage. In part, this occurs by its potentially limiting effect on voluntary intake. In his popular textbook, Van Soest (1982) describes this effect by using an analogy of a skyscraper being demolished; the contents of the building, including furniture, doors, non-load-bearing walls, etc., can be removed prior to demolition, but the structural volume of the building does not change until the building collapses in direct response to a wrecking ball or explosive demolition. The process of rumination has a similar effect on forage fiber, reducing structural volume, thereby fulfilling a critical role in digestion and particulate clearance (passage) from the rumen. External processing of forages (chopping or grinding) has a similar effect and typically reduces rumination time; it also exhibits a positive effect on voluntary intake, primarily in response to the disruption or collapsing of structural cell volume. As an example, dynamic model simulations conducted by Mertens and Ely (1979) suggested that maximum DM intake (**DMI**) of alfalfa and Coastal bermudagrass hays could be increased by >45% (% of bodyweight basis), by the combined processes of grinding and pelleting compared to offering the hays in long-stem form.

### *Growing Dairy Heifers*

A practical example of the effects of forage fiber on voluntary DMI has been illustrated by Hoffman et al. (2008) for Holstein and Holstein × Jersey crossbred dairy heifers offered diets comprised primarily of forages for ad-libitum intakes. Data were compiled from 6,174 and 3,101 daily pen intakes for the Holstein and crossbred heifers (Figures 2 and 3, respectively). In this analysis, the bodyweights of heifers ranged from about 360 to 1400 lbs for Holstein heifers and 350 to 1300 lbs for the crossbreds; therefore, diets reflected the greater energy requirements of younger heifers that were lowered as the heifers grew, primarily by increasing the concentration of NDF within the diet. A prominent observation in this analysis was that NDF intake was relatively static at approximately 1% of bodyweight, regardless of the size of the heifers. There was a slight decline from this threshold for heifers weighing > 1100 lbs, which may have been associated with reduced gut capacity in response to displacement by the developing fetus. While not conclusive, these data conform generally to theories of gut fill intake regulation, as voluntary DMI declined with increasing concentrations of NDF in the diet.

These concepts also can be used to intentionally limit or constrain weigh gains in dairy heifers to normally recommended levels (1.8 to 2.0 lbs/day for Holstein heifers), thereby preventing undesirable over-conditioning. This becomes especially relevant for pregnant heifers with lower energy requirements, particularly when they consume diets containing corn silage that is often readily available, but too energy dense. In another pen-based study conducted at Marshfield, Wisconsin, a control alfalfa-corn silage diet was diluted with eastern gamagrass silage at rates of 9, 18, or 27% of the total dietary DM,

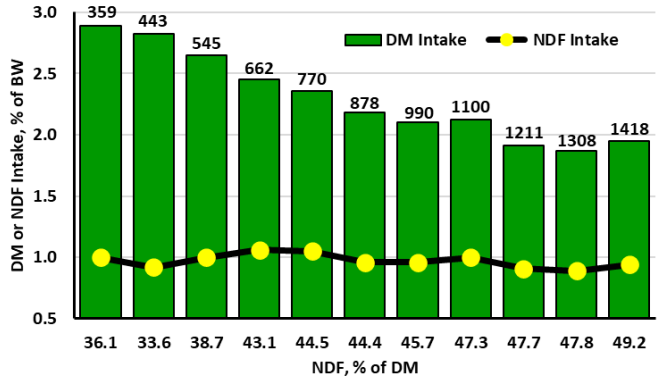


*Figure 2. Declining voluntary DM intakes by Holstein heifers as affected by the concentration of NDF within the diet. Data suggests voluntary DM intakes are constrained by gut fill when NDF intake is approximately 1% of bodyweight. Data labels indicate the mean weight of heifers in each grouping [adapted from Hoffman et al. (2008)].*

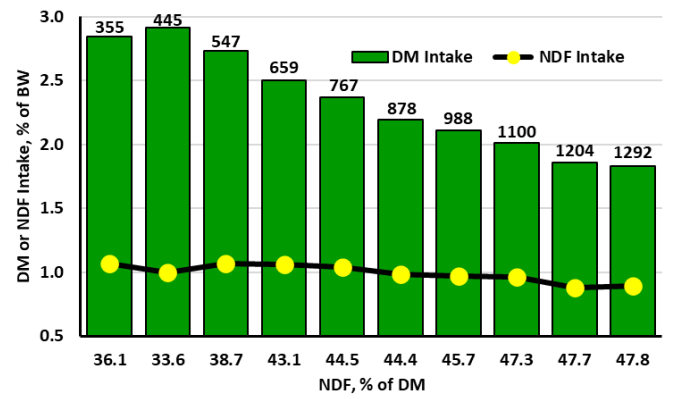


*Figure 3. Declining voluntary DM intakes by Holstein × Jersey crossbred heifers as affected by the concentration of NDF within the diet. Data suggests voluntary DM intakes are constrained by gut fill when NDF intake is approximately 1% of bodyweight. Data labels indicate the mean weight of heifers in each grouping [adapted from Hoffman et al. (2008)].*

yielding dietary NDF concentrations of 39.6, 43.0, 45.6, and 48.7% for the control and three diluted diets, respectively (Coblentz et al., 2012). Eastern gamagrass is a tall-growing, perennial warm-season bunch grass whose NDF concentration was 69.7% in this experiment. As such, it was an ideal forage for adding fiber and diluting energy density within the diets. Voluntary NDF intakes for the alfalfa-corn silage control diet were 0.88% of bodyweight, indicating they may not have been entirely regulated by gut fill, but heifers still gained 2.4 lbs/day, which exceeds normal recommended targets by 0.4 to 0.6 lbs/day. The inclusion of eastern gamagrass silage reduced overall DMI; furthermore, NDF intakes increased to 1.04% of bodyweight at the 27% dilution rate (Figure 4). At that inclusion rate, average daily gains (**ADG**) were reduced to within the normal recommended range (1.87 lbs/day) for Holstein dairy heifers.

A similarly designed trial was conducted in the same research facility, where pregnant dairy heifers were again offered a control alfalfa-corn silage diet that was diluted with either chopped corn fodder, chopped wheat straw, or eastern gamagrass silage (Coblentz et al., 2015). Inclusion rates ranged from about 15 to 26%, depending on the dilutant, yielding dietary concentrations of NDF of 43.3, 50.4, 50.9, and 53.3% for the control, corn fodder, wheat straw, and eastern gamagrass diets, respectively (Figure 5). Dry matter intakes were reduced by all forms of dilution, and NDF intakes were limited at 0.96 to 1.02% of bodyweight in all diluted diets, which is consistent with the observations of Hoffman et al. (2008) illustrated previously in Figures 2 and 3. Intakes of NDF were less for the control diet (0.89% of bodyweight), again suggesting intake was not entirely regulated by gut fill. The use of dilution was evident from the reported ADG, where heifers gained 2.6 lbs/day on the control diet, but gains were reduced to 1.7 to 2.2 lbs/day by dilution with high-fiber forages.

### *Lactating Cows*

Compared to lactating cows, the previous discussions addressing the effects of dietary NDF on voluntary DMI by growing dairy heifers are simplistic examples. The rather one-dimensional nature of that general discussion can be largely attributed to the forage-based nature of the diets that were always offered for ad-libitum intakes. Typically, the assessment of the role NDF plays in regulating the DMI by lactating cows is far more complicated because of the greater nutrient demands of these animals, as well as a myriad of animal/physiologically related factors that are involved in regulating the DMI of high-producing cows. It is beyond the scope of this research synopsis to address the animal-related aspects of DMI

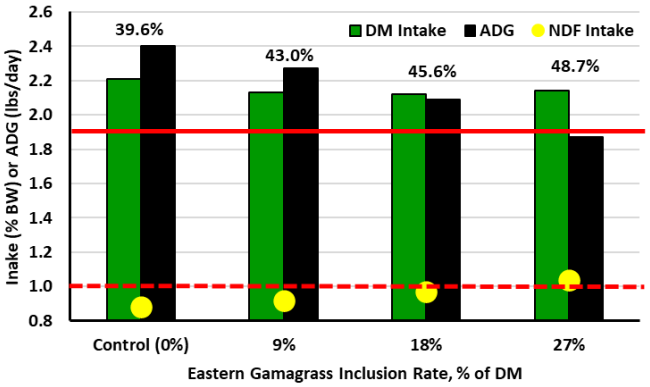


*Figure 4. Dry matter and NDF intakes by pregnant dairy heifers for an alfalfa-corn silage control diet diluted with eastern gamagrass at rates of 9, 18, or 27% of dietary DM showing dilution effects on reducing average daily gains (ADG) and avoiding over-conditioning. The solid horizontal line (—) represents a normal ADG target for Holstein dairy heifers, and the horizontal hashed line (- -) indicates the NDF intake constraint of 1% of bodyweight for dairy heifers suggested by Hoffman et al. (2008). Data labels indicate the concentration of NDF within each diet [adapted from Coblentz et al. (2012)].*

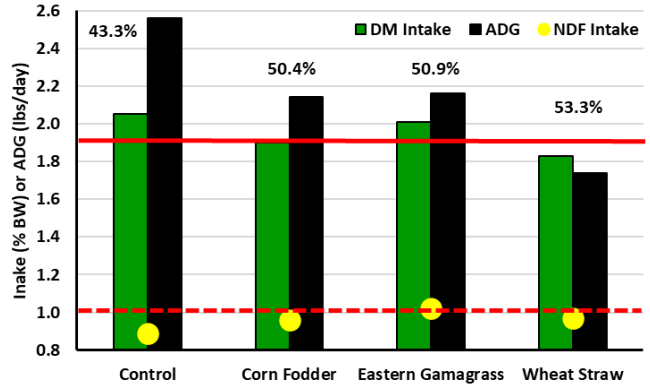


*Figure 5. Dry matter and NDF intakes by pregnant dairy heifers offered an alfalfa-corn silage control diet diluted with corn fodder, eastern gamagrass silage, or wheat straw at rates ranging from 15 to 26% of dietary DM, thereby showing dilution effects on reducing average daily gains (ADG) and avoiding over-conditioning. The solid horizontal line (—) represents a normal ADG target for Holstein dairy heifers, and the horizontal hashed line (- -) indicates the NDF intake constraint of 1% of bodyweight for dairy heifers suggested by Hoffman et al. (2008). Data labels indicate the NDF concentration within each diet [adapted from Coblentz et al. (2015)].*

regulation, which are discussed and referenced in detail within the recently published model of dairy nutrition (NASEM, 2021). However, numerous dietary or ration-based factors also prominently affect DMI by lactating cows. Some of these include forage processing (long-stem vs. chopped or ground forages), dietary NDF concentrations (both total and specifically from forage), digestion rate of NDF, fragility of NDF, rates of ruminal clearance, and the general conditions within the fermentation environment (e.g., ruminal pH).

Obviously, the best way to provide the needed additional energy for high-producing dairy cows is to replace portions of fiber in the diet with energy-dense concentrates. It is well known that this solution also has practical limits, perhaps most notably from the perspective of avoiding various livestock health disorders. Generally, this complicates the direct relationships between concentrations of dietary NDF and subsequent DMI by lactating cows because DMI can ultimately be limited by either energy or fill constraints.

Many years ago, Mertens (1994) described the complexity of this relationship in what was referred to as the NDF-Energy Intake System. A simplified illustration of this system is provided in Figure 6. In that review, the author noted that previous research literature had failed to establish a clear relationship between NDF and DMI for use in ration formulation or forage evaluation. However, those observations did not consider the biphasic nature of DMI constraints as described by the NDF-Energy Intake System, in which either energy demand by the cow

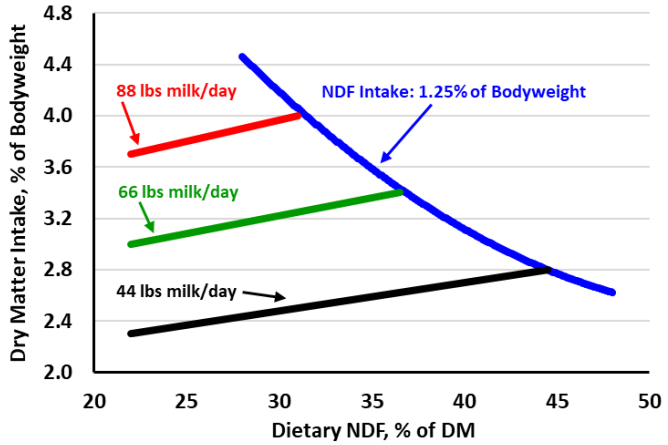


*Figure 6. Simplified NDF-Energy Intake System described by Mertens (1994), where red, green, and black lines represent DM intake constraints based on energy, and the blue line represents intake constraint based on rumen fill.*

or fill are considered first limiting factors. In simplest terms, when the energy needs or demands of the animal have been met, energy limits or restrains DMI, and in those situations, dietary NDF and DMI are positively correlated. Conversely, when fill restricts DMI, NDF and DMI are negatively correlated. The author suggested that gut fill is the controlling factor when daily NDF intake reaches 1.1 to 1.3% of bodyweight, and that maximum DMI occurs at daily NDF intakes of about 1.25% of bodyweight. From Figure 6, it is easy to discern that as milk production increases, dietary NDF must be reduced to maximize DMI.

As noted by Mertens (1994), this simple model may not predict DMI in all situations; specific exceptions were noted when finely ground wet corn gluten feeds were included in the diet that likely did not have the same filling effect as long forages per unit of NDF. This simple model does effectively illustrate when DMI is limited by fill, as well as the significant role NDF plays in regulating DMI in lactating cows. Currently, the significant roles NDF and its associated digestibility (neutral detergent fiber digestibility; **NDFD**) play in intake regulation are illustrated by the ration-based equation for predicting DMI by Holstein cows more than 60 days postpartum (NASEM, 2021), which is as follows:

DMI = 12.0 – (0.107 × forageNDF) + (8.17 × ADF/NDF) + (0.0253 × forageNDFD) – [0.328 × (ADF/NDF – 0.602) × (forageNDFD - 48.3)] + (0.225 × MilkYield) + [0.00390 × (forageNDFD – 48.3) × (MilkYield – 33.1)

In this expression:

i. DMI is expressed in kilograms DM/day;
ii. forageNDF is the forage NDF content of the diet, expressed as a percentage;
iii. ADF/NDF is a proxy for forage fragility, expressed as a fraction;
iv. forageNDFD is the digestibility of forage NDF measured by in vitro or in situ methods, and reported as a percentage; and
v. MilkYield is the yield of milk expressed as kilograms/day.

In part, the expression was restricted to appropriate use only after 60 days postpartum because it was assumed that DMI is likely dominated by metabolic mechanisms (rather than distension) prior to that point.

## Measuring NDF Digestion Kinetics

### *Overview*

The kinetics of NDF digestion can be measured using either in vitro or in situ methodologies, where the final step is extraction in neutral detergent. (*Note: Some evaluations are made on an ash-free basis, which requires an additional ashing procedure.*) The final extraction step in neutral detergent also conveniently removes microbial debris following fermentation (Goering and Van Soest, 1970). In vitro methods can be conducted traditionally, where a single sample is incubated in buffered rumen fluid within a dedicated tube or flask (**CONV**). Although subject to frequent modifications, these procedures have been described in numerous publications dating back more than 60 years (Tilley and Terry, 1963; Goering and Van Soest, 1970). Alternatively, batch procedures pioneered primarily by the ANKOM Technology Corporation (**ANKOM**; Macedon, NY) incubate about 25 samples simultaneously. Samples are secured within porous fiber bags and then incubated within large, sealed glass jars that are agitated (rotated) continuously at 39°C (102°F). Procedures for this approach can be obtained from the manufacturer's website.[1] As might be expected, each approach has advantages and disadvantages. In either system, incubations can be terminated at specific time points, thereby permitting establishment of a decay curve that forms the basis of subsequent kinetic calculations. It also is quite common to measure fiber disappearance (NDFD) at single, specific time points, most commonly after a 48-hour incubation.

In situ procedures also can be used for these same purposes. Usually, ground forage samples are sealed in 10 × 20-cm Dacron bags and suspended into the ventral rumen of cannulated heifers, cows, or steers for predetermined time intervals; partially digested samples either can be withdrawn over time to establish a decay curve, or withdrawn at specific, predetermined time points (e.g., 48 hours, etc.). Unlike most in vitro assessments, in situ decay curves can be established by inserting all samples into the rumen at once and then withdrawing them over time, or by using a staggered insertion approach followed by a common removal time. In either case, in situ bags must be washed (often in a commercial washing machine) to remove solid debris. Microbial debris is effectively removed by the required terminal extraction in neutral detergent. It is important to emphasize that both in vitro and in situ procedures for determining NDFD or any associated kinetics of digestion are generally not standardized, and many procedural variations exist across laboratories.

### *Some General Advantages/Disadvantages of In Vitro and In Situ Methods*

In an extensive review of kinetics of cell wall digestion, Mertens (1993) stated that accurate measurement of the rate of digestion of any substrate requires two conditions:

- the substrate must be a single homogeneous pool and
- only the characteristics of the substrate itself can limit digestion.

Meeting the first condition is problematic for kinetic assessments of digestion/disappearance of DM, organic matter (**OM**), or protein because procedures to remove microbial debris from incubated forage residues are either cumbersome, impractical, or unavailable. This specific problem is avoided in determinations of NDF digestion by the terminal extraction of undigested residues in neutral detergent, which effectively separates residual forage fiber from the microbial debris associated with fermentation. The first condition also necessitates removal (subtraction) of any indigestible portion of the substrate prior to the subsequent calculation of rate kinetics. The second condition identified by Mertens (1993) clearly implies that the fermentation environment itself (in vitro or in situ) cannot be the limiting factor in digestion. Potentially, many factors could violate the second condition; some examples may include inadequate protein or N, suboptimal pH, compromised anaerobic conditions, poor techniques in obtaining/handling rumen inoculum, and many others. Unfortunately, it is not uncommon for in vitro or in situ methods used in specific laboratories to be suboptimal in some of these respects.

One advantage of in situ methodology is that assessment of kinetics is not unlinked or removed from the natural ruminal environment established by the cannulated animal. Intuitively, this advantage

[1] *See: https://www.ankom.com/sites/default/files/2024-08/Method_3_InVitro_D200_D200I.pdf.*

seems particularly attractive, but it also has significant potential limitations, particularly with respect to violation of the second condition proposed by Mertens (1993). In situ evaluations of NDF digestion kinetics are subject to animal-to-animal variation, and may be affected by feeding/eating patterns, or simply whether the basal diet is offered for controlled vs. ad-libitum intakes. Furthermore, some of these violations of the second condition can be injected into kinetic evaluations intentionally, depending on whether the evaluation is intended to be strictly intrinsic or modified by extrinsic objectives. For example, offering basal diets appropriate for a non-lactating beef cow or a lactating dairy cow may be desirable/appropriate to address different targeted research objectives, but neither may be truly optimal for fiber digestion, thereby potentially violating Merten's second condition. In addition, the necessary porous nature of 10 × 20-cm Dacron bags (typically, 50 ± 10 µm) may permit passage of some fine particles into or out of in situ bags during ruminal incubation and/or post-incubation washing, thereby presenting a potential source of error.

A major advantage of in vitro procedures is the ability to exert close control over incubation conditions, which is desirable for measuring intrinsic kinetics of digestion and complying with Merten's second condition. Unlike in situ methodologies, there is no potential for particle loss or movement through porous Dacron bags with CONV in vitro procedures, where loose forage particles are incubated in dedicated flasks. However, there have been recurring concerns about potential particle loss through porous F57 fiber bags with ANKOM procedures (Vogel et al., 1999; Hall and Mertens, 2023) that may be linked (in part) to sample preparation (grinding) methods. A somewhat intuitive disadvantage of in vitro procedures is they are one step removed from the functioning ruminal environment of an animal. In particular, procedures associated with obtaining and handling ruminal inoculum must be carefully monitored to avoid shock to the inoculum. Failure to obtain and/or handle rumen fluid properly often results in greater error between assays, especially at early time intervals. Acknowledgment of this general problem has led to some recent efforts to prime ruminal inoculum prior to assessing NDFD by in vitro methods (Goeser and Combs, 2009; Goeser et al., 2009).

### *Understanding and Defining Kinetic Parameters.*

The underlying assumption in calculation of NDF digestion kinetics is that they follow a first-order model, where the substrate digested at any time is proportional to the amount of potentially digestible matter remaining at that time (Mertens, 1993). Any discussion of kinetics requires a description/definition of the various parameters calculated in association with the first-order kinetic model, which are illustrated in Figure 7 for a regrowth cutting of orchardgrass hay harvested in Arkansas (Ogden et al., 2005).

**Fraction A.** Normally, fraction A is defined as that portion of a forage component that disappears at a rate too fast to be measured, generally meaning it cannot be recovered at 0 hours of incubation. Quantifying fraction A is very important in kinetic assessments of some relevant parts of the forage, such as DM, OM, or CP, where fraction A can approach or exceed 50% of the total amount present. One may wonder how any forage or feedstuff sample could be incubated for 0 hours; however, many kinetic assessments actually include a pseudo-0-hour type of incubation. For example, a 0-hour incubation by in situ methods may include both the common wetting (soaking) in tepid water that usually precedes insertion into the rumen, as well as the hand or machine rinsing that is necessary following ruminal incubation; however, these 0-hour samples are never

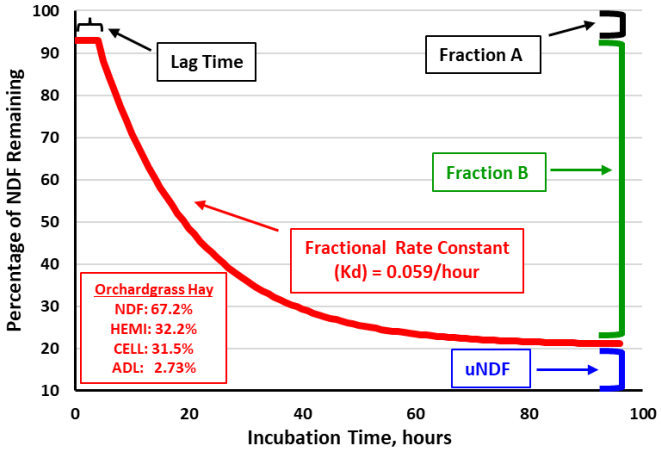


*Figure 7. In situ fiber (NDF) digestion as a function of ruminal incubation time as fitted by nonlinear regression that illustrates various parameters typically fitted by kinetic analysis. Abbreviations for fiber components are: HEMI, hemicellulose; CELL, cellulose; and ADL, acid detergent lignin. The nonlinear regression curve depicts NDF digestion of a regrowth cutting of orchardgrass hay harvested in northern Arkansas [adapted from Ogden et al. (2005)].*

actually inserted into the rumen or subjected to rumen fluid.

Theoretically, forage fiber (measured as insolubility in neutral detergent) is insoluble in water and should be recovered in its entirety at 0 hours of incubation. Although fraction A should theoretically be nil in assessments of NDF degradation kinetics, there are common situations where there can be incomplete recovery at 0 hours. This can be observed for the orchardgrass hay shown in Figure 7, where 7.1% of the NDF (y-intercept = 92.9%) could not be recovered at 0 hours using in situ analysis procedures. In some studies, this anomaly is particularly evident for immature cool-season grasses as noted by Hoffman et al., 1993 and Coblentz et al., 2018, but may subside or disappear entirely as grasses mature, as shown in Figure 8. It remains somewhat unclear why this inconsistency occurs, but it usually comprises a small percentage of the total NDF (< 10%). Recently, Ghimire and Ferreira (2026) have associated this anomaly with losses of fine particles from porous filter bags as affected by grinding method, bag preparation, and/or washing procedures. These scientists hypothesized that the detection of fraction A within the context of NDF degradation kinetics was an artifact of methodology, rather than an intrinsic property of the forage itself. Regardless, from a strict mathematical perspective, any portion of NDF measured or calculated as fraction A is assumed to be digestible, but also makes no contribution to the calculation of a fractional rate constant (**Kd**).

**Fraction B.** In simplest terms, fraction B is that percentage of NDF that is digested at a measurable rate. Any calculation of Kd is based on the digestion of this specific fraction alone. Figure 8 illustrates the changing percentages of various NDF fractions within triticale forages as a function of their growth stage at harvest. It should be obvious that NDF fractions must sum to 100%, and that fraction B declines with plant maturity, specifically in response to increasing proportions of undigestible NDF (**uNDF**) within the forage. As such, fraction B is equal to 100% - uNDF. If fraction A is measured or calculated, it also should be subtracted because it does not contribute mathematically to calculation of Kd. A potential extent of NDF digestion (**pdNDF**) is reported in some studies, which is either equivalent to fraction B or, if fraction A is determined, to fractions B + A. It should also be emphasized that many kinetic assessments of fiber digestion are based on pdNDF, where any potential for fraction A is assumed or measured to be (statistically) nil. Most importantly, since estimation of Kd is specific to fraction B only, an appropriate and accurate determination of uNDF is critical.

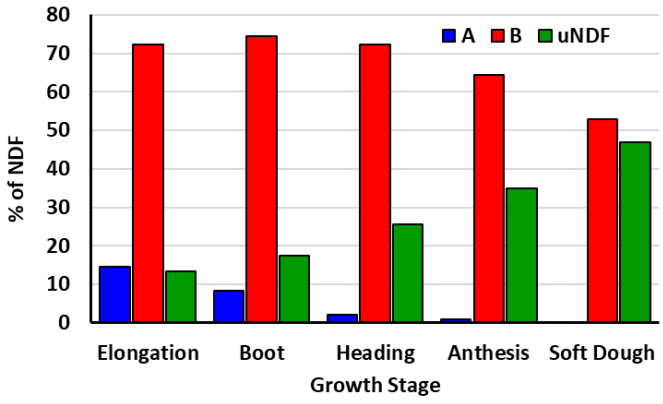


*Figure 8. Example of NDF fractions obtained from in vitro assays of triticale forages as determined by a nonlinear two-pool regression model [adapted from Coblentz et al. (2018)].*

**Undigestible NDF (uNDF).** The absolute percentage of forage fiber that is never capable of being digested by dairy cows can be described as indigestible NDF. However, in practice, this portion of the total fiber matrix must be estimated from the residual fiber remaining after various in situ or in vitro incubation intervals, and referred to as undigestible fiber or NDF (uNDF). Accurate determination of uNDF has been the subject of some controversy and continuing refinement for many years, in part because of its critical role is determining any estimation of digestion rate (Kd). Waldo (1969) is often credited with hypothesizing that cellulose digestion might follow a first-order kinetic model if undigestible residues were subtracted prior to making any calculations leading to quantification of Kd. Subsequently, Smith et al. (1971, 1972) used 72-hour in vitro incubation procedures to quantify undigestible cell walls for a variety of legumes and cool-season grasses, concluding that digestion approached an asymptote at (or before) that termination point, and that digestion was then essentially complete. More recently, Mertens (1993) offered further refinement by summarizing that a 72-hour incubation was satisfactory for determining uNDF if lag time is < 6 hours and Kd > 0.06/hour. If the calculated lag time is > 6 hours and Kd is < 0.06/hour, then a 96-hour incubation might be most appropriate for estimating

uNDF. Other approaches also have been used to quantify uNDF, such as 2.4 × lignin/NDF (Chandler et al., 1980; Van Soest et al., 2000; Van Amburgh and Van Soest, 2003) or the surface-area relationship with lignin (usually assessed as acid detergent lignin; **ADL**) used by NASEM (2001).

Currently, more extended in situ or in vitro incubation times are typically used to estimate uNDF; these often range from 120 to 288 hours (Bender et al., 2016), but are most commonly assessed at about 240 hours (Grant, 2015; Raffrenato et al., 2018; Rafrennato et al., 2019; Valentine et al., 2019). An example summarizing the effects of incubation times on uNDF based on the work of Raffrenato et al. (2018) is illustrated in Figure 9. In that example, the means obtained from 12 diverse forages at extended 240- and 504-hour incubation times did not differ ($P = 0.46$). Furthermore, the use of extended incubation times, such as those at 240 hours, has led to recent speculation that NDF digestion may follow a more complicated three-pool model that includes both fast and slowly digested pools (Coblentz et al., 2018; Raffenato et al., 2019; Barry and Hall, 2025).

It should be noted that determination of uNDF has implications for optimizing a cow's response to dietary forages that go well beyond an accurate determination of Kd. Grant (2015) emphasized that while NDF is a non-uniform or non-ideal feed fraction comprised of various pools, uNDF is a uniform or ideal feed fraction whose digestibility

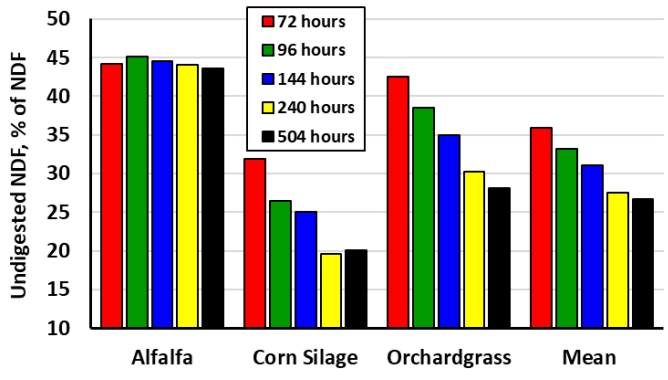


*Figure 9. Undigested NDF (% of NDF) for alfalfa, conventional corn silage, and orchardgrass after 72, 96, 144, 240, or 504 hours of in vitro incubation. The category variable designated as 'mean' on the x-axis represents the mean of 12 forages; on this basis, 240 and 504-hour averages did not differ (P = 0.46). Analytically, NDF was determined with a-amylase, sodium sulfite, and corrected for residual ash [adapted from Raffrenato et al. (2018)].*

coefficient is essentially nil. As such, uNDF is a fiber fraction that influences physical effectiveness, gut fill, and digestion/passage dynamics. The author further postulated that uNDF can be viewed as a 'baseline' of fill that constrains NDF flux, where maximum and minimum amounts of uNDF avoid limits on voluntary intake and maintain proper rumen function/health, respectively.

**Calculation of Digestion Rate.** The two-pool model for fiber digestion following first-order kinetics can be calculated by two common methods: i) natural log transformation of the percentage of fraction B remaining at each incubation time (**ln-linear**); or ii) nonlinear regression. Examples of both approaches will be described subsequently in some detail. While the kinetic parameter estimates may vary between methods for a wide variety of reasons related to computational and statistical differences, Mertens (1993) concluded that both approaches provide acceptable estimates of kinetic parameters provided they are used with valid mathematical descriptions of the decay model and accurate data collected from well-designed experiments.

It should be noted that the determination of a measurable fraction A within the specific context of digestion kinetics of pdNDF raises some potentially contradictory issues. Typically, a discrete lag time is associated with digestion of fiber, which is generally defined as the time interval (hours) required before disappearance of NDF actually begins. Theoretically, NDF fibers are insoluble in water, and should not demonstrate in situ or in vitro disappearance after a pseudo-0-hour incubation. Furthermore, regardless of whether a measurable and statistically relevant fraction A is an artifact of experimental methodology, intrinsic to the forage itself, or some combination of both possibilities, it must be accounted for mathematically in any subsequent calculations of Kd. This issue also begs obvious theoretically and biologically relevant questions, such as how can there be significant disappearance of pdNDF at 0 hours, but still a clear, calculated discrete lag time before further disappearance occurs? Further study, such as that provided by Ghimire and Ferreira (2026), is needed to best establish appropriate procedures for reconciling these methodological/computational issues.

*ln-Linear Method*. The ln-linear method offers a major advantage over nonlinear regression because all calculations can be satisfactorily performed with a good spreadsheet program, such as (but not limited to) the EXCEL product offered by Microsoft Corporation (Redmond, WA). To examine the ln-linear method, consider the data presented in Tables 1 and 2, which respectively summarize in vitro digestions of NDF for triticale forages harvested at the boot and soft-dough stages of growth at Marshfield, Wisconsin (Coblentz et al., 2018). Data are averaged across 5 field replications, each of

*Table 1. Summary of NDF in vitro digestion statistics and calculations for triticale forages harvested at boot stage at Marshfield, Wisconsin [adapted from Coblentz et al. (2018)].*

| Incubation Time | Raw NDF Remaining | Raw NDF Remaining - U | | ln (Raw NDF Remaining – U) | |
|---|---|---|---|---|---|
| | | @ $U_{72}$[1] | @ $U_{240}$[2] | @ $U_{72}$[1] | @ $U_{240}$[2] |
| hours | ---------- *% of NDF* ---------- | | | --------- *ln (% of NDF)* --------- | |
| 0 | 91.80 | 71.06 | 76.22 | 4.264 | 4.334 |
| 3 | 93.22 | 72.48 | 77.64 | 4.283 | 4.352 |
| 6 | 90.41 | 69.67 | 74.83 | 4.244 | 4.315 |
| 9 | 84.29 | 63.55 | 68.71 | 4.152 | 4.230 |
| 12 | 75.78 | 55.04 | 60.20 | 4.008 | 4.098 |
| 18 | 56.53 | 35.79 | 40.95 | 3.578 | 3.712 |
| 24 | 43.99 | 23.25 | 28.41 | 3.146 | 3.347 |
| 36 | 31.00 | 10.26 | 15.42 | 2.329 | 2.736 |
| 48 | 25.32 | 4.58 | 9.74 | 1.521 | 2.276 |
| 72 | 20.74 | . . . | 5.16 | . . . | 1.641 |
| 96 | 18.60 | . . . | 3.02 | . . . | 1.107 |
| 144 | 16.62 | . . . | 1.04 | . . . | 0.035 |
| 240 | 15.58 | . . . | . . . | . . . | . . . |

[1] *Undigested NDF determined after 72 hours of in vitro incubation.*
[2] *Undigested NDF determined after 240 hours of in vitro incubation.*

*Table 2. Summary of NDF in vitro digestion statistics and calculations for triticale forages harvested at the soft dough stage at Marshfield, Wisconsin [adapted from Coblentz et al. (2018)].*

| Incubation Time | Raw NDF Remaining | Raw NDF Remaining - U | | ln (Raw NDF Remaining – U) | |
|---|---|---|---|---|---|
| | | @ $U_{72}$[1] | @ $U_{240}$[2] | @ $U_{72}$[1] | @ $U_{240}$[2] |
| hours | ---------- *% of NDF* ---------- | | | --------- *ln (% of NDF)* --------- | |
| 0 | 100.0 | 44.93 | 55.69 | 3.805 | 4.020 |
| 3 | 100.0 | 44.93 | 55.69 | 3.805 | 4.020 |
| 6 | 99.46 | 44.39 | 55.15 | 3.793 | 4.010 |
| 9 | 96.17 | 41.10 | 51.86 | 3.716 | 3.949 |
| 12 | 93.04 | 37.97 | 48.73 | 3.637 | 3.886 |
| 18 | 83.86 | 28.79 | 39.55 | 3.360 | 3.678 |
| 24 | 77.18 | 22.11 | 32.87 | 3.096 | 3.493 |
| 36 | 65.88 | 10.81 | 21.57 | 2.380 | 3.071 |
| 48 | 61.52 | 6.45 | 17.21 | 1.863 | 2.845 |
| 72 | 55.07 | . . . | 10.76 | . . . | 2.375 |
| 96 | 52.05 | . . . | 7.74 | . . . | 2.046 |
| 144 | 48.22 | . . . | 3.91 | . . . | 1.364 |
| 240 | 44.31 | . . . | . . . | . . . | . . . |

[1] *Undigested NDF determined after 72 hours of in vitro incubation.*
[2] *Undigested NDF determined after 240 hours of in vitro incubation.*

which was assessed 4 times (independently) by in vitro methods using ANKOM procedures with F57 filter bags. In addition, initial and residual NDF concentrations were corrected for residual ash.

The first step noted in Tables 1 and 2 is the calculation of the percentage of NDF remaining at each incubation time. Please note that for boot-stage triticale (Table 1), there is incomplete recovery of NDF at the 0-hour time point, which also occurs at the subsequent 3- or 6-hour time points before digestion begins. As a result, fraction A is calculated as: 100% - 91.8% = 8.2%, and is not included in subsequent rate calculations. Note also that NDF is completely recovered at 0 hours for the triticale harvested at the soft-dough stage of growth (fraction A = 0; Table 2).

One disadvantage of using the ln-linear method for calculating rate kinetics is that defining various parameters can be somewhat arbitrary. One such prominent issue is defining when digestion actually begins; technically, any point occurring before digestion is initiated should be considered as part of the discrete lag time and should not be included in any calculation of Kd. For the example in Table 1, this is relatively easy to discern, as the mean response at 0-, 3-, and 6-hour time points is identical to that obtained at 0 hours (91.8%), and digestion has clearly begun by 9 hours when the percentage of NDF remaining has decreased to 84.3%. Years ago, Mertens and Loften (1980) described the effects of including 0-hour time points (before digestion begins) in assessments of in vitro digestion kinetics of pdNDF for tall fescue hay, concluding that doing so subsequently slowed the regression-based estimate of Kd, reduced calculated lag time, and increased computational residuals and standard errors.

The second step required in these examples is defining and subtracting out uNDF, since it is not part of pdNDF. For purposes of these examples, uNDF is defined as the portion of NDF remaining after 72 hours of incubation (**$uNDF_{72}$**; Smith et al., 1971, 1972), or as the (now common) NDF remaining after an extended 240-hour incubation (**$uNDF_{240}$**). For the boot-stage triticale forages, $uNDF_{72}$ and $uNDF_{240}$ are not identical, representing 20.7 and 15.6% of NDF, respectively, suggesting digestion of NDF was not complete after 72 hours of incubation. After mathematically eliminating $uNDF_{72}$ or $uNDF_{240}$, the remaining percentages of residual NDF are subjected to a natural-log transformation, which then can be regressed against incubation time to yield a slope equivalent to Kd (Figures 10 and 11). Sample calculations are provided below for both triticale forages using $uNDF_{72}$ as an estimate of undigestible NDF.

***Boot-Stage Triticale Using $uNDF_{72}$:***

Fraction A = 100% - 91.8% = 8.2% of NDF

$uNDF_{72}$ = 20.7% of NDF

Fraction B = 100% - [Fraction A (8.2%) + $uNDF_{72}$ (20.7%)] = 71.1% of NDF

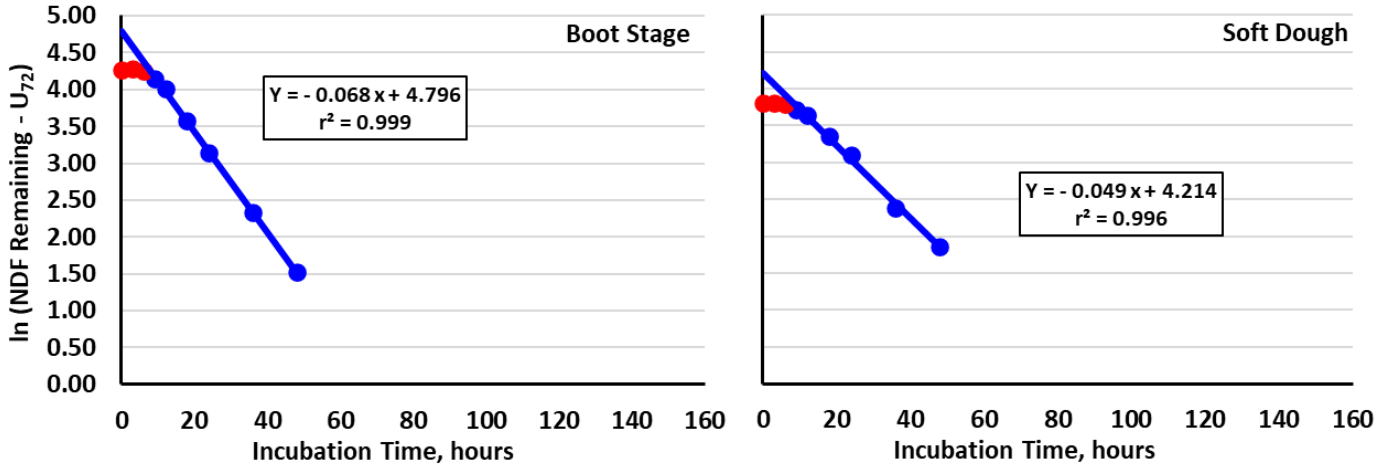


*Figure 10. Regressions of the natural log (ln) of NDF remaining versus in vitro incubation time for triticale forages harvested at the boot (left) and soft dough (right) stages of growth. For these regressions, the pool of potentially digestible NDF was established by using the NDF remaining after 72 hours of incubation as undigestible NDF ($uNDF_{72}$). Red markers (●) indicate data points excluded from the regression because the incubation time was less than the calculated discrete lag time [adapted from Coblentz et al. (2018)].*

Potential Extent = 100% - $uNDF_{72}$ (20.7%) = 79.3% of NDF

Regression Equation: Y = - 0.068 x + 4.796, $r^2$ = 0.999

Slope (Kd) = - 0.068/hour

Lag Time = (4.264 – 4.796) / - 0.068/hour = 7.82 hours,

where 4.264 = natural log of NDF remaining at time = 0 hours, and 4.796 is the y-intercept derived from the regression equation.

***Soft-Dough-Stage Triticale Using $uNDF_{72}$***:

Fraction A = 0

$uNDF_{72}$ = 55.1% of NDF

Fraction B = 100% - $uNDF_{72}$ (55.1%)] = 44.9% of NDF

Potential Extent = 100% - $uNDF_{72}$ (55.1%) = 44.9% of NDF

Regression Equation: Y = - 0.049 x + 4.214, $r^2$ = 0.996

Slope (Kd) = - 0.049/hour

Lag Time = (3.801 – 4.214) / - 0.049/hour = 8.43 hours,

where 3.801 = natural log of NDF remaining at time = 0 hours, and 4.214 is the y-intercept derived from the regression equation.

A comparison of parameter estimates for the triticale forages clearly illustrates the effects of plant maturity on kinetics of pdNDF digestion. Fraction B was reduced from 71.1 to 44.9% of NDF in response to advancing plant maturity, primarily by the associated respective increases in $uNDF_{72}$ (20.7 vs. 55.1% of NDF). Furthermore, the calculation of Kd indicated a substantially slower digestion rate for the more mature, soft-dough stage triticale (0.049 vs. 0.068/hour), as well as a slightly longer lag time (8.43 vs. 7.82 hours). Generally, these are consistent responses to plant maturity observed across most forages. Consider further the implications of analyzing the same data using $uNDF_{240}$ to define undigestible NDF.

***Boot-Stage Triticale Using $uNDF_{240}$***:

Fraction A = 100% - 91.8% = 8.2% of NDF

$uNDF_{240}$ = 15.6% of NDF

Fraction B = 100% - [Fraction A (8.2%) + $uNDF_{240}$ (15.6%)] = 76.2% of NDF

Potential Extent = 100% - $uNDF_{240}$ (15.6%) = 84.4% of NDF

Regression Equation: Y = - 0.031 x + 4.159, $R^2$ = 0.958

Slope (Kd) = - 0.031/hour

Lag Time = 0,

Note that lag time cannot be meaningfully calculated because the ln of NDF remaining at time = 0 hours is greater than the y-intercept, meaning mathematical calculation of lag time results in a negative number [(4.334 - 4.159) / - 0.031/hour = - 5.65 hours]. Lag time cannot really be negative, as this would imply digestion started 5.65 hours before incubation began.

***Soft-Dough-Stage Triticale Using $uNDF_{240}$***:

Fraction A = 0

$uNDF_{240}$ = 44.3% of NDF

Fraction B = 100% - $uNDF_{240}$ (44.3%)] = 55.7% of NDF

Potential Extent = 100% - $uNDF_{240}$ (44.3%) = 55.7% of NDF

Regression Equation: Y = - 0.019 x + 3.956, $R^2$ = 0.968

Slope (Kd) = - 0.019/hour

Lag Time = 0,

Note that lag time cannot be meaningfully calculated because the ln of NDF remaining at time = 0 hours is greater than the y-intercept, meaning mathematical calculation of lag time results in a negative number [(4.020 – 3.956) / - 0.019/hour = - 3.37 hours]. As observed for the boot-stage triticale, lag time cannot really be negative, as this would imply digestion started 3.37 hours before incubation began.

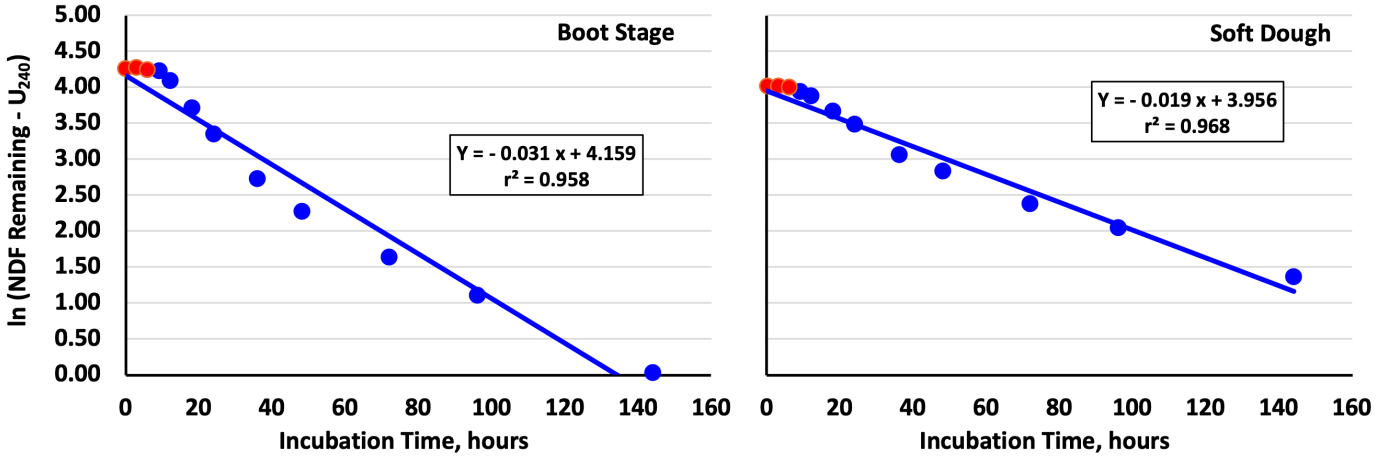


*Figure 11. Regressions of the natural log (ln) of NDF remaining versus in vitro incubation time for triticale forages harvested at the boot (left) and soft dough (right) stages of growth. For these regressions, the pool of potentially digestible NDF was established by using the NDF remaining after 240 hours of incubation as undigestible NDF ($uNDF_{240}$). In these examples, red markers (●) indicate data points at 0, 3, or 6 hours of incubation when there was no discernable change in the recovery of NDF. Since there was no meaningful calculation of lag time in either case (< 0 h), regressions could be recalculated based on all points; however, this does nothing to improve the obvious problems created by the apparent fast and slow digestion pools [adapted from Coblentz et al. (2018)].*

Regression illustrations in Figure 11 that use $uNDF_{240}$ to estimate indigestible NDF are problematic, and depict potential problems created by using $uNDF_{240}$ (or uNDF at other extended time intervals) within ln-linear calculations of Kd. The following are several indicators of problematic results within these examples:

- Although $r^2$ statistics using $uNDF_{240}$ were ≥ 0.958 for the two triticale forages, they represent a decline from the near-perfect (≥ 0.996) values obtaining using $uNDF_{72}$;
- Although Kd remained much faster for boot-stage triticale (0.049 vs. 0.019/hour), estimates of Kd for both forages were reduced by ≥ 54% by using $uNDF_{240}$ compared to $uNDF_{72}$ in the calculations; and
- When using $uNDF_{240}$, it was not possible to calculate a lag time for either forage, which is highly unlikely, particularly for the triticale harvested at the soft-dough stage of growth.

Close inspection of the regression relationships in Figure 11 indicates that linearity is compromised at extended incubation times (≥ 72 hours), thereby biasing or constraining the (negative) magnitude of the slope, and also eliminating the calculation of a meaningful discrete lag time. This response suggests that digestible NDF is not a single, homogenous pool, and that multiple digestion pools may be present. As mentioned previously, a three-pool model comprised of fast, slow, and undigestible pools might be more appropriate for describing the NDF digestion of forages. This possibility is illustrated for the boot-stage triticale in Figure 12, but could be applied equally to both forages. More importantly, these examples indicate strongly that kinetic calculations should not be blindly applied without careful consideration of results. To maintain

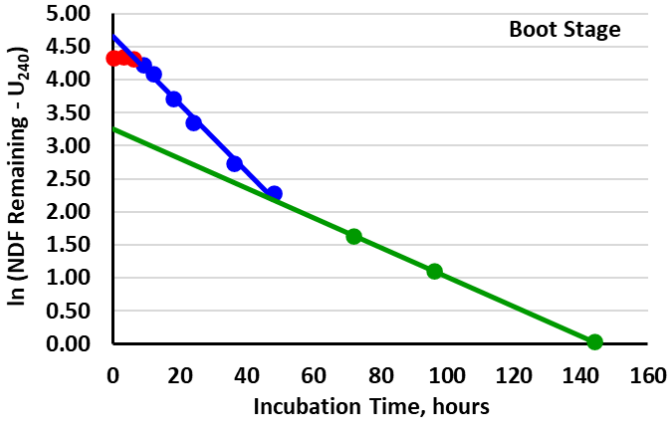


*Figure 12. Evidence for a possible three-pool digestion model for NDF for triticale forages harvested at boot stage at Marshfield, Wisconsin. Red markers (●) indicate data points where the incubation time was less than the calculated discrete lag time. Blue (●) and green (●) markers represent likely fast and slowly digesting NDF pools, respectively. Calculations are based on an undigestible NDF pool established after 240 hours of in vitro incubation ($uNDF_{240}$) [adapted from Coblentz et al. (2018)].*

meaningful two-pool assessments of Kd and lag time for these two triticale forages using $uNDF_{240}$, there are two realistic options, neither of which is entirely satisfactory. These are to: i) revert back to a shorter incubation time for quantifying uNDF, such as that shown in Figure 10 ($uNDF_{72}$) or ii) continue to use $uNDF_{240}$ to most appropriately estimate indigestible NDF, but eliminate points causing the loss of linearity in the regression model. If one is to use the latter option (ii) in these examples, the eliminated points would likely represent NDF digestion at 72, 96, and 144 hours, which would be most closely associated with the slow digestion pool using an alternative three-pool model (Figure 12). A major shortcoming of the former option (i) is that it would be erroneous because it does not include the entire pool of digestible NDF.

*Nonlinear Regression*. The most commonly used alternative to the ln-linear method for describing digestion kinetics is to use nonlinear regression. While there are a number of variations for executing this general regression model (and software by which to conduct the analysis), a typical code using PROC NLIN of SAS (Cary, NC) is provided in the inset to the right. Examples of this analytical approach for the triticale forages harvested at the boot and soft-dough stages of growth are presented in Figure 13. In this example, parameter values and the illustrated curves are based on the mean of nonlinear regression curves for five field replications. The typical model for this type of regression consists of the following two parts or conditions:

- when incubation time (t) is < discrete lag time (L), then NDF remaining at time t (NDF_REM) = fraction B + uNDF
- when t > L, then NDF_REM at time t = fraction B × $e^{[-Kd\,(t-L)]}$ + uNDF.

In this example for triticale forages harvested at the soft-dough stage of growth, the data set is abbreviated to only two field blocks. Normally, in field trials, it is likely that in vitro assessments would be made over 4 to 6 field blocks (replications), or 4 to 6 animals for in situ evaluations. For in situ evaluations, animals are often used as replications for evaluations of single test forages, diets, or dietary components; however, for in situ kinetic evaluations based on field trials, animals also can be intentionally confounded with field replications, essentially blocking on more than one factor at the same time.

*Example code for developing a nonlinear regression of fiber digestion against incubation time using the NLIN procedure in SAS.*

```
Data SoftDough;
  Input fieldblock hour NDF_REM;
  Cards;
  1   0     100.0
  1   3     100.0
  1   6     99.5
  1   9     97.4
  1   12    95.6
  1   18    87.4
  1   24    80.5
  1   36    67.6
  1   48    65.5
  1   72    59.0
  1   96    54.1
  1   144   51.8
  1   240   48.1
  2   0     99.9
  2   3     100.0
  2   6     98.7
  2   9     96.6
  2   12    93.0
  2   18    83.2
  2   24    75.6
  2   36    63.9
  2   48    59.9
  2   72    53.5
  2   96    49.9
  2   144   45.5
  2   240   42.1
  PROC PRINT;
  DATA NEW; SET SoftDough;
  PROC SORT; BY fieldblock;
  PROC  NLIN  BEST  =  10  METHOD  =
  Marquardt; BY fieldblock;

  PARMS     B = 40 to 60 by 2
            Kd = 0.01 to .30 by .01
            L = .1 to 15 by .5
            uNDF = 40 to 60 by 1;

IF HOUR LT L THEN DO;
  MODEL NDF_REM = B + uNDF;
  IF HOUR GE L THEN DO;
  MODEL NDF_REM = B * EXP (-Kd * (HOUR
  - L)) + uNDF;
  BOUNDS  0<B<100,  0<Kd<.31,  0<L<20,
  0<uNDF<75;

RUN;
```

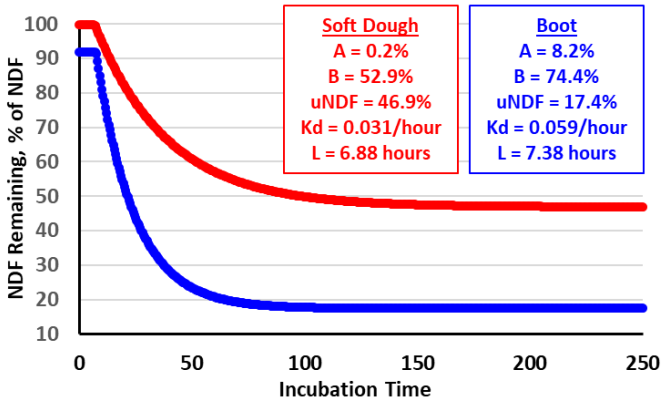


*Figure 13. Nonlinear regression relationships for in vitro digestion of NDF for triticale forages harvested at the boot (—) and soft dough (—) stages of growth. Abbreviations for kinetic parameters are: A, percentage of NDF disappearing at a rate too fast to measure; B, percentage of NDF disappearing at a measurable rate; uNDF, undigestible NDF; Kd, digestion rate; and L, discrete lag time. Digestion curves are based on the mean of five field replications [adapted from Coblentz et al. (2018)].*

An example of this approach would be to evaluate hays obtained from five field replications or blocks in five cannulated cows, where field replication 1 was evaluated in animal 1, field block 2 in animal 2, and so forth.

Several other considerations are important to note. First, the 'BY fieldblock' statement after the PROC SORT and PROC NLIN code lines ensures that digestion kinetics will be fit to individual field replications, rather than across the whole collective data set. Secondly, when using the nonlinear regression method, it is necessary to establish a range over which PROC NLIN should look for solutions to model parameters (PARMS). In the past, this was somewhat problematic. PARMS needed to be close to the final solution to ensure rapid and efficient model convergence. As computing power has increased, this is less important, as noted by the relatively wide ranges for the PARMS in the previous example. The PARMS for the previous example also could be focused or 'fine-tuned' by using the ln-linear method to obtain an estimate of what the final nonlinear parameters might be, or by confining a preliminary nonlinear regression to a single field replication, rather than wasting computer efficiency on a large data set with unknown PARM ranges.

Although it is (far) less likely when computing digestion kinetics of NDF, not all decay curves for various forage components have discernable or meaningful solutions for discrete lag time. This is particularly true for the disappearance of kinetics of crude protein (**CP**) from legumes, such as alfalfa. If the lower limit of the BOUND for lag time (L) is 0, the nonlinear solution may result in an unrealistically small lag time (~ $10^{-6}$ hours), basically indicating that there is no calculable lag. In those circumstances, it may be best to just eliminate lag time from the model. As mentioned previously for the ln-linear method, any model adjustment (i.e., BOUND) that establishes a negative lag time is illogical, implying that digestion or disappearance began before incubation started.

One strong advantage of the nonlinear regression method is that determination of some regression parameters is less dependent on arbitrary (and potentially flawed or biased) judgement during calculations. This is especially true when quantifying lag time and uNDF. To determine uNDF, the nonlinear model establishes a value based on an asymptote, which is theoretically equivalent to residual NDF after an infinite incubation time. There is no need to subtract out uNDF prior to any subsequent calculation of Kd, as that is satisfied within model calculations. However, the selection of extended time points for incubation must be suitable or long enough to clearly establish an asymptote. A common error or problem occurs with the assumption that a terminal 72-hour incubation can correctly establish the asymptotically based limit of NDF digestion. An example of a fictitious digestion curve for which this might be problematic is illustrated in Figure 14, where the data set provided is likely insufficient to establish a truly legitimate asymptote or limit for NDF digestion. The following are a number of possible practical indicators of this type of problem:

- The regression-based estimate of uNDF (theoretically, at an infinite incubation time) approaches 0, approaches a (> 0) minimum BOUND, or is substantially less than the NDF remaining at the final incubation time point;
- There is substantial evidence of NDF digestion between the final two incubation times (ideally, the percentage of NDF remaining should be similar at these two final time points to clearly establish an asymptote); and/or

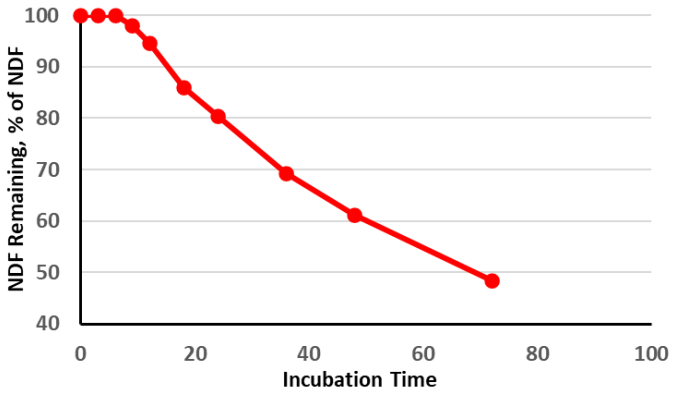


*Figure 14. Fictitious example of in vitro NDF digestion in which the terminal incubation time at 72 hours is unlikely to adequately establish an appropriate value for uNDF by nonlinear regression.*

- The regression-based parameter value of uNDF seems unrealistically low for the forage being evaluated.

**Compendium of NDF Digestion Parameters for Forages.** It must be acknowledged that scientific reports quantifying digestion kinetics of NDF for forages are conducted by laboratory/analytical methods that range widely, and are further complicated by the limited or inconsistent standardization of those methods. Despite these inconsistencies, some general trends or characteristics can be summarized from 335 individual kinetic assessments compiled from reported studies primarily within the United States. A condensed summary is provided in Table 3, and several key points should be considered.

First, overall fractional digestion rates of pdNDF (Kd) ranged generally from 0.020 to 0.120/hour, but estimates > 0.100/hour were relatively rare. Usually, these most-rapid estimates of Kd were associated with high-quality legumes or immature cool-season grasses (annual or perennial). Several estimates of Kd reported by Smith et al. (1971, 1972) for low-NDF forages were particularly rapid, ranging as high as 0.309/hour for ladino clover.

Generally, Kd of pdNDF slowed with forage age or maturity, while uNDF exhibited the opposite response, indicating a smaller percentage of the total NDF pool was potentially digestible as plants became more mature.

Data for perennial cool-season (C3) grasses summarized in Table 3 included studies evaluating orchardgrass, reed canarygrass, Kentucky bluegrass, perennial ryegrass, festolium, quackgrass, timothy, smooth bromegrass, and tall fescue. Some studies evaluated regrowth or fall-stockpiled applications of these forages where there was no potential for reproductive (stem) development, thereby somewhat restricting the ceiling on concentrations of NDF. The mean estimate of Kd for (generally) vegetative grasses with < 50% NDF was 0.090/hour, which declined to 0.043/hour for grasses that were rank or otherwise problematic (> 70% NDF; Figure 15). Between these extremes, which were related primarily to physiological development, uNDF more than doubled for the high-NDF forages (15.5 vs. 33.5% of NDF, respectively; Figure 16).

Cool-season (C3) annual grasses summarized here were cereal-grain forages, including barley, wheat, cereal rye, oat, and triticale. Some of these studies evaluated fall-grown oat, which will elongate without vernalization, but does not lignify heavily under cooler fall growing conditions compared to oat forages that mature during late spring or early summer. In general, the effects of maturity on Kd were similar to those described for C3 perennial grasses (Figure 17); the mean Kd for vegetative cereals (< 45% NDF) was 0.108/hour, which was substantially greater than the rate of 0.031/hour for cereal straws (≥ 70% NDF). The mean Kd for the boot and soft-dough stages of growth, which are typical harvest targets for these forages, were intermediate in magnitude, but also distinctly different from each other (0.062 and 0.040/hour,

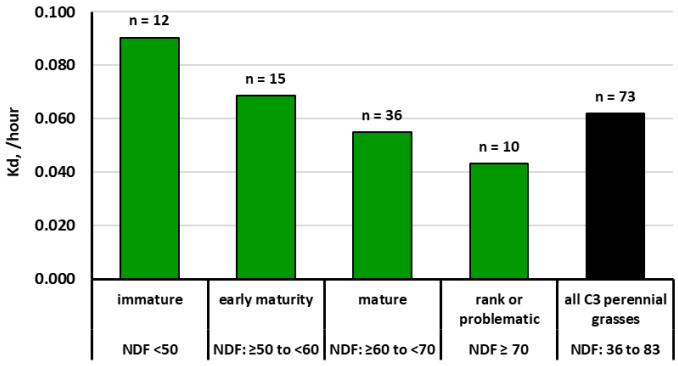


*Figure 15. Summary of digestion rates (Kd) of potentially digestible NDF (pdNDF) for perennial cool-season grasses determined by in vitro or in situ procedures and calculated by ln-linear or nonlinear regression methods. Descriptions of maturity were established from data within the individual published studies using NDF as a proxy for forage maturity or plant age. See Table 3 for more information.*

*Table 3. Summary of NDF digestion kinetics gleaned from various studies determined by in situ or in vitro methods and calculated by ln-linear or nonlinear regression techniques. See list of Supplemental References for list of research contributing data to this table.*

| Description | NDF Range | *N* | A[1] | B[2] | pdNDF[3] | uNDF[4] | Kd[5] | Lag Time[6] | Mean NDF |
|---|---|---|---|---|---|---|---|---|---|
| | *% of DM* | *no.* | ---------- | ---------- | *% of NDF* | ---------- | */hour* | *hours* | *% of NDF* |
| ***C3 Perennials*** | | | | | | | | | |
| immature | < 50 | 12 | 10.9 | 79.1 | 84.5 | 15.5 | 0.090 | 1.58 | 44.9 |
| early maturity | ≥ 50 to < 60 | 15 | 8.9 | 75.8 | 80.0 | 20.0 | 0.069 | 4.16 | 55.0 |
| mature | ≥ 60 to < 70 | 36 | 10.5 | 65.2 | 68.4 | 31.6 | 0.055 | 3.61 | 65.1 |
| rank or problematic | ≥ 70 | 10 | 3.9 | 64.8 | 66.5 | 33.5 | 0.043 | 3.23 | 75.9 |
| all grasses | 36 to 83 | 73 | 9.2 | 69.6 | 73.2 | 26.8 | 0.062 | 3.51 | 61.2 |
| ***C3 Annuals*** | | | | | | | | | |
| mostly vegetative | < 45 | 18 | 8.1 | 83.9 | 90.1 | 9.9 | 0.108 | 2.31 | 39.1 |
| elongation, booting, early heading | ≥ 45 to < 55 | 19 | 7.5 | 81.0 | 86.5 | 13.5 | 0.062 | 2.43 | 49.6 |
| anthesis, grain fill | ≥ 55 to < 70 | 22 | 5.1 | 58.3 | 61.8 | 38.2 | 0.040 | 4.92 | 61.9 |
| mostly straws | ≥ 70 | 6 | 4.1 | 57.5 | 58.2 | 41.8 | 0.031 | 4.10 | 77.4 |
| all grasses | 33 to 81 | 65 | 6.8 | 72.0 | 76.5 | 23.5 | 0.064 | 3.35 | 53.4 |
| ***C4 Perennials*** | | | | | | | | | |
| early mature | < 70 | 18 | 5.6 | 62.0 | 63.9 | 36.1 | 0.042 | 3.58 | 67.5 |
| mature | ≥ 70 | 32 | 7.0 | 51.9 | 56.5 | 43.5 | 0.039 | 2.71 | 75.5 |
| all grasses | 59 to 83 | 50 | 6.7 | 55.5 | 59.2 | 40.8 | 0.040 | 2.87 | 72.6 |
| ***Legumes*** | | | | | | | | | |
| immature | < 40 | 25 | 6.1 | 53.2 | 54.4 | 45.6 | 0.109 | 1.63 | 32.3 |
| mid-maturity | ≥ 40 to < 46 | 13 | 6.2 | 44.6 | 47.4 | 52.6 | 0.084 | 2.01 | 42.3 |
| mature | ≥ 46 | 11 | 7.2 | 48.6 | 49.1 | 50.1 | 0.081 | 1.09 | 48.6 |
| all forages | 18 to 53 | 49 | 6.3 | 49.9 | 51.6 | 48.4 | 0.096 | 1.61 | 38.6 |
| ***Heated Alfalfa/Orchardgrass Hays*** | | | | | | | | | |
| prestorage | | 2 | 15.0 | 47.5 | 62.5 | 37.6 | 0.099 | 1.52 | 50.8 |
| minor heating, < 122°F | | 4 | 14.9 | 47.9 | 62.8 | 37.2 | 0.092 | 1.66 | 52.4 |
| moderately heated, ≥ 122 to < 140°F | | 5 | 16.2 | 48.8 | 65.1 | 34.9 | 0.079 | 1.68 | 56.7 |
| highly heated, ≥ 140 to 158°F | | 6 | 20.8 | 47.1 | 67.9 | 32.1 | 0.067 | 2.50 | 59.9 |
| severely heated/fire hazard, ≥ 158°F | | 3 | 22.1 | 45.4 | 67.6 | 32.4 | 0.066 | 2.69 | 58.4 |
| all forages | | 20 | 18.1 | 47.5 | 65.6 | 34.4 | 0.078 | 2.06 | 56.5 |
| ***Heated Bermudagrass Hays*** | | | | | | | | | |
| heating degree days[7] = 5 | | 1 | 4.0 | 66.5 | 70.5 | 29.5 | 0.048 | 3.1 | 78.7 |
| heating degree days = 119 | | 1 | 4.0 | 68.4 | 72.4 | 27.6 | 0.040 | 2.6 | 79.0 |
| heating degree days = 201 | | 1 | 3.1 | 70.7 | 73.8 | 26.2 | 0.034 | 2.8 | 79.8 |
| heating degree days = 273 | | 1 | 3.5 | 68.8 | 72.2 | 27.8 | 0.033 | 3.2 | 80.9 |
| heating degree days = 401 | | 1 | 3.3 | 66.8 | 70.1 | 29.9 | 0.037 | 4.6 | 82.0 |
| all forages | | 5 | 3.6 | 68.2 | 71.8 | 28.2 | 0.038 | 3.3 | 80.1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ***Warm Season Annuals*** | | | | | | | | |
| corn silage | 50 | 14.3 | 60.2 | 60.5 | 39.5 | 0.030 | 7.54 | 43.4 |
| corn silage (without macro in situ procedures) | 18 | 14.3 | 71.5 | 72.3 | 27.7 | 0.033 | 7.54 | 39.7 |
| corn stover | 1 | NR[8] | 67.7 | 67.6 | 32.4 | 0.044 | 4.35 | 73.0 |
| crabgrass | 7 | 5.9 | 72.5 | 78.4 | 21.6 | 0.078 | 1.56 | 58.7 |
| sorghum | 4 | NR | 53.7 | 53.7 | 46.3 | 0.041 | 5.55 | 58.7 |
| sorghum × sudangrass | 14 | 11.3 | 62.1 | 69.4 | 30.7 | 0.046 | 9.28 | 68.4 |
| pearl millet | 2 | NR | 70.8 | 70.8 | 29.3 | 0.025 | NR | 50.4 |

*[1] Mean of those studies reporting a fraction A, which disappears at a rate too fast to be measured.*
*[2] Mean of the percentage of NDF on which rate calculations were based, either fraction B (when fraction A was reported) or pdNDF (when fraction A was not reported).*
*[3] pdNDF, potentially digestible NDF, calculated as: 100% - uNDF.*
*[4] uNDF, undigestible NDF.*
*[5] Kd, digestion rate (/hour).*
*[6] Mean of those studies reporting a lag time.*
*[7] Heating degree days incurred during storage.*
*[8] NR, not calculated or reported.*

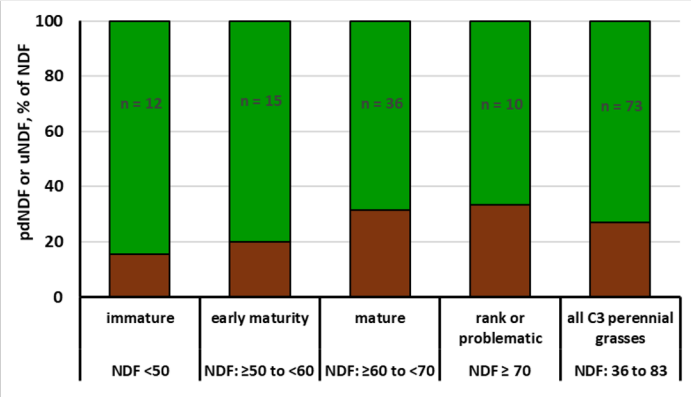


*Figure 16. Summary of potentially digestible NDF (pdNDF; green bars, ) and undigestible NDF (uNDF; brown bars, ) for perennial cool-season grasses determined by in vitro or in situ procedures and calculated by ln-linear or nonlinear regression methods. Descriptions of maturity were established from data within the individual published studies using NDF as a proxy for forage maturity or plant age. See Table 3 for more information.*

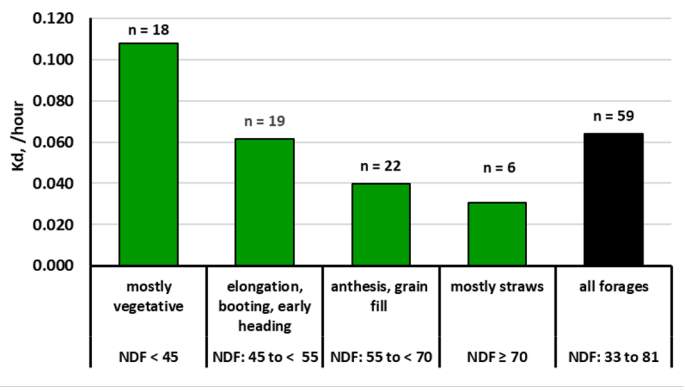


*Figure 17. Summary of digestion rates (Kd) of potentially digestible NDF (pdNDF) for cool-season annual grasses determined by in vitro or in situ procedures and calculated by ln-linear or nonlinear regression methods. Descriptions of maturity were established from data within the individual published studies using NDF as a proxy for forage maturity or plant age. See Table 3 for more information.*

respectively). The physiological process of grain fill had little or no effect on consistent declines in Kd in response to advancing plant maturity. Undigestible NDF (uNDF) comprised only 9.9% of NDF in vegetative plants, but increased to 38.2% of NDF at the soft-dough stage of growth. Cereal straws that are often included in diets to provide additional fiber, or in some cases to limit voluntary intake, had only slightly greater uNDF (41.8% of NDF) than cereals harvested at the soft dough stage of growth (Figure 18), further supporting the premise that grain fill has little effect on digestion kinetics of pdNDF for cereal-grain forages. This concept has been discussed in general terms by Allen and Mertens (1988), who concluded that the digestibility of whole-feeds (or whole-plant forages in this specific context) is influenced by the nearly complete digestion of non-fiber constituents, whose variability in ruminant feedstuffs accounts for the poor relationship between digestibilities of DM and fiber.

Legumes summarized in Table 3 were perennials, mostly alfalfa, but also included a few evaluations of ladino clover, birdsfoot trefoil, red clover, and crownvetch. For relatively immature legumes (< 40% NDF), the mean Kd (0.109/hour; Figure 19) was comparable to vegetative cereal grains, but the subsequent effects of maturation were only moderate; the mean Kd for mature legumes (≥ 46% NDF) remained considerably more rapid (0.081/hour) than observed for mature C3 grasses (annual or perennial). Despite the rapid estimates of Kd, a distinguishing characteristic associated with legumes was the large percentages of uNDF inherent within these forages (Figure 20). Unlike immature C3 grasses, the mean uNDF for immature legumes accounted for nearly half of the total NDF pool (45.6%), which increased to > 50% of the NDF pool at more advanced maturities. Despite the large contributions uNDF makes to the total NDF pool, digestion of pdNDF remains uniquely rapid, regardless of NDF concentrations or growth stage.

Table 3 includes 50 evaluations of perennial warm-season (C4) grasses, primarily bermudagrass, switchgrass, and eastern gamagrass. Some studies specifically evaluated these forages for use in fall-stockpiling applications. Compared to both annual

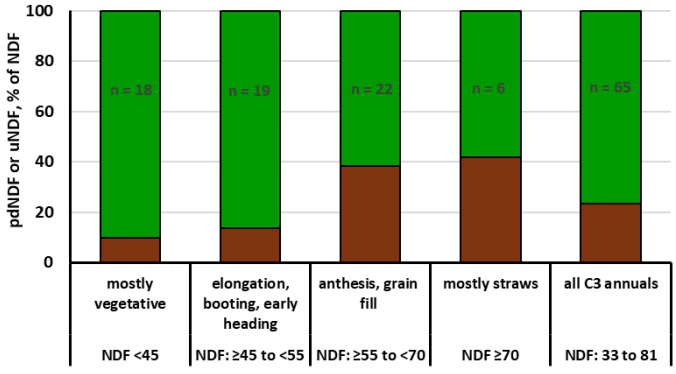


*Figure 18. Summary of potentially digestible NDF (pdNDF; green bars, ) and undigestible NDF (uNDF; brown bars, ) for cool-season annual grasses determined by in vitro or in situ procedures and calculated by ln-linear or nonlinear regression methods. Descriptions of maturity were established from data within the individual published studies using NDF as a proxy for forage maturity or plant age. See Table 3 for more information.*

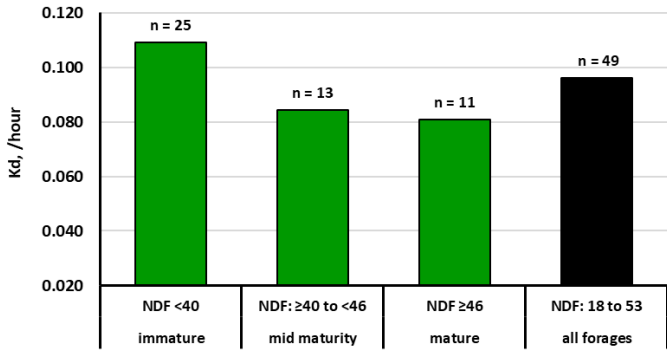


*Figure 19. Summary of digestion rates (Kd) of potentially digestible NDF (pdNDF) for legumes determined by in vitro or in situ procedures and calculated by ln-linear or nonlinear regression methods. Descriptions of maturity were established from data within the individual published studies using NDF as a proxy for forage maturity or plant age. See Table 3 for more information.*

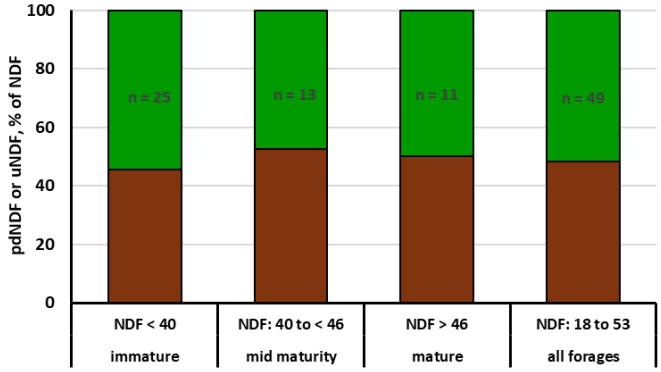


*Figure 20. Summary of potentially digestible NDF (pdNDF; green bars, ) and undigestible NDF (uNDF; brown bars, ) for legumes determined by in vitro or in situ procedures and calculated by ln-linear or nonlinear regression methods. Descriptions of maturity were established from data within the individual published studies using NDF as a proxy for forage maturity or plant age. See Table 3 for more information.*

and perennial C3 grasses, the effects of increasing NDF concentration (used as a proxy indicator for maturity) had minimal impact on Kd (Figure 21). For early maturity forages (NDF < 70%; mean = 67.5%), the average Kd was relatively slow (0.042/hour). At greater NDF concentrations (NDF ≥ 70%; mean = 75.5%), Kd slowed only modestly (0.039/hour). For these two groups, the mean concentrations of uNDF were large (Figure 22), and increased with maturity (36.1 to 43.5% of NDF). As such, uNDF concentrations for mature perennial C4 grasses are often comparable to those of cereal-grain straws. Generally, perennial C4 grasses can be characterized by their high concentrations of NDF and uNDF, as well as their slow digestion rates that are relatively independent of maturity, age, or concentrations of NDF when compared to temperate (C3) forage types. As such, they should be viewed primarily as maintenance-type forages for livestock with relatively low nutrient demands, such as pregnant, non-lactating beef cows.

Any analysis of NDF digestion kinetics for annual warm-season grasses is complicated by the inclusion of species with exceptionally heavy grain loads (corn silage), inclusion of brown midrib (***bmr***) mutants, as well as the limited and inconsistent representation of many species. The most important forage within this general category is corn silage, which yielded a mean Kd of 0.030/hour coupled with a mean uNDF of 39.5% of NDF (*N* = 50 assessments; Table 3). After excluding the contributions of Johnson et al. (2003) that utilized unique macro in situ procedures, the mean uNDF was less (27.7% of NDF), but the mean Kd was largely unaffected (0.033/hour). Most other annual C4 species largely reflected the relatively slow estimates of Kd observed for perennial C4 grasses (0.025 to 0.046/hour), except for common crabgrass harvested weekly from early-July through August in northwest Arkansas (Ogden et al. 2005). In that experiment, Kd was notably more rapid (0.069 to 0.086/hour) than observed for other C4 annuals, and demonstrated only a marginal relationship with concentrations of NDF (Figure 23). Distinguishing characteristics of these crabgrass forages across these sampling dates were the relative stability of NDF concentrations (55.5 to 61.9%), and the limited lignification of stem tissue, which ranged from 1.75 to 3.39% of stem DM.

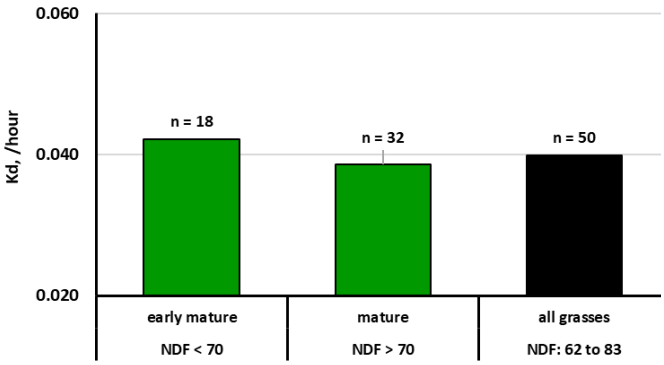


*Figure 21. Summary of digestion rates (Kd) of potentially digestible NDF (pdNDF) for perennial C4 grasses determined by in vitro or in situ procedures and calculated by ln-linear or nonlinear regression methods. Descriptions of maturity were established from data within the individual published studies using NDF as a proxy for forage maturity or plant age. See Table 3 for more information.*

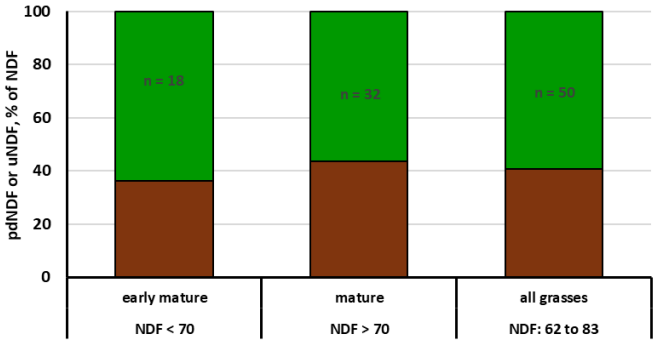


*Figure 22. Summary of potentially digestible NDF (pdNDF; green bars, ) and undigestible NDF (uNDF; brown bars, ) for perennial C4 grasses determined by in vitro or in situ procedures and calculated by ln-linear or nonlinear regression methods. Descriptions of maturity were established from data within the individual published studies using NDF as a proxy for forage maturity or plant age. See Table 3 for more information.*

Two studies have related Kd with various increments of spontaneous heating during storage of hays, both indicating that Kd slowed for digestion of pdNDF in response to this type of heating during bale storage. Coblentz and Hoffman (2009) evaluated this relationship for large-round bales of alfalfa-orchardgrass hays that incurred mild to severe heating, with some maximum internal bale temperatures in excess of 158°F (70°C). Mean in situ values for Kd declined from 0.099/hour for prestorage hays incurring no heating to 0.066/hour for severely heated hays (Figure 24). Similar declines in Kd also have been reported by McBeth et al.

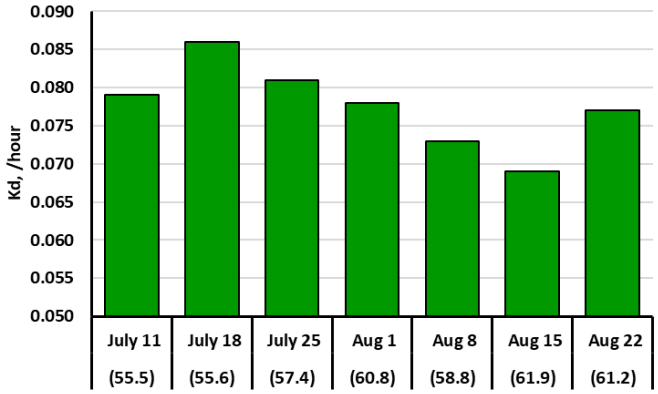


*Figure 23. Relationship between in-situ digestion rate of NDF (Kd) and harvest date obtained from common crabgrass forages harvested weekly between mid-July and late-August in northwest Arkansas. A negative linear trend (P = 0.051) was detected between Kd and the equally spaced harvest dates. Associated concentrations of NDF (% of DM) for each harvest date are shown parenthetically [adapted from Ogden et al. (2005)].*

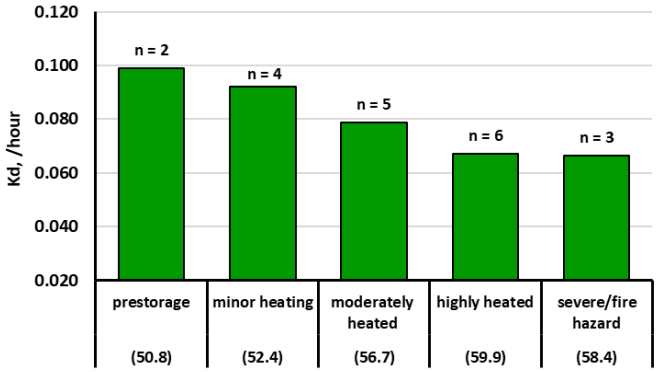


*Figure 24. Effects of spontaneous heating within large-round bales of alfalfa-orchardgrass hay on subsequent in situ NDF digestion rates (Kd). Associated concentrations of NDF (% of DM) for each heating category are shown parenthetically. Category descriptions are somewhat arbitrary based on distribution of treatments, where maximum internal bale temperatures were: minor heating, < 122°F (50°C); moderately heated, 122 to < 140°F (50 to < 60°C); highly heated, 140 to < 158°F (60 to < 70°C); and severely heated/fire hazard, > 158°F (> 70°C) [adapted from Coblentz and Hoffman (2009)].*

(2003) for heated bermudagrass hays packaged in small rectangular bales (Figure 25).

**Some Specific Factors Affecting NDF Digestion Kinetics.** As mentioned in the *Some General Advantages/Disadvantages of In Vitro and In Situ Methods* section found on page 5, many factors can affect the in vitro or in situ assessments of intrinsic digestion kinetics. Among these are inadequate or inappropriate N or CP, trace nutrients, pH, redox potential, anaerobic conditions, and/or microbial numbers within the fermentation medium, such that digestion is limited by factors external to the feedstuff being evaluated (Grant and Mertens, 1992a; Mertens, 1993). Because of poor technique or procedures, many in vitro or in situ studies may not meet these standards for a truly intrinsic evaluation of specific forages and ruminant feeds. It also must be emphasized that inadequacies for intrinsic assessment are often implemented intentionally. For example, an assessment of in situ NDF digestion kinetics of dormant native range grasses might logically be performed in steers consuming a basal diet of poor-quality hay that contains inadequate CP. In other words, experimental conditions may be logical and appropriate to meet specific research objectives, but they may not meet the standards required for a truly intrinsic assessment. It is beyond the scope of this research synopsis to provide a detailed assessment of all limiting factors that can occur during in situ or in vitro evaluations of NDF digestion kinetics; however, research related to some

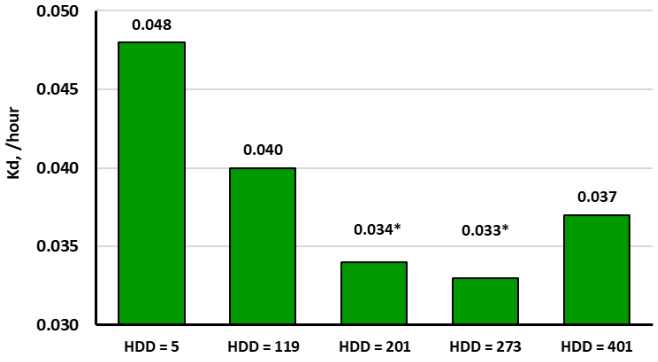


*Figure 25. Relationship between NDF digestion rate (Kd) and heating degree days > 35°C (95°F) (HDD) incurred during storage of bermudagrass hays packaged in small rectangular bales produced in northwest Arkansas. Treatments differing from little heating (HDD = 5) are denoted with an asterisk (*). Conversion of HDD categories to a Fahrenheit basis are 9, 214, 362, 491, and 722 HDD, respectively [adapted from McBeth et al. (2003)].*

common concerns, particularly the confounding factors of starch and pH, can be summarized herein.

It has long been understood that consumption of readily fermentable carbohydrates, such as starch, causes depressions in ruminal pH, and this can result in impaired digestion of NDF. In a detailed review, Hoover (1986) concluded bluntly that whenever fermentation of carbohydrates causes ruminal pH to decrease to 6.0 or less, fiber digestion will be decreased. This response is often associated with various deleterious effects on fibrolytic bacteria.

Several studies have evaluated these concerns for in vitro incubation systems, examining the effects of starch and pH both independently and in tandem. Mertens and Loften (1980) evaluated the effects of corn and wheat starch on digestion kinetics of NDF for alfalfa, tall fescue, and orchardgrass hays, as well as pelleted Coastal bermudagrass. In their in vitro evaluations, each forage substrate was altered by the addition of 0, 40, 60, or 80% wheat or corn starch. This study evaluated only the addition of starch, as pH was maintained at 6.8 within each fermentation vessel. Furthermore, kinetic parameters were calculated by both ln-linear and non-linear regression methods.

The effects of starch inclusion on Kd as determined by ln-linear regression for each forage type are summarized in Figure 26, where the mean Kd across all starch inclusion rates was most rapid for alfalfa (0.0779/hour) and tall fescue (0.0841/hour) hays, and slowest for orchardgrass hay (0.0621/hour). The mean Kd for pelleted Coastal bermudagrass (0.0717/hour) was intermediate between the noted extremes and differed statistically from neither. The mean pdNDF for the two cool-season grass hays (73.8% of NDF) was greater than exhibited by pelleted Coastal bermudagrass (63.2% of NDF). Alfalfa hay exhibited the least pdNDF (47.7% of NDF), which is consistent with the poorer availability of NDF from legumes summarized in Table 3. Figure 27 summarizes both Kd and pdNDF averaged across all forage types as a function of starch inclusion rate. Overall, Kd did not differ on the basis of starch inclusion (overall mean = 0.0742/hour), but pdNDF declined from 67.8 to 61.4% of NDF as starch was added to the in vitro fermentations. Some caution is advised with respect to interpretation, as pdNDF was determined on the basis of a 72-hour fermentation, which may not have adequately defined the fermentation endpoint for all treatment combinations. It is notable that when calculations were made by nonlinear regression methods that mathematically determine an asymptotic digestion limit, there was no statistical difference in pdNDF across starch inclusion levels. Calculated lag times based on ln-linear regression methods ranged from 1.13 to 3.22 hours, which increased linearly with starch percentage; however, lag times obtained for individual starch inclusion percentages did not differ ($P > 0.05$). Conclusions drawn from these results were that starch added to fermentation vessels affected digestion kinetics primarily by increasing lag times, which does not explain the many reported reductions in fiber digestibility associated with including starch in the diets of ruminant livestock. One explanation of this apparent discrepancy was that acidity was tightly controlled at pH = 6.8 within these in vitro fermentation vessels, but can easily be depressed to less than pH = 5.5 by the digestion of the starch within the rumen of livestock.

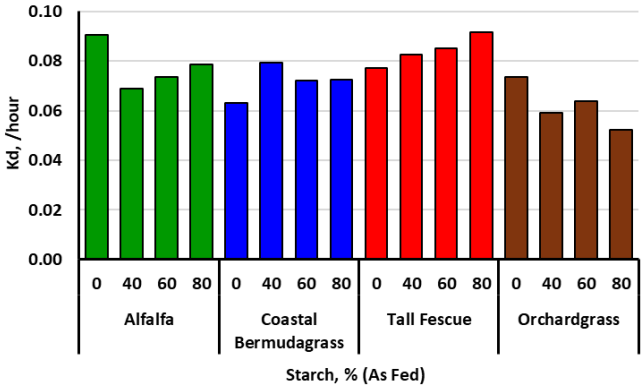


*Figure 26. The effects of wheat or corn starch on in vitro digestion rates (Kd) of NDF for alfalfa, tall fescue, and orchardgrass hays, as well as Coastal bermudagrass pellets. Digestion rates were calculated by ln-linear regression techniques. Averaged across all starch inclusion levels, the mean Kd for alfalfa (0.0779/hour) and tall fescue (0.0841/hour) hays were most rapid, while orchardgrass hay was the slowest (0.0621/hour); the mean Kd for Coastal bermudagrass pellets (0.0670/hour) was intermediate and differed statistically from neither extreme [adapted from Mertens and Loften (1980)].*

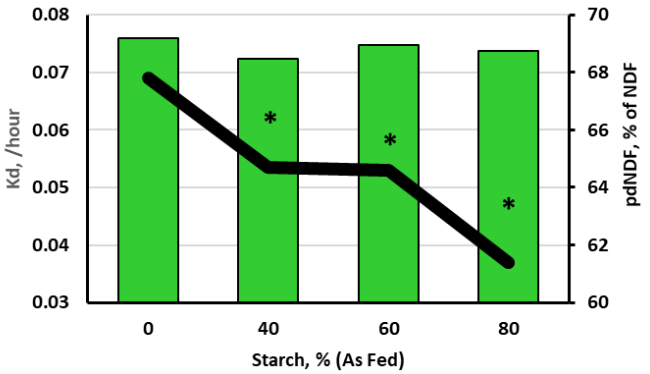


*Figure 27. Effects of inclusion rate of wheat or corn starch on in vitro digestion rate (Kd; ▮) of Coastal bermudagrass pellets and alfalfa, tall fescue, and orchardgrass hays (collectively) determined by ln-linear regression techniques. Digestion rate (Kd) was not affected statistically; means for potentially digestible NDF (pdNDF; —) that differed from the control (starch = 0) are denoted with an asterisk (*). The mean pdNDF at the 80% starch inclusion rate also differed from those at intermediate inclusion rates [adapted from Mertens and Loften (1980)].*

The effects of pH (without starch) on the in vitro digestion kinetics of NDF for alfalfa hay, smooth bromegrass hay, and corn silage were assessed by Grant and Mertens (1992b). The pH of the in vitro inoculum was buffered normally (pH = 6.8) or reduced to pH = 5.8 with citric acid. Attempts to use phosphoric acid to adjust pH were less suitable because of pH drift within the fermentation media, particularly at low pH (5.8). Therefore, the experimental conditions for this experiment examined solely pH effects, without including starch as an additional substrate. The primary effects of pH were observed for discrete lag times, which were longer at pH = 5.8 for all three forages when calculated by nonlinear regression, and for smooth bromegrass and corn silage when ln-linear regression techniques were used (Figure 28). Moreover, these effects were substantial in magnitude; averaged across three forages and two regression methods, lag times were nearly twice as long at pH = 5.8 compared to pH = 6.8 (10.61 vs. 5.93 hours). It was suggested that this strong effect of depressed pH was associated with a required adaptation of the microbial population within the inoculum to a low (more acidic) pH environment, which included the possible inhibition of ruminal cellulolytic bacteria. The effects of pH on Kd for these forages were less definitive. The Kd of pdNDF digestion was slower for corn silage at pH = 5.8 compared to pH = 6.8 when calculated by both nonlinear (0.041 vs. 0.067/hour) and ln-linear (0.037 vs. 0.053/hour) regression methods (Figure 29). Digestion rate (Kd) was not affected in alfalfa or smooth bromegrass hays, irrespective of regression method. However, it was noted that across all experimental conditions (forages and pH), Kd was about 20% faster when calculated by nonlinear methods compared to the ln-linear approach.

Another study conducted by Grant and Mertens (1992c) investigated the dual effects of starch substrate and pH on the in vitro fermentation of alfalfa hay, smooth bromegrass hay, and corn silage. When raw corn starch was added to the fermentation vessels, a target concentration of 30% NDF for the entire substrate pool (starch + forage) was established. Calculations were based on the ln-linear regression method using a 96-hour incubation to define the extent of digestion. Results of the study are summarized for discrete lag time (Figure 30) and Kd (Figure 31), and generally indicate that low pH in

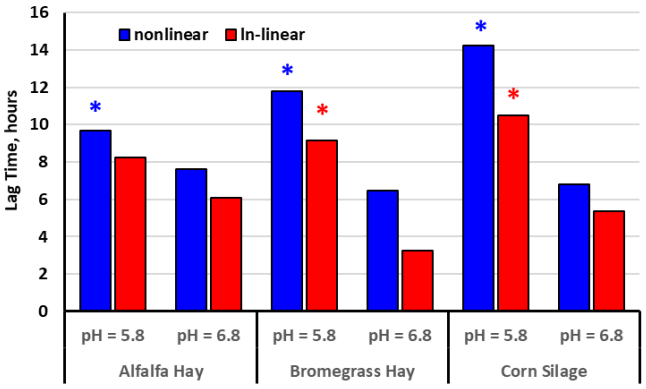


*Figure 28. Effects of pH on discrete lag times as calculated for three forages by nonlinear and ln-linear regression methods. Inclusion of an asterisk at pH = 5.8 denotes a statistical difference (P < 0.05) from the corresponding value at pH = 6.8 [adapted from Grant and Mertens (1992b)].*

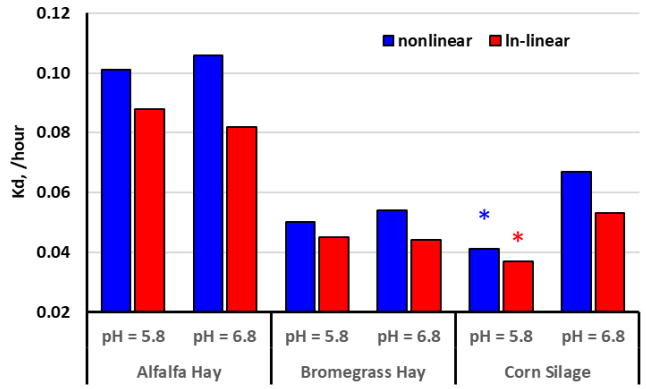


*Figure 29. Effects of pH on NDF digestion rate (Kd) as calculated for three forages by nonlinear and ln-linear regression methods. Inclusion of an asterisk at pH = 5.8 denotes a statistical difference (P < 0.05) from the corresponding value at pH = 6.8 [adapted from Grant and Mertens (1992b)].*

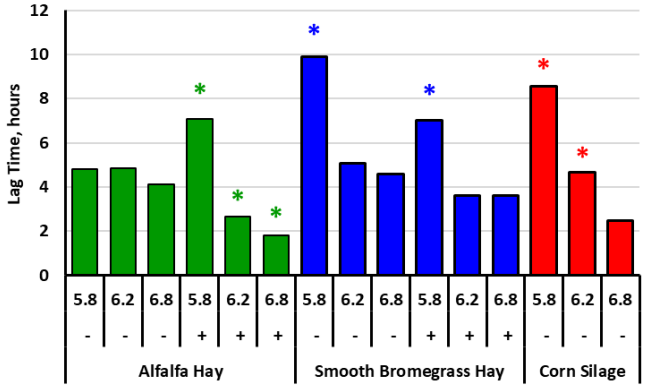


*Figure 30. Effects of starch addition and pH adjustment on discrete lag time for in vitro digestion of NDF from alfalfa hay, smooth bromegrass hay, and corn silage. Raw corn starch was added (+) or omitted (-) to the substrate pool in fermentation vessels; when raw corn starch was added, the entire substrate pool was adjusted to a concentration of 30% NDF. An asterisk (*) indicates that Kd differed (P < 0.05) from the value exhibited at pH = 6.8 without additional starch [adapted from Grant and Mertens (1992c)].*

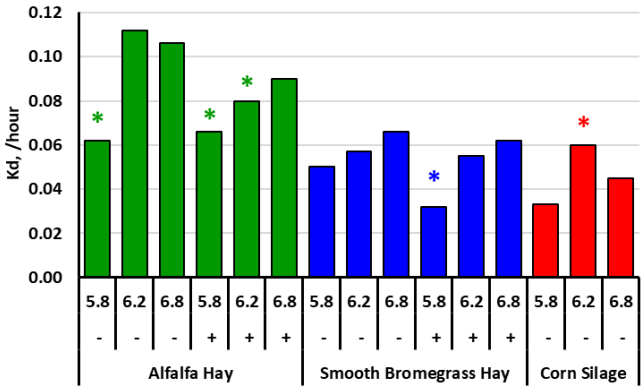


*Figure 31. Effects of starch addition and pH adjustment on digestion rate (Kd) for in vitro digestion of NDF from alfalfa hay, smooth bromegrass hay, and corn silage. Raw corn starch was added (+) or omitted (-) to the substrate pool in fermentation vessels; when raw corn starch was added, the entire substrate pool was adjusted to a concentration of 30% NDF. An asterisk (*) indicates that Kd differed (P < 0.05) from the value exhibited at pH = 6.8 without additional starch [adapted from Grant and Mertens (1992c)].*

the fermentation media decreased (slowed) Kd and increased discrete lag times. Furthermore, the presence of starch enhanced these effects for some forage substrates. For example, alfalfa hay incubated alone (without additional starch) exhibited discrete lag times that were not affected by pH (overall mean = 4.59 hours), but they were shorter at pH 6.2 and 6.8 (mean = 2.23 hours) compared to the most acidic conditions (pH = 5.5; lag = 7.06 hours) in the presence of additional starch (Figure 30).

In a procedurally similar study, Grant (1994) further examined the effects of starch type (raw corn starch, raw sorghum starch, or pure corn starch) on the in vitro kinetics of NDF digestion for alfalfa hay. Starch sources were selected based on sharply differing in vitro disappearance rates, where sorghum starch exhibited the slowest rate (0.055/hour) followed by raw corn starch (0.079/hour) and pure corn starch (0.102/hour). For this study the terminal incubation interval was set at 96 hours, and kinetic parameters were fitted using the ln-linear regression method. Effects of starch type and media pH on Kd and discrete lag times are summarized in Figure 32, while effects on pdNDF also are summarized in Figure 33. Results suggested that responses to media pH were not linear, as the mean discrete lag time increased from 2.85 to 4.44 hours (56%) between pH 6.8 and 6.2, but from 4.44 to 11.34 hours (155%) when pH was lowered further from 6.2 to 5.5. Conversely, the mean Kd at pH 5.5 (0.028/hour) was sharply reduced compared to pH 6.2 (0.058/hour) or pH 6.8 (0.060/hour). Similarly, the potential extent of NDF digestion was sharply reduced at pH 5.5 (32.8% of NDF) compared to pH 6.2 (52.7%) or pH 6.8 (54.1%). Generally, these responses were somewhat muted with the addition of raw sorghum starch, which exhibited a slower starch digestion rate than the raw or pure corn starches. While the reduction of pH within the fermentation media yielded similar trends, irrespective of starch inclusion, starch sources affected Kd and discrete lag times somewhat differently, thereby yielding significant interactive effects.

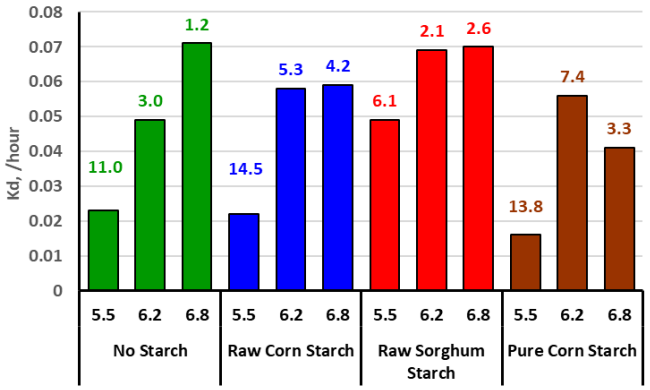


*Figure 32. Effects of starch type and media pH (5.5, 6.2, or 6.8) on the in vitro digestion rate (Kd) of NDF for alfalfa hay (40.3% NDF). Addition of various starch types were formulated such that the overall substrate concentration of NDF was about 28%. Data labels reflect the associated discrete lag times (hours) for each treatment. The potential extent of NDF digestion was based on a 96-hour incubation [adapted from Grant (1994)].*

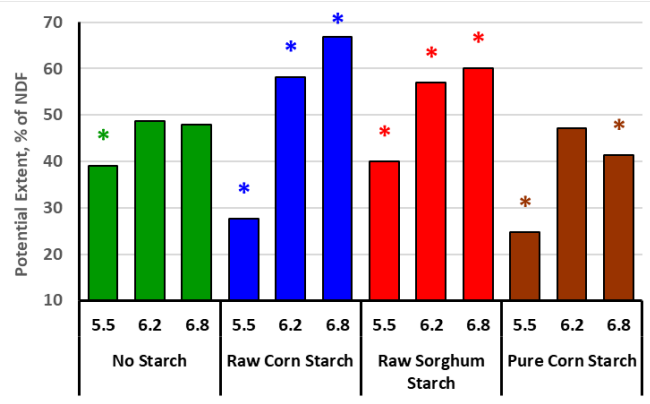


*Figure 33. Effects of starch type and media pH (5.5, 6.2, or 6.8) on the potential extent of in vitro NDF digestion for alfalfa hay (40.3% NDF). Addition of various starch types were formulated such that the overall substrate concentration of NDF was about 28%. Insertion of an asterisk as a data label indicates the treatment differed from no starch/pH = 6.8 treatment at the P < 0.05 level of confidence [adapted from Grant (1994)].*

**Kinetics of Plant Parts.** A number of studies have evaluated the digestion kinetics of NDF for specific plant parts (e.g. leaf, leaf sheath, stem, etc.); however, it is important to remember that the overall digestibility of forage fiber is also affected strongly by the proportion of each plant tissue type in the total forage NDF pool. While it is true that assessments of whole-plant digestive kinetics generally reflect the responses for individual plant tissue types considered proportionally, this generalization is not absolute. It assumes no interactive effects, and ignores the inherent variability within in vitro and in situ procedures. A further complication in this regard is the inherent differences in forages. For example, compositional and kinetic differences between leaf and stem tissues for alfalfa or other legume forages are usually quite large and distinct, whereas those for perennial warm-season grasses are (by comparison) far more muted. Regardless, a few generalizations are illustrated from several published experiments (Cherney et al., 1983; Buxton, 1989; Coblentz et al., 1998; Ferreira et al., 2022).

First, kinetic parameters differ distinctly for leaf and stem tissues of legumes. Figure 34 illustrates the effects of plant part (leaf, stem, and whole plant) on Kd and uNDF for alfalfa and red clover forages harvested in Kansas during September (Coblentz et al., 1998). Kinetic parameters were determined by in situ incubations and nonlinear regression procedures. For alfalfa, Kd differed sharply between leaf and stem tissues (0.091 vs. 0.053/hour), as did uNDF (24.6 vs. 61.2% of NDF). These responses reflect the sharp respective differences in concentrations of NDF (25.4 vs. 59.4% of DM) and ADL (3.7 vs. 11.1% of DM) for these plant parts, as well as their primarily metabolic and structural functions. Given that the alfalfa forage was 65.1% leaf tissue, whole-plant determinations of Kd (0.075/hour) and uNDF (46.8% of NDF) were intermediate between those observed for the two individual tissue types, generally reflecting proportional contributions from both. For red clover, which exhibited a greater percentage of leaf (84.9%), uNDF was similar in comparisons of leaf and whole-plant issues (26.2 vs. 27.9% of NDF), which was consistent with the expected small contributions from stem tissue (47.2% of NDF). However, while Kd for whole-plant red clover (0.072/hour) was similar to that of whole-plant alfalfa, the estimate was notably slower than either of its individual tissue types that also differed sharply (0.114 or 0.089/hour). Given the complexities of kinetic assessments and their associated inherent variabilities, such anomalies may be confusing, disconcerting, or illogical, but they are not necessarily uncommon.

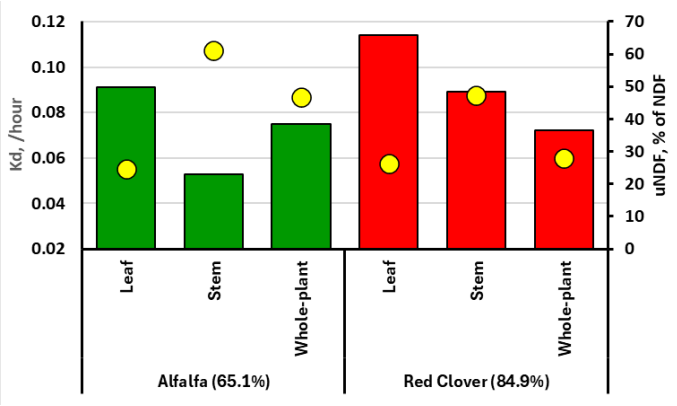


*Figure 34. In situ determinations of NDF digestion rate (Kd) and undigestible NDF (uNDF) for leaf, stem, and whole-plant tissues of alfalfa and red clover, where Kd is represented by bars ( or ) and uNDF by yellow circles ( ). Calculated values for Kd and uNDF were determined by nonlinear regression, and are keyed to the left and right y-axis, respectively. Forages were harvested in Kansas during September when alfalfa and red clover were 65.1 and 84.9% leaf, respectively [adapted from Coblentz et al. (1998)].*

Additionally, digestive characteristics of fiber from cool-season forages differ by plant part and are affected negatively by maturity. A study conducted in Minnesota by Cherney et al. (1983) examined two cereal-grain forages (oat and barley) harvested either 7 or 28 days after the first emergence of inflorescence. In vitro kinetic parameters were determined for leaf blade, leaf sheath, stem, and inflorescence tissues via ln-linear regression procedures using a 72-hour digestion as the terminal incubation interval. Rates of digestion (Kd) and uNDF are summarized for leaf blade, leaf sheath, and stem in Figures 35 and 36. Within maturity rating, the Kd for leaf blades was faster than observed for the leaf sheath and stem, while Kd was generally similar between leaf sheath and stem tissues. Furthermore, Kd generally slowed with plant maturity; averaged across the two forage species, the 21-day harvest delay resulted in a slower rate of pdNDF digestion for leaf blade (0.103 vs. 0.111/hour), leaf sheath (0.076 vs. 0.082/hour), stem (0.065 vs. 0.086/hour), and inflorescence (0.039 vs. 0.078/hour). For uNDF, there were very consistent trends with respect to both plant part and maturity. Averaged across forages and harvest dates, uNDF was greatest for stem tissue

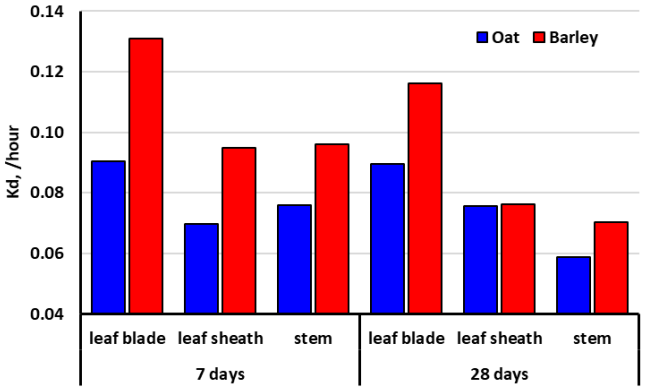


*Figure 35. In vitro digestion rate of NDF (Kd) for individual plant parts of oat and barley forages harvested either 7 or 28 days after the first emergence of inflorescence in Minnesota. Digestion rate was calculated by ln-linear regression procedures with a terminal incubation interval of 72 hours [adapted from Cherney et al. (1983)].*

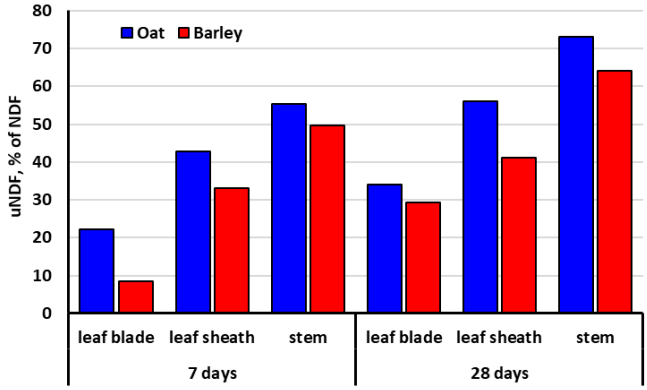


*Figure 36. In vitro undigestible NDF (uNDF) for individual plant parts of oat and barley forages harvested either 7 or 28 days after the first emergence of inflorescence in Minnesota. Digestion kinetics were calculated by ln-linear regression procedures with a terminal incubation interval of 72 hours [adapted from Cherney et al. (1983)].*

(60.6% of NDF), followed by leaf sheath and leaf blade (43.2 and 23.5% of NDF, respectively), and included consistent increases with harvest delay for each of these plant parts (Figure 36). In addition, it was further concluded that grain fill had little effect on the fiber digestion kinetics exhibited by these forages.

Another study conducted by Buxton (1989) further illustrated the effects of maturity on the in vitro NDF digestion of stem tissue obtained from various temperate legumes and grasses. Kinetic parameters were determined by nonlinear regression procedures using a 72-hour incubation as the terminal time point (Table 4). During Year 2 of the

*Table 4. Effects of maturity on in vitro digestion kinetics of NDF from stem tissues of temperate legumes and grasses. Kinetic parameters were calculated by nonlinear regression, where a 72-hour incubation was the terminal incubation time. Data for smooth bromegrass, orchardgrass, alfalfa, and birdsfoot trefoil represent the mean of two cultivars, while red clover was represented by only a single cultivar[1] [adapted from Buxton (1989)].*

| Forage | Year | Maturity[2] | NDF | uNDF[3] | Kd[4] |
|---|---|---|---|---|---|
| | | | *% of DM* | *% of NDF* | */hour* |
| Smooth Bromegrass | 1 | seed ripening | 64.2 | 66.0 | 0.035 |
| | 2 | stem elongation | 65.7 | 30.6 | 0.074 |
| | 2 | seed ripening | 66.4 | 63.9 | 0.030 |
| Orchardgrass | 1 | seed ripening | 68.9 | 65.9 | 0.036 |
| | 2 | stem elongation | 60.0 | 33.3 | 0.080 |
| | 2 | seed ripening | 72.0 | 66.1 | 0.035 |
| Alfalfa | 1 | mid seed ripening | 73.6 | 67.9 | 0.074 |
| | 2 | early flower | 58.2 | 55.7 | 0.085 |
| | 2 | mid seed ripening | 74.9 | 68.4 | 0.048 |
| Birdsfoot Trefoil | 1 | mid seed ripening | 74.4 | 64.5 | 0.107 |
| | 2 | early flower | 53.9 | 52.1 | 0.080 |
| | 2 | mid seed ripening | 72.6 | 62.5 | 0.061 |
| Red Clover | 1 | early seed ripening | 58.3 | 50.1 | 0.039 |
| | 2 | late bud | 32.9 | 45.0 | 0.120 |
| | 2 | early seed ripening | 60.9 | 56.3 | 0.060 |

*[1] Cultivars: smooth bromegrass, Barton and Rebound; orchardgrass, Napier and Orion; alfalfa, Magnum and Spredor 2; birdsfoot trefoil, Empire and Viking; and red clover, Arlington.*
*[2] Maturity ratings as described by Buxton (1989).*
*[3] uNDF = undigestible NDF, as determined by nonlinear regression.*
*[4] Kd, in vitro digestion rate of NDF.*

trial, forages were harvested at two differing maturities, which were (generally) stem elongation and seed ripening for grasses (smooth bromegrass and orchardgrass) and at early flower and seed ripening for legumes (alfalfa, birdsfoot trefoil, and red clover). The more mature forages consistently exhibited slower Kd and substantially greater uNDF, regardless of forage type (legume or grass). Averaged across both grasses for Year 2 of the trial, the mean Kd for mature grasses (0.033/hour) was less than half that of grasses harvested during stem elongation (0.077/hour). Sharp maturity effects also were observed for uNDF, where mature grasses exhibited much greater uNDF (65.0% of NDF) compared to grasses harvested during stem elongation (32% of NDF). Similar trends in response to maturity also were observed for the legumes; for example, the Kd for immature (early flowering) alfalfa stems (0.085/hour) was nearly twice as fast as observed subsequently during seed ripening (0.048/hour).

Another generality is that perennial warm-season grasses are somewhat unique. For perennial warm-season grasses, it is possible to discern differences in fiber digestion kinetics between leaf and stem tissues, but they tend to be considerably more muted compared to those observed for the cool-season forages described previously. Coblentz et al. (1998) evaluated leaf and stem tissues of eastern gamagrass at three maturities, defined as boot, anthesis (flowering), and mature forage. It is noteworthy that only a low percentage of tillers developed reproductively; therefore, percentages of leaf were quite high across all maturities (69.5 to 78.1% of DM). Leaf tissue was quite fibrous in nature; their concentrations of NDF ranged narrowly across the three noted maturity levels (69.2 to 76.8%). For stem tissue, concentrations of NDF exhibited a similarly narrow range (69.0 to 81.1%) that differed only marginally from leaf tissue harvested at the same stages of growth. For this study, in situ kinetic parameters were fitted by nonlinear regression procedures with a terminal incubation interval of 96 hours. Digestion rate (Kd) and uNDF for leaf, stem, and whole-plant tissues are summarized in Figure 37. While Kd declined with maturity, it was characteristically slow for all maturities, which is a consistent trait observed for other perennial warm-season grasses. Digestion rate ranged from 0.054 to 0.043/hour for leaf tissue, and slightly slower (0.045 to 0.035/hour) for stem. Conversely, uNDF increased

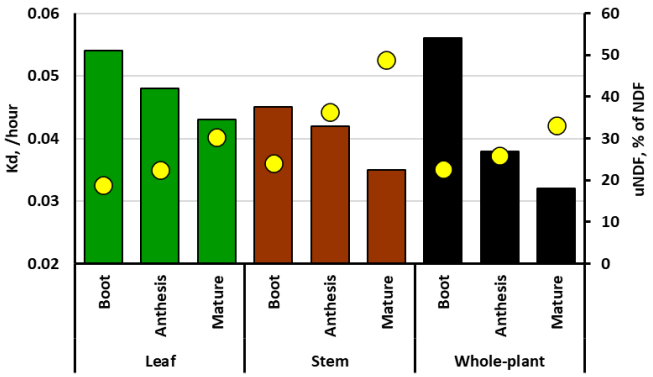


*Figure 37. In situ determinations of NDF digestion rate (Kd) and undigestible NDF (uNDF) for leaf, stem, and whole-plant tissues of eastern gamagrass harvested in Kansas at three stages of growth. The Kd is represented by bars ( , , or ) and uNDF by yellow circles ( ). Calculated values for Kd and uNDF were determined by nonlinear regression with a 96-hour terminal incubation time, and are keyed to the left and right y-axis, respectively [adapted from Coblentz et al. (1998)].*

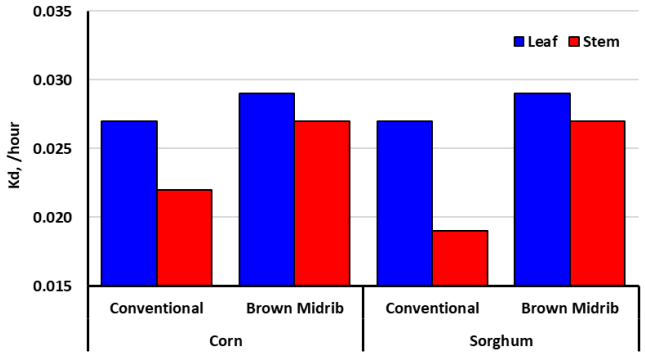


*Figure 38. In situ determinations of NDF digestion rate (Kd) for leaf and stem tissues obtained from corn and sorghum forages either with or without the brown midrib mutation. Corn was harvested at ¼ to ½ milkline, while sorghum was harvested at the milky to soft-dough stages of growth. Digestion rate was calculated by nonlinear regression procedures with a 240-hour terminal incubation time [adapted from Ferreira et al. (2022)].*

sharply with plant maturity, reaching maxima of 30.4 and 48.8% of NDF for leaf and stem tissues, respectively, at physiological maturity.

Finally, it is important to note how the brown midrib (*bmr*) trait affects digestive characteristics of warm-season annuals. Recently Ferreira et al. (2022) reported in situ kinetic assessments of NDF digestion for several warm-season annual forages. Results for corn and sorghum are summarized for Kd (Figure 38) and uNDF (Figure 39) based on nonlinear regression procedures with a 240-hour terminal incubation interval. For this experiment, both conventional cultivars, as well as those possessing a *bmr* mutation

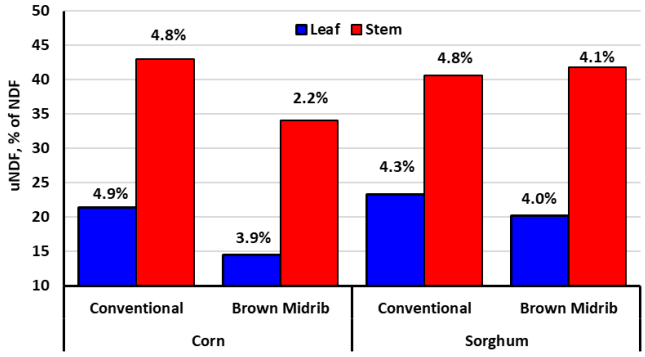


*Figure 39. In situ determinations of undigestible NDF (uNDF) for leaf and stem tissues obtained from corn and sorghum forages either with or without the brown midrib mutation. Corn was harvested at ¼ to ½ milkline, while sorghum was harvested at milky to soft-dough stages of growth. Undigestible NDF (uNDF) was calculated by nonlinear regression procedures with a 240-hour terminal incubation time. Data labels indicate the associated concentrations of acid detergent lignin (ADL) [adapted from Ferreira et al. (2022)].*

were evaluated, where corn was harvested at ½ to ¼ milk line, and the sorghum at the milky to soft-dough stage of growth. For both leaf and stem tissues, Kd was characteristically slow for warm-season grasses, ranging from 0.019 to 0.029/hour. Rates did not vary statistically within individual tissue types on the basis of forage species or *bmr* mutation. In contrast, there were sharp reductions in uNDF observed for corn cultivars possessing the *bmr* mutation, which was likely related to the reduced concentrations of ADL typically observed within *bmr* mutants. For both corn and sorghum cultivars, uNDF was sharply less for leaf tissue compared to stem, where overall respective means were 19.9 and 39.9% of NDF.

**Comparisons of Nonlinear and ln-Linear Regression Methods.** In his comprehensive review of digestion kinetics of cell walls, Mertens (1993) concluded that both ln-linear and nonlinear regression procedures will provide acceptable estimates of kinetic parameters when used with valid mathematical descriptions of the decay model, and when accurate data is collected from well-designed experiments. A similar conclusion was reported by Moore and Cherney (1986) after conducting in vitro digestion evaluations of cell wall and cell wall components obtained from alfalfa, tall fescue, sorghum × sudangrass, and wheat-straw forages. That stated, nonlinear regression procedures are generally the preferred approach because they result in the smallest residual sums of squared deviations from the decay model (Mertens, 1993). Other practical advantages and disadvantages of each regression approach have been discussed previously (see the ***ln-Linear Method*** and ***Nonlinear Regression*** sections found on pages 9 and 13, respectively).

While both regression methods may produce acceptable parameter estimates, this does not mean that the kinetic parameters produced by each method will be identical. Mertens (1993) strongly advised careful scrutiny in any situation when there are large discrepancies between parameter estimates derived from the two regression approaches, and particularly when long lag times are expected prior to initiation of digestion. This has been specifically illustrated in detail for orchardgrass hays by Mertens and Loften (1980). Similarly, cautions about systematic bias resulting from use of nonlinear regression procedures modeling digestions with long lag times have been noted by Moore and Cherney (1986), which may yield reduced estimates of Kd and lag time. While acknowledging these cautions, several studies have used both regression techniques to estimate kinetic parameters; these include assessments by Moore and Cherney (1986; Figure 40), Grant and Mertens (1992b; Table 5), and Bender et al. (2016; Table 6). Typically, parameter estimates from the two regression techniques have not been directly compared statistically; however, Grant and Mertens (1992b) noted that their estimates of Kd were about 20% faster when using nonlinear regression procedures. In the work by Moore and Cherney (1986), Kd calculated by the two regression methods varied inconsistently across forage

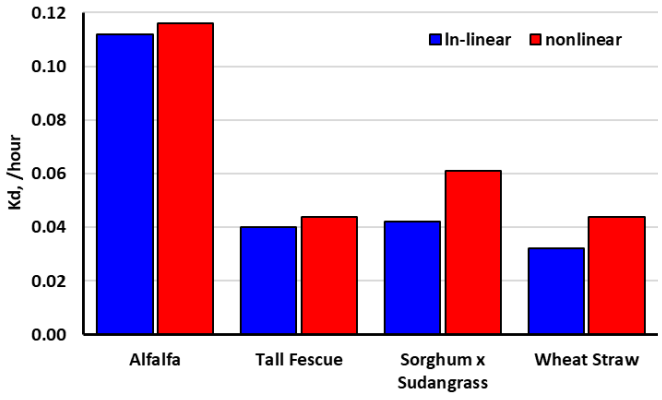


*Figure 40. Comparisons of in vitro rates (Kd) of cell wall digestion for four diverse forages as determined by ln-linear and nonlinear regression procedures, where the terminal incubation time was 96 hours [adapted from Moore and Cherney (1986)].*

*Table 5. Effects of regression technique [nonlinear (NL) or ln-linear (ln-L)] and media pH on parameter estimates of in vitro NDF digestion of alfalfa hay, smooth bromegrass hay, and corn silage [adapted from Grant and Mertens (1992b)].*

| | | Kd[1] | | uNDF[2] | | Lag time | |
|---|---|---|---|---|---|---|---|
| **Forage** | **Media pH** | **NL** | **ln-L** | **NL** | **ln-L** | **NL** | **ln-L** |
| | | *----- /hour -----* | | *% of initial DM* | | *---- hours ----* | |
| Alfalfa Hay | 5.8 | 0.101 | 0.088 | 24.5 | 24.4 | 9.7* | 8.2 |
| | 6.8 | 0.106 | 0.082 | 24.1 | 24.2 | 7.6 | 6.1 |
| Smooth Bromegrass Hay | 5.8 | 0.050 | 0.045 | 30.8 | 31.7* | 11.8* | 9.2* |
| | 6.8 | 0.054 | 0.044 | 25.9 | 27.7 | 6.5 | 3.3 |
| Corn Silage | 5.8 | 0.041* | 0.037* | 11.4 | 15.8* | 14.2* | 10.5* |
| | 6.8 | 0.067 | 0.053 | 11.6 | 11.3 | 6.8 | 5.4 |

** Denotes a statistical difference (P < 0.05) between media pH = 5.8 and 6.8.*

*[1] Kd, in vitro digestion rate of NDF.*

*[2] uNDF, undigestible NDF expressed as a percentage of the initial substrate DM. Kinetics were based on a 96-hour terminal incubation interval.*

*Table 6. The effects of incubation length and regression technique (nonlinear or ln-linear) for in vitro and in situ evaluations of NDF digestion of 13 corn silages [adapted from Bender et al. (2016)].*

| **Regression Type** | **Technique** | **Incubation Length** | **Kd[1]** | **uNDF[2]** | **Lag time** |
|---|---|---|---|---|---|
| | | *Hours* | */hour* | *% of NDF* | *hours* |
| nonlinear | in situ | 120 | 0.021 | 28.7 | 8.7 |
| | | 288 | 0.019 | 24.5 | 8.5 |
| | in vitro | 120 | 0.024 | 34.3 | 9.0 |
| | | 288 | 0.022 | 29.8 | 8.8 |
| ln-linear | in situ | 120 | 0.023 | 32.1 | 9.7 |
| | | 288 | 0.021 | 25.7 | 9.4 |
| | in vitro | 120 | 0.028 | 37.8 | 10.3 |
| | | 288 | 0.024 | 31.2 | 9.8 |

*[1] Kd, digestion rate of NDF.*

*[2] uNDF, undigestible NDF as expressed as a percentage of initial NDF.*

substrates, but estimates of Kd considered across all forage substrates were about 17% faster when fitted by nonlinear regression procedures, which is illustrated graphically in Figure 40.

**Three-Pool Models of NDF Digestion.** As discussed in the ***ln-Linear Method*** section and illustrated in Figure 12, the relatively recent use of extended (~ 240-hour) incubation intervals to better define uNDF has highlighted long-established concerns about NDF actually being comprised of multiple digestible pools. This would violate the essential condition of a single homogenous digestible pool that is required for measuring intrinsic rates of digestion (Mertens, 1993). When ln-linear regression procedures are used to calculate first-order kinetic parameters, the presence of an additional slowly digested pool of NDF frequently results in significant bias, leading to slower estimates of Kd, as well as lag times < 0 hours, which are illogical even though they can be calculated (Figure 11). Concerns about multiple digestible pools have existed for many years. One early example of using a three-pool (fast, slow, and undigestible) model to describe the digestibility of NDF for alfalfa and Coastal bermudagrass was developed by Mertens and Ely (1979) and summarized in Table 7. In that evaluation, estimates of Kd for the fast-digesting pool ($\mathbf{Kd_{fast}}$) were about five times faster than observed for the slow-digesting pool ($\mathbf{Kd_{slow}}$). In these cases, the slowly digested pools for alfalfa and Coastal bermudagrass (9.6 and

*Table 7. Chemical composition and kinetic parameters for alfalfa and Coastal bermudagrass forages based on a three-pool model of NDF digestion [adapted from Mertens and Ely (1979)].*

| Item | Alfalfa | Coastal Bermuda-grass |
|---|---|---|
| NDF, % of DM | 46.6 | 70.0 |
| ADF, % of DM | 36.3 | 34.7 |
| Perm. Lign.[1], % of DM | 9.7 | 5.4 |
| $pdNDF_{fast}$,[2] % of NDF | 42.4 | 51.7 |
| $Kd_{fast}$,[3] /hour | 0.101 | 0.092 |
| $pdNDF_{slow}$,[4] % of NDF | 9.6 | 15.2 |
| $Kd_{slow}$,[5] /hour | 0.019 | 0.022 |
| uNDF,[6] % of NDF | 48.0 | 33.1 |

[1] *Permanganate lignin.*
[2] *$pdNDF_{fast}$, potentially digestible NDF from fast digestion pool.*
[3] *$Kd_{fast}$, digestion rate of fast NDF pool.*
[4] *$pdNDF_{slow}$, potentially digestible NDF from slow digestion pool.*
[5] *$Kd_{slow}$, digestion rate of slow NDF pool.*
[6] *uNDF, undigestible NDF.*

15.2% of NDF, respectively) comprised about 20% of the total digestible NDF (pdNDF).

Recently, Raffrenato et al. (2019) reported a detailed study that evaluated several forage types (conventional or *bmr* corn silages, grasses, straws and hays, and alfalfa) for proportions of NDF within fast, slow and undigestible pools when a 240-hour incubation was used to establish uNDF. Rates of digestion ($Kd_{fast}$ and $Kd_{slow}$) also were estimated using the following nonlinear regression model:

$$NDF(t) = pdNDF_{fast} \times e^{-Kdfast\ (t - L)} + pdNDF_{slow} \times e^{-Kdslow\ (t - L)} + uNDF$$

For this three-pool expression, NDF(t) = NDF remaining at time t, $pdNDF_{fast}$ = potentially digestible NDF (fast pool; % of NDF); $Kd_{fast}$ = digestion rate of fast pool (/hour); t = incubation interval (hours); L = discrete lag time; $pdNDF_{slow}$ = potentially digestible NDF (slow pool; % of NDF); $Kd_{slow}$ = digestion rate of slow pool (/hour); and uNDF = undigestible NDF (% of NDF). [Note: a single value for lag time (L) was applied to both fast and slowly digestible NDF pools]. Pool sizes and corresponding estimates of Kd are summarized for various forage types in Figures 41 and 42, respectively. Particularly noteworthy are the greater proportion of total NDF associated with the fast pool ($pdNDF_{fast}$) for *bmr* compared to conventional corn silages, as well as the large percentages of uNDF observed for alfalfa, which reflects its elevated level of lignification, particularly within stem tissues. Across these forage types, estimates of $Kd_{slow}$ varied, but averaged about 20% (range = 17.0 to 27.6%) of the rate observed for the digestion of the corresponding fast pools.

Using a similar three-pool modeling approach, Coblentz et al. (2018) examined the effects of plant maturity on in vitro digestion of NDF from triticale forages harvested in Wisconsin. 'Forerunner' triticale was established in September of 2015, and then harvested at the stem elongation, boot, heading, anthesis, and soft-dough stages of growth during the following spring and summer. A summary of the effects of plant maturity on various pool sizes of NDF is shown in Figure 43, which includes small portions of NDF that were not recovered after 0 hours of incubation (fraction A). As discussed in the section on ***Fraction A*** (pg 6), this incomplete

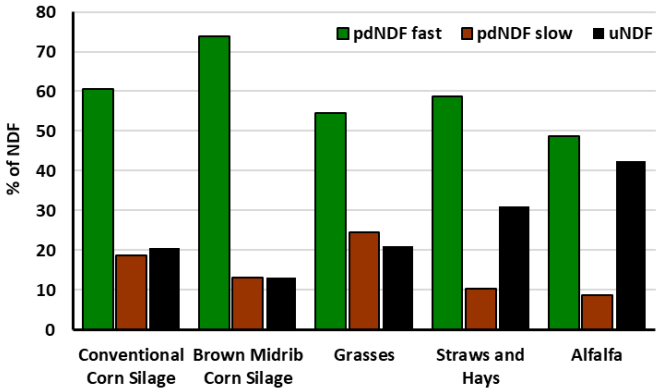


*Figure 41. Summary of fast ($pdNDF_{fast}$), slow ($pdNDF_{slow}$), and undigestible NDF (uNDF) digestion pools (% of total NDF) for several forage types as reported by Raffrenato et al. (2019).*

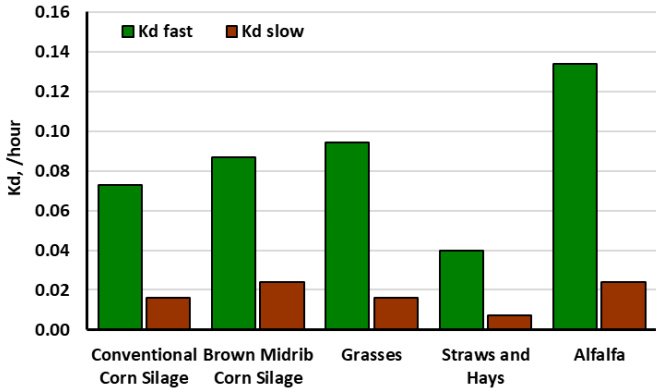


*Figure 42. Summary of digestion rates (Kd) for fast ($pdNDF_{fast}$) and slow ($pdNDF_{slow}$) NDF digestion pools for several forage types as reported by Raffrenato et al. (2019).*

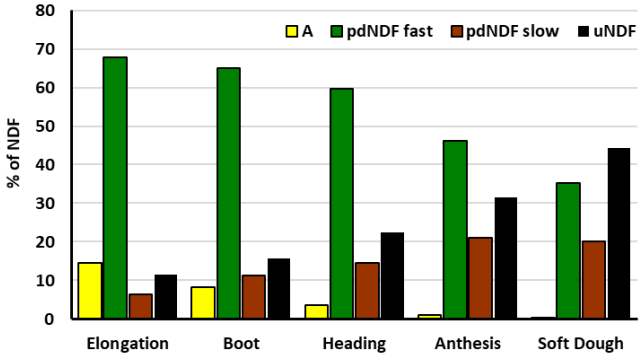


*Figure 43. Summary of various NDF pools derived from in vitro digestion of triticale forages harvested at Marshfield, Wisconsin, from May through July of 2016. Pools of NDF were determined through a nonlinear three-pool regression model; to limit the number of parameters to be estimated by the regression model, uNDF was determined directly via a 240-hour incubation. Pools of NDF are defined as: A, percentage of NDF not recovered at 0 hours of incubation; $pdNDF_{fast}$, percentage of NDF within the fast-digesting pool; $pdNDF_{slow}$, percentage of NDF within the slow-digesting pool; and uNDF, undigestible NDF [adapted from Coblentz et al. (2018)].*

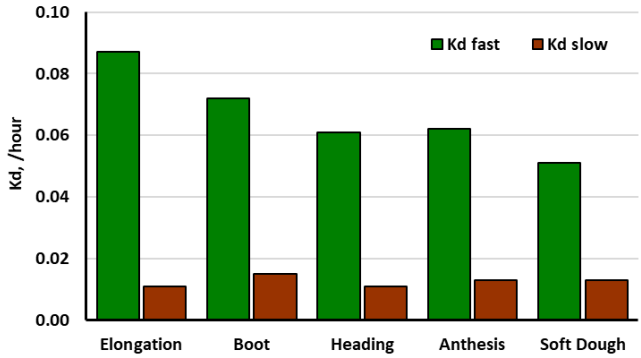


*Figure 44. In vitro digestion rates of fast ($Kd_{fast}$) and slow ($Kd_{slow}$) digestion pools of NDF for triticale forages harvested at Marshfield, Wisconsin, from May through July of 2016 [adapted from Coblentz et al. (2018)].*

recovery is not uncommon with in vitro or in situ studies and may be most notable when evaluating immature cool-season grasses (Hoffman et al., 1993), and/or when using porous in situ or F57 filter bags (Ghimire and Ferreira, 2026). In this specific case, fraction A accounted for 14.5% of NDF during stem elongation, but declined to essentially nil by the time triticale flowered (anthesis). Other effects of maturity on pool sizes were somewhat predictable; the fast-digesting pool of NDF declined sharply with plant maturity, while the slowly digested and uNDF pools increased. The soft dough stage of growth has often been discussed as a possible recommended silage harvest target for cereal-grain forages; it is noteworthy that the slowly digested and uNDF pools comprised about 65% of the total NDF at this growth stage, indicating that i) NDF digestibility is poor at this growth stage, ii) nutritional benefits of these relatively mature forages are derived primarily from production/accumulation of the non-fibrous carbohydrates associated with grain fill, and iii) the physiological process of grain fill may have a strongly positive effect on the digestibility of DM, but NDF digestibility is largely unaffected.

Rates of pdNDF digestion for the fast pool ($Kd_{fast}$) declined with plant maturity from 0.087/hour during stem elongation to 0.051/hour at the soft dough stage of growth (Figure 44). However, estimates of $Kd_{slow}$ were largely unaffected by plant maturity, ranging narrowly from 0.011 to 0.015/hour across the five growth stages. Consistent with the previously discussed reports by Mertens and Ely (1979) and Raffrenato et al. (2019), the overall mean $Kd_{slow}$ was 18.9% of that observed for $Kd_{fast}$. Furthermore, the range for this comparison varied from 12.6 to 25.5% at individual growth stages, which tended to increase with plant maturity, largely in response to the aforementioned declining estimates of $Kd_{fast}$.

While the three-pool approach to NDF digestion may make theoretical sense considering the strong evidence that the pdNDF pool of most forages is not homogeneous, numerous issues exist that may limit the practical application of this analytical approach for routine diet formulation. Prominent among these are the time and expense requirements for conducting kinetic assessments that include enough time points to calculate valid parameter estimates. For example, a primary objective (not discussed herein) of the work published by Raffrenato et al. (2019) was to assess the specific timing and (minimal) number of incubation times required to yield meaningful assessments of various pool sizes, as well as $Kd_{fast}$ and $Kd_{slow}$. Efforts of this type are further complicated by the inherently different physiologies and compositions of forages, which may require procedural (timing) adjustments based on forage type (e.g., cool- vs. warm-season grasses). Barry and Hall (2025) also summarized the differing opinions/concerns related to the number of incubation time points that are required per parameter estimate. As an example, the nonlinear three-pool model described by Raffrenato et al. (2019) required six parameter estimates for model

convergence. Common suggestions for the required number of incubation time points typically range from 3 to 5 per parameter, which would necessitate 18 to 30 observations per kinetic evaluation for a three-pool model. This is clearly prohibitive even with a single observation per time point. Using multiple observations per time point may reduce the number of time points needed for meaningful calculation of parameter estimates, but will not necessarily reduce the number of individual incubations, time, and expense that is required. Where possible, limiting the number of parameters to be estimated by the regression model may be useful. Towards this end, Coblentz et al. (2018) specifically mentioned using a single lag time fit for both fast and slow-digesting NDF pools, and determining uNDF directly from a 240-hour incubation, rather than fitted by a nonlinear regression model as possibilities to aid model convergence.

Barry and Hall (2025) evaluated both intra- and inter-laboratory variability of parameter estimates for 12 forages evaluated by in vitro digestion at two commercial laboratories. Forages included 6 alfalfas, 3 cool-season grasses (orchardgrass, reed canarygrass, and oat), and 3 warm-season grasses (bermudagrass, bahiagrass, and corn stover). Each forage was incubated in duplicate for 0, 3, 6, 12, 18, 24, 30, 48, 72, 120, or 240 hours during each laboratory run, which was subsequently repeated a second time. The absolute values of the difference between laboratory estimates of pdNDF for each individual forage derived from two- and three-pool models are summarized for alfalfa and grass forages in Figures 45 and 46, respectively. Several key points are obvious from a quick examination of these summaries: i) the difference between laboratories for pdNDF derived from a two-pool model was relatively consistent within each forage type, and less than observed for either pdNDF pool when data were fitted to the three-pool model; and ii) excepting the oat silage, agreement between laboratories was generally much better for grasses than observed for the alfalfas. Better agreement between laboratories also was observed for estimates of Kd derived from the two-pool regression model compared to $Kd_{fast}$ and $Kd_{slow}$ determined from the three-pool model (Figures 47 and 48). On an intra-laboratory basis, agreement between incubation runs also was notably better for parameter estimates derived from the two-pool model compared to the more complex approach (Table 8). As noted by Barry and Hall (2025), the greater variability in parameter estimates derived from the three-pool modeling approach raises questions about whether 11 time points (22 observations/run) was adequate to properly estimate critical regression parameters. Regardless, the time, expense, computing power, and statistical expertise required for these evaluations may be prohibitive for routine use, and likely will require development of theoretically sound shortcuts, such as those investigated by Raffrenato et al. (2019).

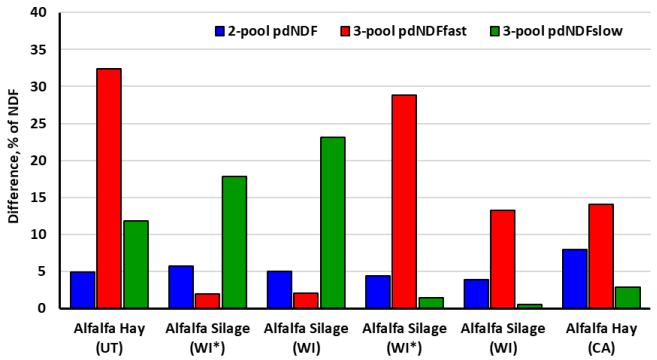


*Figure 45. Absolute value of the difference between laboratories for estimates of potentially digestible NDF (pdNDF) determined by in vitro digestion of six alfalfa forages and fitted to two- and three-pool models. Pools of pdNDF include fast- and slow-digesting pools when fitted by a three-pool model. State of origin for each forage is included parenthetically, and inclusion of an asterisk (*) indicates the forage was a low-lignin cultivar [adapted from Barry and Hall (2025)].*

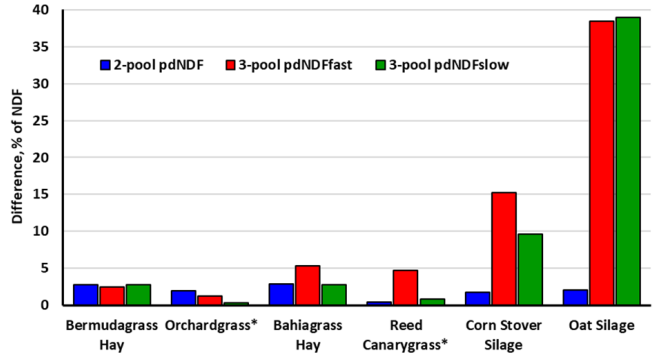


*Figure 46. Absolute value of the difference between laboratories for estimates of potentially digestible NDF (pdNDF) determined by in vitro digestion of six grass forages and fitted to two- and three-pool models. Pools of pdNDF include fast- and slow-digesting pools when fitted by a three-pool model. Inclusion of an asterisk (*) indicates the forage was dried grass, harvested without being conserved as hay or silage [adapted from Barry and Hall (2025)].*

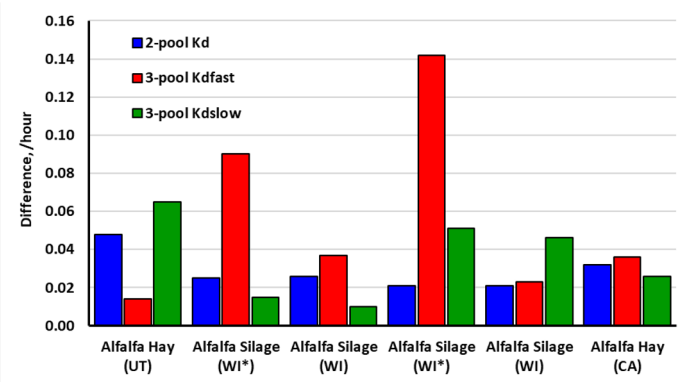


*Figure 47. Absolute value of the difference between laboratories for estimates of NDF digestion rate (Kd) determined by in vitro digestion of six alfalfa forages and fitted to two- and three-pool models. Rates of digestion (Kd) include rates for fast- and slow-digesting pools when fitted by a three-pool model. State of origin for each forage is included parenthetically, and inclusion of an asterisk (*) indicates the forage was a low-lignin cultivar [adapted from Barry and Hall (2025)].*

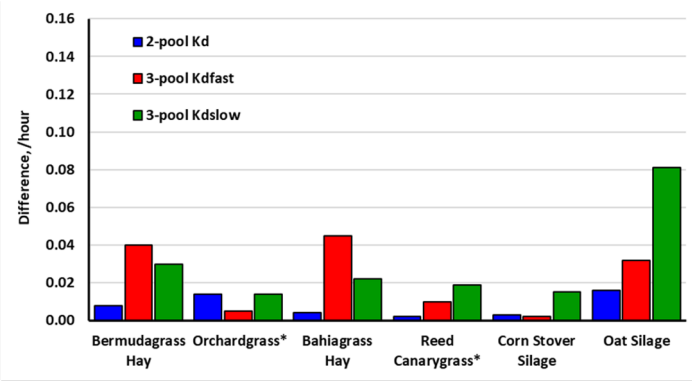


*Figure 48. Absolute value of the difference between laboratories for estimates of NDF digestion rate (Kd) determined by in vitro digestion of six grass forages and fitted to two- and three-pool models. Rates of digestion (Kd) include rates for fast- and slow-digesting pools when fitted by a three-pool model. Inclusion of an asterisk (*) indicates the forage was dried grass, harvested without being conserved as hay or silage [adapted from Barry and Hall (2025)].*

*Table 8. Absolute value of differences between laboratory runs for parameter estimates determined by two individual commercial laboratories based on two- and three-pool models of in vitro NDF digestion for 12 forages[1] [adapted from Barry and Hall (2025)].*

| | Laboratory 1 | | Laboratory 2 | |
|---|---|---|---|---|
| **Parameter Estimate** | **Mean[2]** | **Absolute Value[3] (Run 1 – Run 2)** | **Mean[2]** | **Absolute Value[3] (Run 1 – Run 2)** |
| ***2-Pool Model*[4]** | | | | |
| pdNDF, % of NDF | 61.6 | 2.5 | 56.4 | 0.4 |
| uNDF, % of NDF | 38.4 | 2.5 | 39.6 | 0.4 |
| Kd, /hour | 0.074 | 0.012 | 0.085 | 0.006 |
| Lag, hours | 1.89 | 1.67 | 2.78 | 0.92 |
| ***3-Pool Model*[5]** | | | | |
| $pdNDF_{fast}$, % of NDF | 37.4 | 18.7 | 43.6 | 7.5 |
| $pdNDF_{slow}$, % of NDF | 33.3 | 8.6 | 23.3 | 7.8 |
| uNDF, % of NDF | 29.3 | 14.6 | 29.1 | 1.7 |
| $Kd_{fast}$, /hour | 0.122 | 0.099 | 0.135 | 0.044 |
| $Kd_{slow}$, /hour | 0.039 | 0.036 | 0.020 | 0.011 |
| Lag, hours | 1.89 | 2.16 | 3.07 | 0.67 |

*[1] Forages included six alfalfa hays or silages, bermudagrass hay, dried orchardgrass, bahiagrass hay, dried reed canarygrass, corn stover silage, and oat silage.*
*[2] Mean of two within-laboratory runs.*
*[3] Absolute value of within-laboratory difference between runs.*
*[4] 2-pool abbreviations: pdNDF, potentially digestible NDF; uNDF, undigestible NDF; Kd, digestion rate of pdNDF; and lag, discrete lag time.*
*[5] 3-pool abbreviations: $pdNDF_{fast}$, potentially digestible NDF within the fast pool; $pdNDF_{slow}$, potentially digestible NDF within the slow pool; uNDF, undigestible NDF; $Kd_{fast}$, digestion rate of the fast NDF pool; $Kd_{slow}$, digestion rate of the slow NDF pool; and lag, discrete lag time.*

While the increased variabilities associated with three-pool parameter estimates are concerning, Barry and Hall (2025) also addressed another valid question: do the additional complexities discussed for the three-pool modeling approach provide a clear benefit when integrated with the competing process of rate of ruminal passage (**Kp**)? To address this question, the predicted ruminal digestibility of NDF (**RDNDF**) was calculated using a wide range of Kp (0.02, 0.03, 0.04, 0.05, 0.06, or 0.07/hour), which would generally be a range inclusive of expectations for livestock consuming coarse (high-NDF) forages at maintenance levels of intake (~ 0.02/hour), as well as high-producing dairy cows at substantially elevated voluntary intakes (~ 0.06/hour). Predicted RDNDF was calculated by the following formulas:

two-pool model: $RDNDF = pdNDF \times (Kd/(Kp + Kd)$

three-pool model: $RDNDF = [pdNDF_{fast} \times (Kd_{fast}/(Kp + Kd_{fast})] + [pdNDF_{slow} \times (Kd_{slow}/(Kp + Kd_{slow})]$

Comparisons of predicted RDNDF at various rates of Kp are summarized for the alfalfas and grasses in Figure 49. The following key points can be summarized from this figure:

- The predicted RDNDF always was reduced by increasing Kp, indicating there was reduced digestion as ruminal residence time was shortened.
- The predicted RDNDF was greater for grasses compared to the alfalfas, thereby reflecting (in part) the greater lignification normally observed within legume forages. Further caution is advised with respect to interpretation. The physical attributes of alfalfa (greater fragility) contribute to more rapid ruminal clearance rates (Kp) than observed for most grasses; therefore, any direct comparison of these forage types at the same Kp is somewhat illogical.
- Within the statistical analysis of RDNDF reported by Barry and Hall (2025), the main effect of digestion model, as well as interactions of other effects with digestion

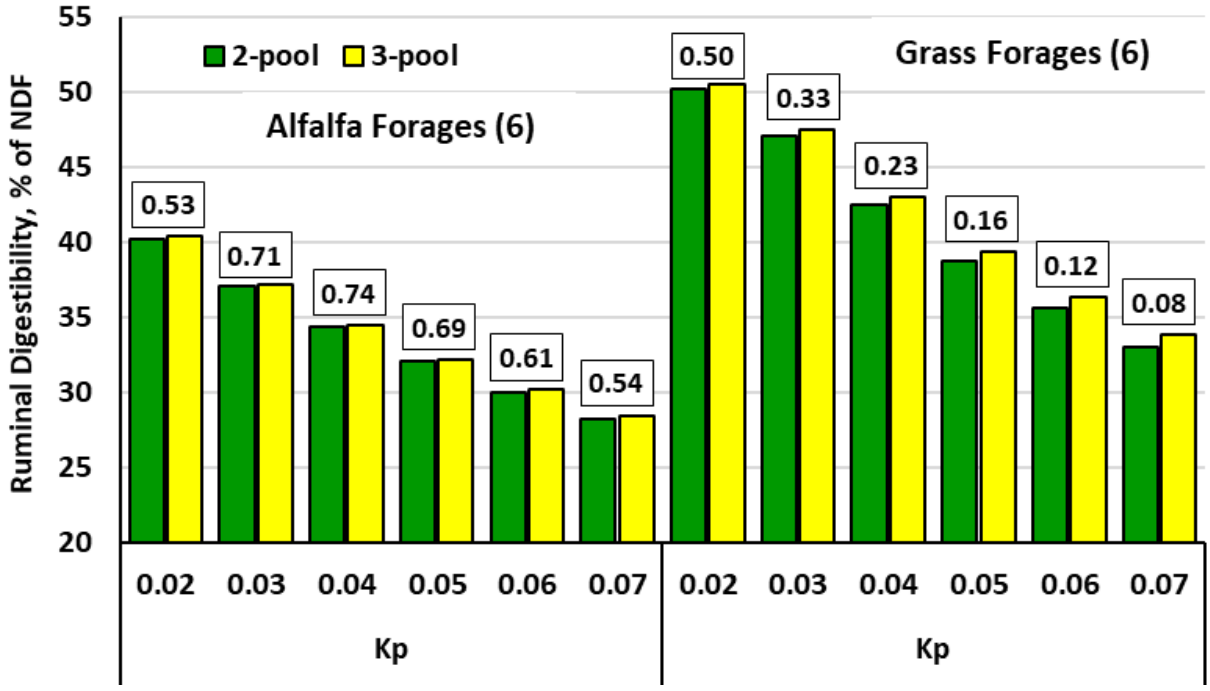


*Figure 49. Predictions of ruminal digestibility of NDF as affected by ruminal passage rate (Kp), in vitro digestion model (two- or three-pool), and forage type. Digestion regression model had no effect on predicted ruminal NDF digestion for either alfalfa or grass forages. Data labels indicate the P-value for comparisons of two- and three-pool models on predicted ruminal digestion at each value of Kp [adapted from Barry and Hall (2025)].*

model were not statistically significant ($P > 0.05$), thereby indicating that digestion model had little effect on predicted values.

- Although there may be a sound theoretical basis for using a three-pool model of fiber digestibility, concluding statements by Barry and Hall (2025) indicated that little difference was observed between two- and three-pool digestion kinetic models for predicting RDNDF, and that no obvious advantage was conferred by using the more complex three-pool model for this diverse 12-forage sample set.

## Single Timepoint Estimates of NDF Digestibility

### *Appropriate Length of In Vitro Incubation*

Given the examples and associated discussion described in Figures 7 and 13, an appropriate question that might be asked is: what is the most appropriate incubation interval for in vitro analysis of fiber digestion? In reality, the answer is somewhat complicated. Early procedurally related references (Tilley and Terry, 1963; Goering and Van Soest, 1970) firmly established a 48-hour incubation interval in buffered rumen fluid as the methodological standard, which largely has been retained today. Tilley and Terry (1963) specifically mentioned the promising possibility of objectively evaluating large numbers of individual plant (forage) genotypes that may have similar digestibilities, in part because the results would be affected only by the accuracy and precision of the method itself. In other words, excellent technique and attention to procedural detail may allow direct comparisons of relatively similar forages or the same forage as affected by maturation, thereby supporting forage breeding programs or other forage-management experiments. Additionally, 48-hour incubations leading to determination of NDFD have been important components of energy calculations in recent nutritional models for dairy cows (NASEM, 2001; 2021).

One prominent issue resulting in significant discussion, and maybe even controversy, is the relative appropriateness of a 48-hour incubation for assessing the NDFD of forages consumed by lactating dairy cows. As described by Hoffman et al. (2003), in vitro assessments of NDFD describe fiber digestion at a maintenance level of intake (no milk production or growth). In recent decades, it has been suggested that a 30-hour incubation may be more appropriate for a lactating dairy cow, thereby initiating some debate and controversy. Within that context, it should be intuitive that estimates of NDFD increase with incubation time, as illustrated for triticale forages harvested at various maturities and subsequently incubated in vitro for 24, 30, 48, or 240 hours (Coblentz et al. 2018; Figure 50). In that illustration, the mean NDFD considered across all six maturities was 41.7% of NDF with a 24-hour incubation compared to 79.7% of NDF when the same forages were incubated for 240 hours. In this specific example, the differential between 30- and 48-hour incubations was 13.2 percentage units of NDF. Because of the more rapid NDF digestion rates typically associated with alfalfa or other legume forages compared to most grasses (Table 3), the differential between NDFD estimates determined on the basis of 30- and 48-hour incubations is often larger for grasses than for legumes (Hoffman et al., 2003; Table 9).

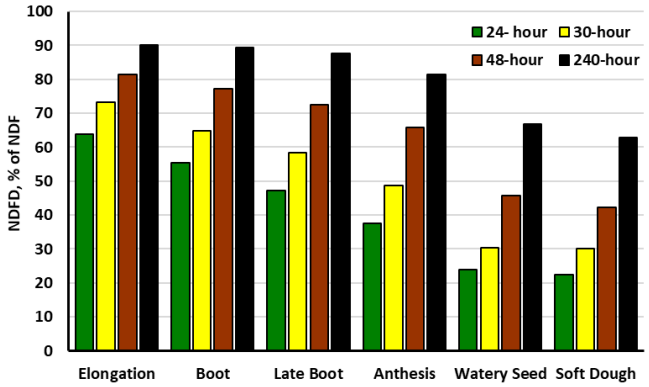


*Figure 50. Single-time-point assessments of in vitro neutral detergent fiber digestibility (NDFD; % of NDF) for triticale forages harvested during 2017 at Marshfield, Wisconsin [adapted from Coblentz et al. (2018)].*

A major drawback of shorter incubation times in laboratory determinations of NDFD is that the error or inherent variability associated with the measurement increases, and estimates of NDFD become less repeatable as incubation times are shortened. This can be observed visually (Figure 51) for the triticale forages described previously in Figure 50; standard deviations of the mean NDFD for each incubation interval generally declined as incubation time was extended, and they were clearly

*Table 9. Difference between in vitro neutral detergent-fiber digestibility (NDFD) determinations after 30- and 48-hour incubations for various forage classes [adapted from Hoffman et al. (2003)][1].*

| | | NDFD, % of NDF | | |
|---|---|---|---|---|
| **Forage Type** | **NDF, % of DM** | **30-Hour** | **48-Hour** | **Difference** |
| Grass Silage | 49.3 | 54.4 | 74.0 | 19.6 |
| | 68.0 | 30.9 | 44.0 | 13.1 |
| | 66.3 | 28.9 | 34.8 | 5.9 |
| | 71.3 | 23.0 | 28.8 | 5.8 |
| | | | ***Mean Difference*** | ***11.1*** |
| Grass Hay | 46.7 | 61.6 | 78.1 | 16.5 |
| | 59.5 | 59.2 | 68.6 | 9.4 |
| | 67.1 | 35.1 | 48.3 | 13.2 |
| | 67.1 | 36.8 | 38.4 | 1.6 |
| | 68.1 | 21.7 | 31.1 | 9.4 |
| | | | ***Mean Difference*** | ***10.0*** |
| Legume Silage | 27.1 | 56.7 | 59.6 | 2.9 |
| | 37.1 | 47.2 | 53.8 | 6.6 |
| | 49.0 | 31.8 | 40.0 | 8.2 |
| | 48.6 | 26.4 | 36.4 | 10.0 |
| | 51.3 | 34.8 | 35.1 | 0.3 |
| | | | ***Mean Difference*** | ***5.6*** |
| Legume Hay | 37.3 | 55.9 | 58.9 | 3.0 |
| | 47.4 | 47.3 | 46.7 | -0.6 |
| | 50.5 | 41.4 | 43.0 | 1.6 |
| | 51.6 | 38.9 | 39.7 | 0.8 |
| | | | ***Mean Difference*** | ***1.2*** |
| Corn Silage | 35.0 | 49.6 | 63.4 | 13.8 |
| | 37.6 | 32.5 | 50.1 | 17.6 |
| | 37.8 | 38.8 | 48.4 | 9.6 |
| | 38.5 | 61.2 | 71.0 | 9.2 |
| | 39.0 | 59.5 | 59.8 | 0.3 |
| | 39.0 | 49.7 | 62.0 | 12.3 |
| | 39.9 | 43.7 | 47.1 | 3.4 |
| | 41.6 | 46.5 | 51.9 | 5.4 |
| | | | ***Mean Difference*** | ***9.0*** |
| Small Grain Silage | 45.8 | 73.3 | 79.1 | 5.8 |
| | 52.3 | 67.8 | 77.5 | 9.7 |
| | 62.4 | 32.2 | 34.0 | 1.8 |
| | 63.4 | 40.6 | 47.4 | 6.8 |
| | 69.9 | 43.0 | 67.1 | 24.1 |
| | | | ***Mean Difference*** | ***9.6*** |

[1] *Differentials expressed herein are for illustrative purposes only and are not intended for use in lieu of actual laboratory determined values.*

the least following a 240-hour in vitro incubation. In this specific illustration, it should be noted that standard deviations were based on four field replications; therefore, the reported deviations from the mean included variability derived from the NDFD assay itself, as well as some variability between field replications. A major contributor to the problematic variability observed for shorter incubation intervals (< 48 hours) is the generally inconsistent nature of ruminal fluid at the time of inoculation. In part, this may be related to suboptimal handling procedures that can result in shock to

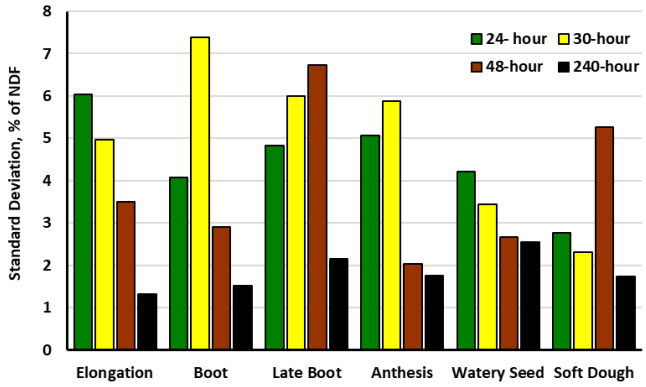


*Figure 51. Standard deviations for single-time-point assessments of in vitro neutral detergent fiber digestibility (NDFD; % of NDF) for triticale forages harvested during 2017 at Marshfield, Wisconsin. Standard deviations were based on four field replications [adapted from Coblentz et al. (2018)].*

ruminal microbes. Typically, this issue is mediated at longer incubation intervals, as illustrated in Figure 51.

In the recently released NASEM (2021) nutritional model, this issue with error/variability was further discussed with reference to the work of Lopes et al. (2015), which indicated that 48-hour in vitro incubations were more accurate at estimating in vivo measures of NDF digestibility than 30-hour in vitro incubations. Two key findings can be noted from this work. First, the total tract NDF digestibility of 21 dairy diets could not be related to NDFD estimates based on a 30-hour incubation in vitro ($P$ = 0.54), but corresponding values based on a 48-hour incubation were statistically significant (in vivo NDF digestibility = [0.61 × 48-h NDFD] + 12.03; $r^2$ = 0.30, $P$ = 0.01). Secondly, it is important to emphasize that 48-hour NDFD estimates were consistently greater than the total tract NDF digestibilities assessed in lactating dairy cows. It is well established that as dairy cows exhibit greater ad-libitum intakes and subsequently produce more milk, rates of ruminal passage (Kp) and passage through the total digestive system become more rapid. This results in a discounted or depressed digestibility of NDF, which was illustrated in the linear regression relationship reported by Lopes et al. (2015). Based on that relationship for 21 dairy production diets, a 48-hour NDFD of 60% would be discounted to 48.6% of NDF on a total-tract basis. This issue is critical to proper estimates of energy availability within many nutritional models, such as NASEM (2001, 2021). Previously, the NASEM (2001) model discounted or adjusted digestibility based on the level of intake and energy density of the diet; these adjustments were commonly referred to as 1×, 2×, 3×, 4×, or 5× levels of maintenance intake. The current (NASEM, 2021) nutritional model takes a different approach, establishing a base condition for a cow consuming a diet containing 26% starch with a level of intake set at 3.5% of bodyweight. This discounted NDF digestibility can be calculated as:

base NDF digestibility (%) = (0.61 × 48-hour NDFD) + 12

Further adjustments or discounts can then be made for elevated intakes and starch inclusion. These can be calculated as:

digested NDF (%) = base NDF digestibility (as calculated previously) – [0.59 × (% Starch – 26)] – [1.1 × DMI (% of body weight) - 3.5]

### *Common Estimates of 48-Hour In Vitro NDF Digestibility.*

Research studies have routinely reported NDFD based on 48-hour in vitro incubations of forages of the same or different species grown under a wide range of experimental conditions. Similarly, NDFD is routinely analyzed and reported as part of forage test analyses conducted by private fee-for-service laboratories. NASEM (2021) has compiled mean values for various forage types that are summarized in Table 10. This compendium includes tests conducted using traditional laboratory-based methods, as well as (secondary) near infrared reflectance (**NIRS**) procedures, which were not distinguished for this application.

When considering the data summarized in Table 10, it becomes obvious that laboratory values of NDFD are generally related inversely to plant maturity, as well as concentrations of NDF and ADL, which often are considered as pseudo-proxies for plant maturity. In part, this has been discussed previously with respect to kinetic parameters (see the ***Compendium of NDF Digestion Parameters for Forages*** section on page 15). While these relationships describe the normal generalized case, particularly when considered on a within-species basis, they do not transcend all forages considered collectively. Under certain circumstances, this generalization also can be flawed on a within-species basis. First, consider Figure 52, which illustrates the relationships between the mean value of NDFD for

*Table 10. Neutral detergent-fiber digestibility (NDFD; % of NDF) as determined from 48-hour incubations summarized from data provided by various fee-for-service laboratories (NASEM, 2021). Both traditional laboratory-based chemistry and near-infrared reflectance data were included within these summaries.*

| | | NDF,[1] % of DM | | ADL,[1] % of DM | | NDFD[1], % of NDF | | |
|---|---|---|---|---|---|---|---|---|
| **Forage** | **Description** | **Mean** | **SD[2]** | **Mean** | **SD[2]** | ***N*[3]** | **Mean** | **SD[4]** |
| ***Legumes*** | | | | | | | | |
| Hay | Immature | 37.7 | 3.69 | 6.59 | 0.776 | 6,645 | 51.4 | 8.48 |
| | mid-maturity | 41.1 | 4.84 | 6.64 | 1.148 | 49,252 | 52.4 | 9.10 |
| | Mature | 46.6 | 3.34 | 8.12 | 0.812 | 677 | 43.4 | 5.07 |
| Silage | Immature | 38.7 | 3.71 | 6.55 | 1.016 | 26,119 | 53.3 | 7.94 |
| | mid-maturity | 43.2 | 3.94 | 7.42 | 1.283 | 48,021 | 49.4 | 7.18 |
| Pea Hay | NA[5] | 43.4 | 6.65 | 5.78 | 1.452 | 2 | 58.5 | 0.71 |
| Pea Silage | NA | 52.5 | 7.04 | 6.42 | 1.644 | 6 | 57.7 | 8.66 |
| Peanut Hay | NA | 45.8 | 6.34 | 8.48 | 2.031 | 14 | 40.1 | 6.98 |
| Soybean Hay | NA | 40.3 | 7.37 | 7.23 | 1.305 | 37 | 50.5 | 9.44 |
| Soybean Silage | NA | 45.3 | 6.59 | 7.81 | 1.482 | 38 | 47.7 | 7.43 |
| ***Cool-Season Grasses*** | | | | | | | | |
| Hay | mid-maturity | 58.0 | 4.08 | 4.17 | 0.786 | 82 | 67.8 | 8.16 |
| | mature | 66.7 | 4.29 | 5.97 | 1.238 | 1,031 | 55.8 | 9.04 |
| Silage | NA | 62.1 | 4.90 | 5.82 | 1.263 | 790 | 63.6 | 5.71 |
| ***Grass/Legume Mixtures*** | | | | | | | | |
| Hay | NA | 58.2 | 5.67 | 6.70 | 1.521 | 1,069 | 54.5 | 7.25 |
| Hay, Mostly Grass | mid-maturity | 54.7 | 4.18 | 4.28 | 0.969 | 196 | 67.5 | 9.54 |
| | mature | 62.0 | 4.82 | 6.08 | 1.511 | 7,379 | 47.5 | 9.18 |
| Hay, Mostly Legume | immature | 43.9 | 4.80 | 6.92 | 1.103 | 420 | 49.9 | 6.46 |
| Silage | NA | 51.2 | 4.94 | 5.90 | 1.423 | 574 | 61.1 | 7.19 |
| Silage, Mostly Grass | NA | 57.7 | 4.62 | 5.62 | 1.240 | 2,810 | 55.2 | 8.36 |
| Silage, Mostly Leg. | NA | 45.9 | 3.82 | 6.60 | 1.198 | 1,247 | 56.2 | 5.94 |
| ***Cool-Season Annual Grasses*** | | | | | | | | |
| Barley Hay | NA | 54.5 | 5.75 | 4.07 | 1.293 | 32 | 61.3 | 9.32 |
| Barley Silage | vegetative | 56.6 | 5.42 | 5.19 | 1.209 | 193 | 57.5 | 6.19 |
| | mid-maturity | 52.9 | 5.19 | 4.96 | 0.858 | 253 | 52.1 | 4.81 |
| Oat Hay | NA | 59.0 | 6.04 | 4.71 | 1.475 | 2,598 | 56.0 | 6.55 |
| Oat Silage | immature | 45.8 | 4.28 | 3.89 | 1.072 | 136 | 59.3 | 9.27 |
| | mid-maturity | 57.4 | 5.40 | 5.39 | 1.138 | 878 | 54.1 | 6.34 |
| Rye (Fresh) | immature | 42.9 | 4.04 | 3.14 | 0.810 | 2 | 83.6 | 10.71 |
| Rye Hay | immature | 47.0 | 6.35 | 3.44 | 1.190 | 209 | 85.5 | 6.98 |
| | mid-maturity | 57.3 | 7.59 | 4.84 | 1.377 | 64 | 59.8 | 13.10 |
| | mature | 66.8 | 5.33 | 6.21 | 1.156 | 33 | 45.9 | 8.72 |
| Rye Silage | mid-maturity | 58.0 | 6.67 | 4.93 | 1.249 | 1,751 | 61.9 | 6.99 |
| | mature | 66.2 | 3.76 | 5.71 | 0.933 | 29 | 66.3 | 4.44 |
| Triticale Hay | NA | 60.0 | 7.46 | 4.84 | 1.345 | 182 | 63.7 | 9.29 |
| Triticale Silage | mid-maturity | 52.2 | 3.80 | 4.31 | 1.105 | 221 | 57.5 | 5.44 |
| | mature | 58.6 | 4.42 | 4.33 | 1.274 | 425 | 58.5 | 5.53 |
| Triticale and Pea Silage | NA | 55.7 | 4.27 | 5.46 | 1.125 | 70 | 65.1 | 4.82 |
| Wheat Hay | vegetative | 58.0 | 6.03 | 4.79 | 1.393 | 58 | 59.3 | 8.28 |
| | headed | 52.8 | 5.19 | 4.91 | 0.946 | 18 | 57.2 | 8.36 |

*Table 10 (cont'd).*

| Forage | Description | NDF,[1] % of DM Mean | NDF,[1] % of DM SD[2] | ADL,[1] % of DM Mean | ADL,[1] % of DM SD[2] | NDFD[1], % of NDF *N*[3] | NDFD[1], % of NDF Mean | NDFD[1], % of NDF SD[4] |
|---|---|---|---|---|---|---|---|---|
| ***Cool-Season Annual Grasses (cont'd)*** | | | | | | | | |
| Wheat Silage | vegetative | 56.6 | 5.50 | 4.79 | 1.139 | 1,034 | 59.0 | 6.12 |
| | headed | 51.1 | 4.25 | 4.99 | 0.704 | 41 | 61.4 | 3.81 |
| Wheat Straw | NA | 76.9 | 4.72 | 8.19 | 1.333 | 362 | 41.8 | 6.09 |
| ***Warm-Season Perennial Grasses*** | | | | | | | | |
| Bermudagrass Hay | NA | 65.4 | 3.79 | 5.41 | 1.199 | 383 | 54.2 | 7.89 |
| Bermudagrass Silage | mid-maturity | 64.1 | 4.57 | 5.90 | 1.261 | 110 | 63.5 | 4.84 |
| | mature | 70.8 | 3.28 | 7.69 | 1.091 | 7 | 52.3 | 2.29 |
| ***Warm-Season Annual Grasses*** | | | | | | | | |
| Corn Silage | typical | 40.9 | 4.75 | 3.05 | 0.564 | 130,789 | 52.0 | 6.25 |
| | immature | 42.6 | 4.45 | 3.15 | 0.569 | 58,478 | 53.4 | 6.07 |
| | mature | 39.3 | 4.14 | 2.97 | 0.528 | 69,227 | 50.8 | 6.09 |
| Corn Ear/Husk[6] | low fiber | 18.1 | 3.08 | 1.75 | 0.386 | 182 | 50.6 | 15.62 |
| | high fiber | 24.9 | 4.07 | 2.01 | 0.446 | 893 | 46.8 | 11.98 |
| Sorghum[7] Hay | NA | 63.0 | 5.93 | 5.00 | 1.465 | 1,165 | 60.8 | 8.71 |
| Sorghum[7] Silage | immature | 56.7 | 3.40 | 4.92 | 0.918 | 151 | 58.5 | 6.47 |
| | mature | 61.6 | 4.27 | 5.15 | 1.125 | 416 | 63.6 | 6.67 |
| Sorghum[8] Hay | NA | 48.1 | 6.69 | 4.54 | 0.865 | 724 | 57.5 | 6.11 |
| Sorghum[8] Silage | mid-maturity | 53.1 | 3.94 | 5.03 | 0.858 | 392 | 55.3 | 5.98 |
| | mature | 45.7 | 4.46 | 4.25 | 0.779 | 1,248 | 56.2 | 6.83 |
| Millet Hay | NA | 61.9 | 5.58 | 5.64 | 1.629 | 138 | 64.0 | 7.31 |
| Millet Silage | NA | 59.7 | 5.90 | 5.48 | 1.680 | 61 | 61.2 | 6.98 |
| S x S[9] Hay | NA | 61.0 | 6.68 | 4.94 | 1.407 | 1,549 | 54.4 | 8.58 |
| S x S[9] Silage | NA | 59.5 | 5.16 | 5.25 | 1.254 | 2,201 | 54.9 | 7.91 |
| Sudangrass Hay | mature | 65.8 | 3.30 | 5.08 | 1.004 | 110 | 55.8 | 6.36 |
| Sudangrass Silage | mid-maturity | 60.7 | 4.73 | 4.99 | 1.079 | 47 | 64.6 | 5.44 |
| | mature | 66.6 | 3.96 | 6.34 | 1.336 | 18 | 61.0 | 5.31 |

[1] *Abbreviations: NDF, neutral detergent fiber; ADL, acid detergent lignin; and NDFD, NDF digestibility.*
[2] *SD, standard deviation.*
[3] *N, number of observations in the NDFD mean.*
[4] *SD, standard deviation of NDFD means.*
[5] *NA, not available or specified.*
[6] *Forages were ensiled.*
[7] *Forage types.*
[8] *Grain types.*
[9] *S x S, Sorghum x Sudangrass.*

each of the 62 forage groups listed in Table 10 and their associated respective concentrations of NDF and ADL. It is relatively easy to discern that there is no meaningful relationship in either case. If the independent variable is refined further and expressed as the percentage that ADL contributes to total NDF pool (ADL/NDF × 100%), the overall relationship for the 62 forage groups improves only marginally [NDFD (%) = - 1.27 x + 70.1; $r^2$ = 0.26]. It must be noted that this type of simplified, generalized analysis has obvious limitations, which originate (in part) from the huge differences in representation within forage groups ($N$ = 2 to 130,789), somewhat arbitrary maturity designations/groupings, different contributing laboratories/methodologies, as well as the potentially wide-ranging growing/climatic conditions included within the same forage groupings. While acknowledging these limitations, there remain several notable reasons for the

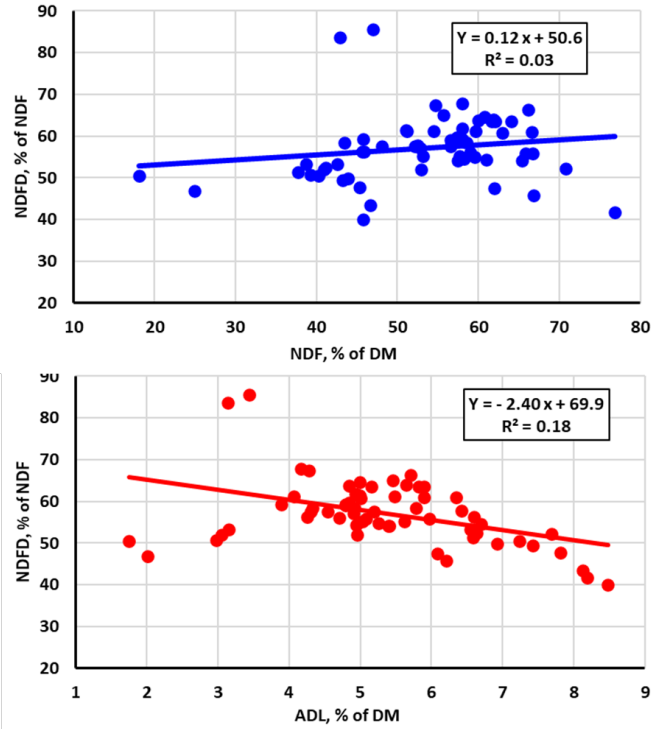


*Figure 52. Collective relationships between neutral detergent-fiber digestibility (NDFD, % of NDF) and concentrations of neutral detergent fiber (NDF; top) and acid detergent lignin (ADL; bottom) for the various forage groups summarized from fee-for-service laboratories by NASEM (2021) and also listed in Table 10.*

collectively poor relationships between NDFD and common laboratory proxies for maturity.

First, there are stark differences between legumes and grasses. Generally, legumes contain lower concentrations of NDF than grasses, but the NDF within those legumes is more heavily lignified. To illustrate this, the mean ADL/NDF percentage for the 10 legume groupings shown in Table 10 was 16.4% of NDF, while those for cool-season perennial and annual grasses were roughly half that percentage (8.5 and 8.7%, respectively). It also should be emphasized that ADL is not a static entity, either in terms of its composition or its configuration and/or linkages within the cell wall. Despite their heavy lignification, particularly within stem tissues, pdNDF digestion rates (Kd) for legumes are typically faster than observed for most cool-season annual or perennial grasses (Figures 15, 17, and 19). However, the noted heavier lignification commonly observed in legume forages limits the percentage of total NDF that can be digested. As such, the mean 48-hour NDFD for the 10 legume groupings shown in Table 10 was 50.4%, while the comparable NDFD means for annual and perennial cool-season grasses were 62.4 and 60.3% of NDF, respectively. Typical differences in Kd between legumes and grasses also can complicate interpretation of relationships between 48-hour determinations of NDFD and maturity. Generally, the rapid estimates of Kd for legumes ensure that NDF digestion for legume forages is nearly complete or complete when the in vitro assay is terminated at 48 hours; however, the slower Kd observed for most cool-season grasses suggests that significant digestion of NDF can occur beyond the 48-hour termination point (Figures 7 and 13).

Additionally, grain-bearing forage crops dilute NDF within the total mass. Many plant species that are harvested at full maturity as grain crops also can be utilized as forages, normally at (less mature) growth stages that would precede a harvest as grain. Among these species, the most notable is corn, but also grain sorghum and various cereal grains are harvested routinely as forages. While these crops are all technically grasses, they are unique because of the large percentages of total plant DM that can be partitioned into grain, which contains comparatively little NDF or ADL. For example, a coarsely ground corn grain contains only 9.8 ± 1.50% NDF and 1.37 ± 1.972% ADL (NASEM, 2021). This partitioning of highly digestible carbohydrate into the filling ear or grain head distorts the normal (often expected) relationships between NDFD and analytical proxies for plant maturity (NDF or ADL). This occurs by the simple dilution of forage fiber components with non-fiber carbohydrates (**NFC**; usually starch), resulting in reduced or static concentrations of NDF, ADF, or ADL at advanced growth stages.

To illustrate this concept for corn silages, Ferreira and Mertens (2005) examined 32 corn silages obtained from a commercial feed analysis laboratory that were selected based on their diversity with respect to particle size and chemical constituents. Table 11 summarizes the correlations of DM, starch, and NFC with concentrations of typical fiber-component metrics within the silages. Increasing concentrations of DM, starch, and NFC all would reflect advancing plant maturity and greater percentages of the total plant DM pool partitioned into grain. To confirm this, both DM ($r$ = 0.48) and NFC ($r$ = 0.93) were positively correlated with starch with a high degree of confidence ($P$ < 0.01). Concentrations of NFC would include starch, but

*Table 11. Correlations between concentrations of dry matter (DM), starch, and non-fiber carbohydrate (NFC) and fiber components as determined from 32 corn silages (Ferreira and Mertens, 2005).*

| | ---------- Fiber Component[1] ------- | | |
|---|---|---|---|
| Item | NDF | ADF | ADL |
| DM | - 0.49 ** | - 0.55 ** | - 0.37 * |
| Starch | - 0.89 ** | - 0.84 ** | - 0.65 ** |
| NFC | - 0.96 ** | - 0.91 ** | - 0.74 ** |

**, ** Correlations were statistically significant at P < 0.05 and P < 0.01, respectively.*

*[1] Fiber components: NDF, neutral detergent fiber; ADF, acid detergent fiber; and ADL, acid detergent lignin.*

also sugars, pectins, and organic acids, thereby serving as a crude estimate of available carbohydrate with important relevance to appropriate recommendations for rapidly fermentable matter in dairy rations. Concentrations of DM, starch, and NFC, all of which could be considered pseudo-proxies for grain content, were negatively correlated with concentrations of fiber components, leading to concluding statements that accumulation of grain dilutes fiber concentrations determined on a whole-plant basis.

As initially suggested, 48-hour in vitro determinations of NDFD often can be related very closely to direct assessments of plant maturity, such as those evaluated by linear models that express growth stage as a continuous variable. Figure 53 summarizes the relationships between 48-hour in vitro estimates of NDFD with a linear, numerical model for growth stage for triticale forages harvested during 2016 and 2017 at Marshfield, Wisconsin (Coblentz et al. (2018). These linear relationships exhibited coefficients of determination ($r^2$) that were ≥ 0.92, indicating that the close relationship between NDFD and numerical quantification/assignment of plant maturity is largely independent of grain fill. However, when fiber-related proxies are substituted for direct measures of plant maturity, the resulting relationships are strongly disrupted by grain fill (Figures 54 and 55). Prior to the initiation of grain fill, fiber-related proxies were excellent linear predictors of NDFD, yielding coefficients of determination ($r^2$) ≥ 0.93 for NDF and ≥ 0.81 for ADL. Within this context considered over two years, NDFD declined by about 1.1 percentage units for every 1 percentage unit increase in NDF concentration; similarly, NDFD declined by about 8.6 percentage units for every 1 percentage unit increase in ADL. These strong relationships were completely distorted with the initiation of grain fill (see circled data points in Figures 54 and 55) because concentrations of NDF or other fiber components are diluted by grain development, but NDFD continues to decline in response to advancing plant maturity.

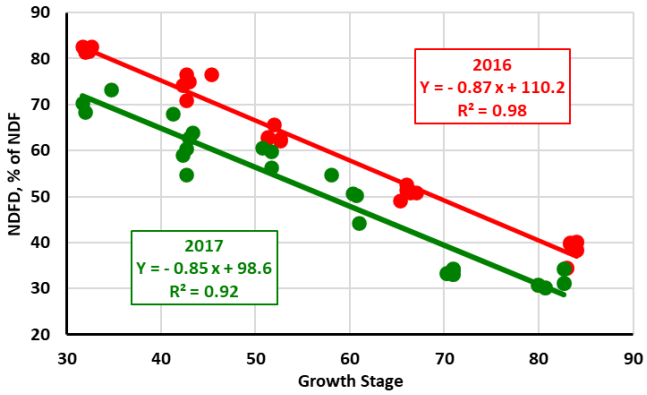


*Figure 53. Linear regressions relating 48-hour neutral detergent fiber digestibility (NDFD, % of NDF) for triticale forages with a linear model for growth stage at harvest (Stauss, 1994). Stages of growth are identified as stem elongation (30 to 39), booting (40 to 49), heading (50 to 59), anthesis (60 to 69), seed development (70 to 79), and seed ripening (80 to 89). Forages were harvested during the spring and early summer during 2016 and 2017 at Marshfield, Wisconsin [adapted from Coblentz et al. (2018)].*

Another noteworthy consideration is the unique characteristics of perennial warm-season grasses. A notable characteristic of perennial warm-season grasses is their uniquely high concentrations of NDF, especially at relatively immature growth stages. Two examples of this characteristic are illustrated in Figures 56 and 57 for eastern gamagrass forages grown in Wisconsin (Coblentz et al. 2010) and bermudagrass harvested in southern Georgia (Mandebvu et al., 1999). Although most perennial warm-season grasses grow in southern states, where they are well-adapted to warmer ambient temperatures, some species (notably warm season perennial grasses native to North America, such as eastern gamagrass) can survive and flourish outside of that region. Figure 56 summarizes concentrations of both NDF and ADL in eastern gamagrass as a function of harvest date in central Wisconsin. It must be emphasized that spring growth is delayed significantly in Wisconsin because of longer winters, cooler spring temperatures, juxtaposed against this species' warm-season physiology. As such, the June 1st harvest date shown in Figure 56 represents a very

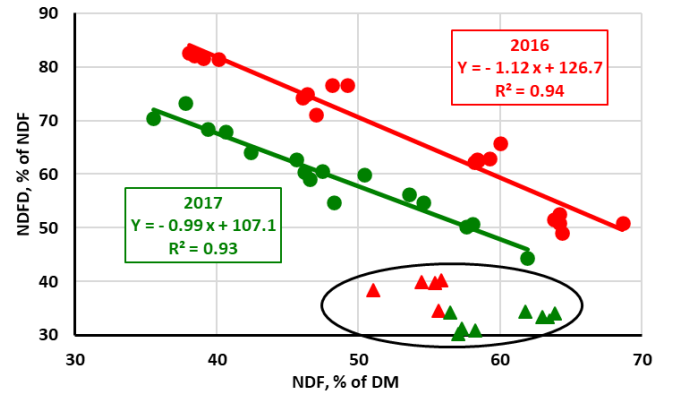


*Figure 54. Relationships between 48-hour estimates of neutral detergent fiber digestibility (NDFD, % of NDF) and corresponding concentrations of neutral detergent fiber (NDF, % of DM) for triticale forages harvested during 2016 and 2017 at Marshfield, Wisconsin. Linear regressions include only forages harvested prior to the onset of grain fill (●, ●), but excludes those harvested after grain fill began (▲, ▲; circled). These summaries illustrate the distortion that occurs when using NDF as a proxy for plant maturity when evaluating fiber digestibility for grain crops [adapted from Coblentz et al. (2018)].*

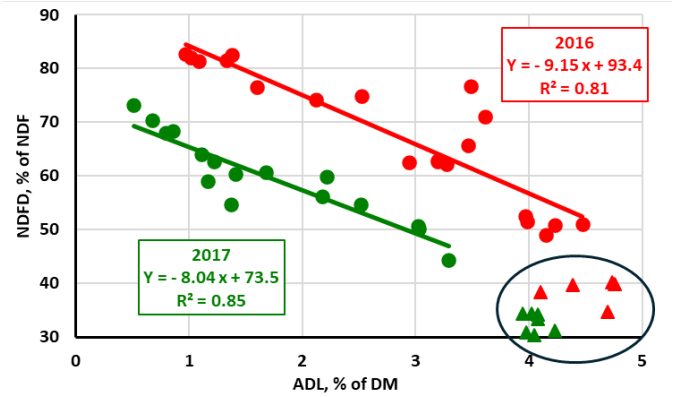


*Figure 55. Relationships between 48-hour estimates of neutral detergent fiber digestibility (NDFD, % of NDF) and corresponding concentrations of acid detergent lignin (ADL, % of DM) for triticale forages harvested during 2016 and 2017 at Marshfield, Wisconsin. Linear regressions include only forages harvested prior to the onset of grain fill (●, ●), but excludes those harvested after grain fill began (▲, ▲; circled). These summaries illustrate the distortion that occurs when using ADL as a proxy for plant maturity when evaluating fiber digestibility for grain crops [adapted from Coblentz et al. (2018)].*

immature forage; however, the corresponding concentration of NDF on that date was 67.3% of DM, which is more than 25 percentage units greater than a perennial cool-season grass harvested at the same location and maturity, roughly a month earlier. The immature nature of this harvested forage is further confirmed by the low concentration of ADL (1.8% of

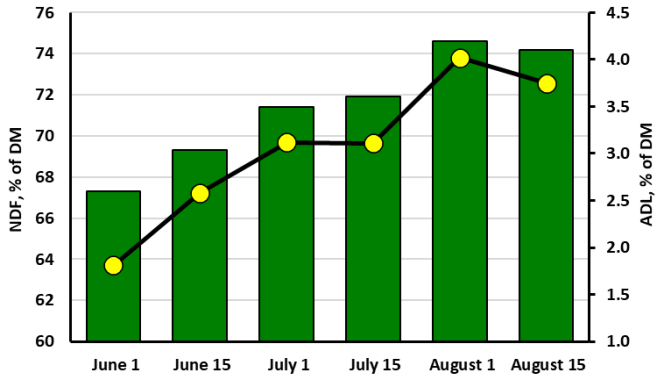


*Figure 56. Relationships between neutral detergent fiber (NDF; ■) or acid detergent lignin (ADL; ●) and harvest date for eastern gamagrass forages grown at Marshfield, Wisconsin [adapted from Coblentz et al. (2010)].*

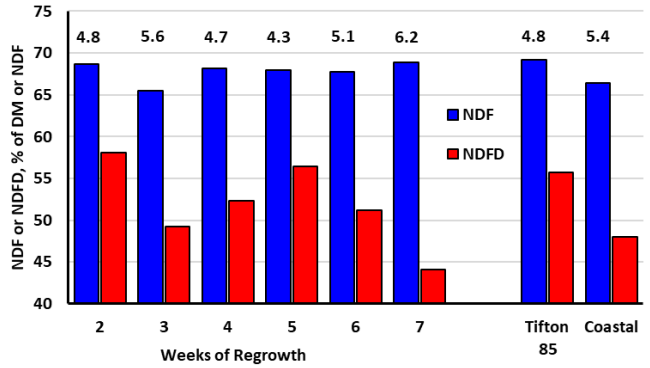


*Figure 57. Relationships between neutral detergent fiber (NDF) or neutral detergent fiber digestibility (NDFD) and weeks of regrowth for bermudagrass forages grown at Tifton, Georgia. Data labels indicate corresponding concentrations of acid detergent lignin (ADL) [adapted from Mandebvu et al. (1999)].*

DM), yielding an ADL/NDF percentage of only 2.7%.

Similarly, Mandebvu et al. (1999) demonstrated high concentrations of NDF for two cultivars of bermudagrass (Tifton 85 and Coastal) harvested after 2, 3, 4, 5, 6, or 7 weeks of regrowth following an initial harvest (Figure 57). Concentrations of NDF remained relatively static across harvest dates, with no statistical difference observed between the initial (2-week) regrowth and forages harvested after 4, 5, 6, or 7 weeks. As observed for the eastern gamagrass forages summarized in Figure 56, immature regrowth exhibited a very high concentration of NDF (68.7% of DM). Despite the static response of NDF to the regrowth interval, NDFD generally declined over time, particularly in response to changing concentrations of ADL. Given the characteristically slow rates (Kd) of NDF digestion typically observed

for both warm-season annuals and perennials (< 0.050/hour; Table 3), the 48-hour termination of in vitro incubation may affect the magnitude of NDFD values negatively, and more so than often observed for both legumes and most perennial cool-season grasses.

Finally, it is crucial to note the effects of lignin, *bmr* mutations, and etherified ferulic acid crosslinking on fiber digestibility. The work of Mandebvu et al. (1999) also illustrated an additional complexity to the generalized inverse relationship between NDFD and plant maturity. As discussed previously, neither NDF nor ADL are static entities, either in terms of concentration or composition. In the referenced work, Tifton 85 bermudagrass exhibited consistently greater concentrations of NDF, but less ADL and ether-linked ferulic acid than Coastal bermudagrass. When considered across all regrowth intervals, this resulted in greater in vitro NDFD for Tifton 85 (55.7 vs. 48.0% of NDF), despite its concomitantly greater concentrations of NDF (69.2 vs. 66.4% of DM), hemicellulose (38.5 vs. 37.2% of DM), and cellulose (27.6 vs. 25.3% of DM).

Moreover, these types of relationships have been detected in other grass species. Casler and Jung (1999) sought to identify smooth bromegrass genotypes with unconfounded divergence in concentrations of (Klason) lignin and etherified ferulic acid, such that their independent effects on NDFD could be determined. Both lignin and etherified ferulic acid exhibited negative effects on NDFD determined by a 96-hour in vitro incubation; however, there was not a clear difference in the magnitude that each entity affected NDFD. It was concluded that both the concentration of the lignin in the cell wall, as well as the amount of ferulic acid crosslinking lignin to cell-wall polysaccharides via ether bonds, were both under genetic control and contribute to regulation of ruminal digestion of cell walls. In a follow-up experiment, Casler et al. (2008) examined high-etherified vs. low-etherified and high vs. low (Klason) lignin groups of selected clones from smooth bromegrass, reed canarygrass, and orchardgrass. Forages were grown over two years, and initial growth was harvested on four dates ranging up to 50 days after April 30th. Linear regressions of 96-hour in vitro NDFD on concentrations of Klason lignin, esterified ferulates, and etherified ferulates are summarized in Table 12. Overall, there were inverse relationships between 96-hour NDFD and both Klason lignin and etherified ferulates; however, the relationship with esterified ferulates was positive, and may have been an artifact of a negative correlation between esterified and etherified ferulates established previously by Jung and Casler (1991). It was further established that while etherified ferulates and Klason lignin are positively correlated, their effects on 96-hour NDFD are clearly independent of each other, offering two potential opportunities for improvement of ruminal fiber digestion. It also was observed that concentrations of Klason lignin had no effect on 24-hour determinations of NDFD, but these short-term in vitro digestions were affected negatively by etherified ferulates for each forage species evaluated.

The relationships described above, coupled with those of *bmr* mutations (see the ***Kinetics of Plant Parts*** section on page 23), also have been demonstrated for corn silages (Jung et al., 2011). Five inbred corn lines were evaluated including a parental control line (W23), two near-isogenic mutant low-ferulate lines (W23-LF1, W23-LF2), B73, and BF73 possessing the *bmr* mutation (BF73-*bmr*). Compositional characteristics of the five corn silages are summarized in Table 13 and verify lower concentrations of ferulate esters and ethers within the

*Table 12. Linear regressions of 96-hour in vitro digestion of NDF (% of NDF) on Klason lignin (% of NDF), esterified ferulates (% of NDF), and etherified ferulates (% of NDF) for three perennial cool-season grasses (smooth bromegrass, reed canarygrass, and orchardgrass) harvested at four growth stages over two years [adapted from Casler et al. (2008)].*

| | ----- Klason Lignin ---- | | | ---- Esterified Ferulates | | | ---- Etherified Ferulates | | |
|---|---|---|---|---|---|---|---|---|---|
| **Species** | **Slope** | **SD[1]** | **$R^2$** | **Slope** | **SD[1]** | **$R^2$** | **Slope** | **SD[1]** | **$R^2$** |
| Smooth Bromegrass | - 2.74 | 0.77 | 0.34 | 76.4 | 18.7 | 0.41 | - 25.4 | 6.4 | 0.40 |
| Reed Canarygrass | - 2.77 | 0.68 | 0.37 | 50.8 | 24.4 | 0.13 | - 50.6 | 4.8 | 0.80 |
| Orchardgrass | - 1.25 | 0.59 | 0.14 | 107.7 | 19.4 | 0.52 | - 52.1 | 6.6 | 0.69 |

[1] *SD, standard deviation.*

*Table 13. Composition and in vitro NDF digestibility of isogenic corn silages containing low ferulates or a bmr mutation. Analyses were based on composite samples obtained throughout digestion trials with lambs and a lactation trial with Holstein cows and did not include field replication [adapted from Jung et al. (2011)].*

| | Corn Line | | | | |
|---|---|---|---|---|---|
| **Component** | **W23** | **W23-LF1** | **W23-LF2** | **B73** | **B73-*bmr*** |
| ***Composition*** | | | | | |
| NDF, % of DM | 45.0 | 48.1 | 47.3 | 47.0 | 47.4 |
| Cell wall (CW),[1] % of DM | 45.1 | 50.4 | 50.0 | 45.9 | 46.1 |
| ADL, % of DM | 4.9 | 6.7 | 4.9 | 5.3 | 4.0 |
| Klason lignin, % of CW | 16.1 | 16.6 | 15.9 | 15.3 | 12.9 |
| Ferulate esters, % of CW | 0.83 | 0.69 | 0.72 | 0.88 | 0.84 |
| Ferulate ethers, % of CW | 0.92 | 0.70 | 0.67 | 0.95 | 0.90 |
| *p*-coumarate esters, % of CW | 1.76 | 1.79 | 1.72 | 1.70 | 1.01 |
| ***In vitro NDF Digestibility***, % of NDF | | | | | |
| 24-hour incubation | 34.4 | 28.1 | 35.0 | 29.3 | 40.5 |
| 48-hour incubation | 46.5 | 41.4 | 46.2 | 46.3 | 61.2 |
| 96-hour incubation | 60.2 | 57.2 | 60.7 | 59.4 | 75.0 |
| ***In vitro Cell Wall Digestibility,*** % of CW | | | | | |
| 24-hour incubation | 36.7 | 39.7 | 51.4 | 37.4 | 61.3 |
| 48-hour incubation | 63.0 | 61.6 | 62.7 | 56.4 | 72.2 |
| 96-hour incubation | 68.3 | 68.1 | 70.4 | 67.3 | 81.8 |

*[1] Sum of all cell wall polysaccharide component sugars (glucose, xylose, arabinose, galactose, mannose, rhamnose, fucose, and uronic acids), Klason lignin, ferulates, and esterified p-coumarates.*

cell walls of low-ferulate lines (W23-LF1, W23-LF2), as well as reduced ADL and Klason lignin within the BF73-*bmr* silage. In this study, the clearest improvements in NDFD were observed in association with the *bmr* mutation for the B73 corn line, yielding improvements of 11.2 to 15.6 percentage units of NDF, which tended to increase with length of incubation (24, 48, or 96 hours). The benefits of low etherified ferulate were most evident for 24-hour incubations, where in vitro digestion of total cell wall polysaccharides for the W23-LF2 line exceeded those of W23 and W23-LF1 by 14.7 and 11.7 percentage units, respectively. Similar responses were observed for 24-hour digestions of individual cell wall polysaccharides, such as glucose, xylose, and arabinose.

Another notable example of the effects of the *bmr* (*bm3*) mutation on determinations of NDFD in corn silages was reported by Oba and Allen (2000); relevant compositional metrics, as well as in vitro determinations of NDFD after 30-hour incubations are summarized in Table 14. Two nearly isogenic corn silage hybrids were compared that either were conventional (Cargill 6208FQ) or possessed the *bmr* mutation (*bm3*; Cargill 657). Corn silage with the *bmr* mutation exhibited statistically reduced ($P < 0.05$) concentrations of fiber components (NDF, ADF, and ADL), of which reductions in ADL were most noteworthy (2.0 vs. 1.3% of DM). As a result, the 30-hour NDFD was improved by 9.4 percentage units (55.9 vs. 46.5% of NDF), thereby illustrating a common response to *bmr* mutations.

*Table 14. Nutrient composition of near isogenic corn silage hybrids (Cargill 657 and Cargill 6208FQ) harvested in Michigan. The Cargill 657 hybrid contained a bmr (bm3) mutation. Each comparison differed significantly at a minimum $P < 0.05$ level of confidence [adapted from Oba and Allen (2000)].*

| **Nutritional Component** | **Cargill 657 (*bmr*)** | **Cargill 6208FQ (Control)** |
|---|---|---|
| DM, % | 29.9 | 30.1 |
| NDF, % of DM | 41.4 | 42.9 |
| ADF, % of DM | 20.2 | 22.4 |
| ADL, % of DM | 1.3 | 2.0 |
| 30-hour NDFD, % of NDF | 55.9 | 46.5 |

### *Some Sources of Variation Among Methods, Laboratories, and Procedures.*

Because in vitro determinations of NDFD have become critical components of routine forage testing and estimation of energy, limiting the variability in results is important. This is true both within and across laboratories and is further complicated by the procedural variations used by individual laboratories. A number of studies have attempted to address these concerns in an effort to quantify and then improve both the accuracy and precision of NDFD determinations. While it is beyond the scope of this research synopsis to systematically summarize all such research efforts, a few specific studies are worthy of discussion.

**Early Method Comparisons**. One somewhat dated study (Wilman and Adesogan, 2000) sought to compare ANKOM and CONV incubation methods, as well as ruminal inoculum obtained from two sources (sheep or cattle). The ANKOM methods utilized F57 filter bags, where 21 samples were incubated collectively within rotating incubation jars for 48 hours. For this study, the final extraction in neutral detergent was accomplished by boiling filter bags containing undigested forage particles in neutral detergent for 1 hour in 600 mL beakers with condensers applied to each beaker. The CONV methods (Tilley and Terry, 1963; Van Soest et al. 1966) utilized 100-mL polypropylene tubes as incubation vessels, where each forage sample was incubated in a dedicated tube, such that each sample was not constrained within the dedicated incubation tube containing the inoculum. For this study, rumen fluid donors (sheep and cattle) were maintained on hay and pasture diets, respectively; two test forages, Italian ryegrass (54.1% NDF) and alfalfa (42.7% NDF), were subjected to the in vitro incubation procedures.

One portion of the study evaluated the effects of sieve (milling) size (0.5, 1.0, or 1.5 mm) on subsequent values of NDFD; this aspect of the evaluation was designed primarily to address concerns about particulate matter potentially migrating into and/or out of the F57 filter bags. Finer milling was postulated to enhance the possibility of particle movement out of filter bags; however, results showed that there was no discernable milling effect on NDFD, suggesting that the 25-µm pore size associated with the F57 filter bags was small enough to retain forage particles adequately (Figure 58). Considered across all three sieve sizes, CONV incubations exhibited a 7.8-percentage unit greater NDFD than samples incubated with ANKOM methodology in which 0.5-gram forage samples were restrained within the F57 filter bags during in vitro incubation.

In this study, rumen fluid obtained from sheep donors maintained on a basal diet of hay (species unspecified) consistently yielded greater NDFD values compared to rumen fluid obtained from cattle. This was true irrespective of the forage species being evaluated, individual plant-part substrates, or incubation method (ANKOM or CONV; Figures 59 and 60, respectively). Overall, these differences

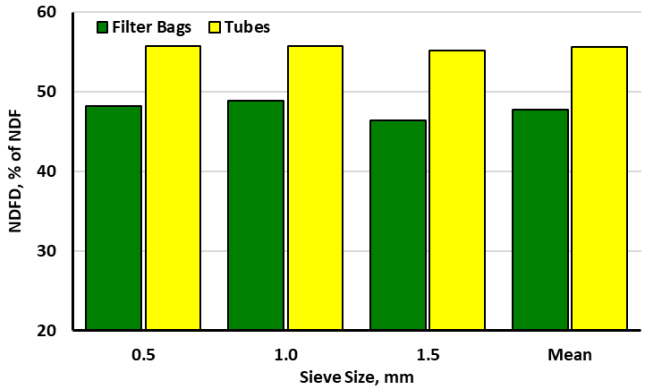


*Figure 58. Effects of sieve (milling) size on the in vitro neutral detergent fiber digestibility (NDFD, % of NDF) of Italian ryegrass and alfalfa forages using ANKOM Technology Corporation (F57 filter bags; Daisy$^{II}$ incubator) and traditional (tubes) methods. Data represents the mean of two forages and in vitro digestion using inoculum obtained from both sheep and cattle and evaluated during successive weeks [adapted from Wilman and Adesogan (2000)].*

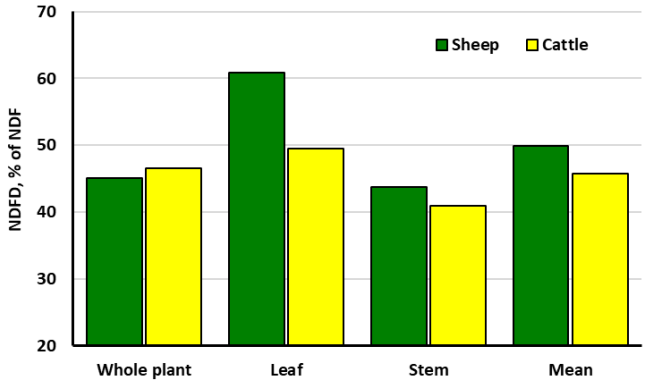


*Figure 59. Effects of inoculum donor (sheep or cattle) on the in vitro neutral detergent fiber digestibility (NDFD, % of NDF) of Italian ryegrass and alfalfa plant parts using ANKOM Technology Corporation methods (F57 filter bags; Daisy$^{II}$ incubator). Data represents the mean of two forages [adapted from Wilman and Adesogan (2000)].*

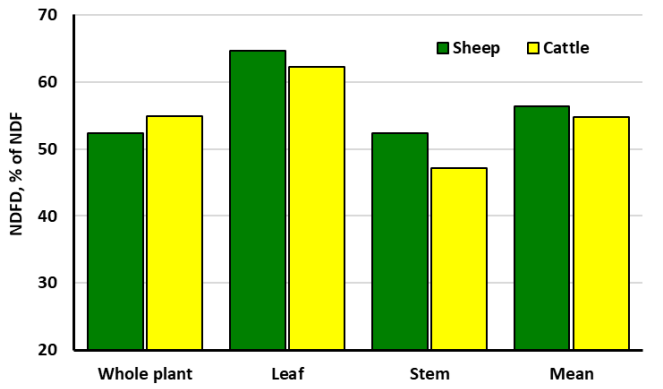


*Figure 60. Effects of inoculum donor (sheep or cattle) on the in vitro neutral detergent fiber digestibility (NDFD, % of NDF) of Italian ryegrass and alfalfa plant parts using traditional 100-mL incubation vessels/procedures (Tilley and Terry, 1963; Van Soest et al., 1966). Data represents the mean of two forages (Italian ryegrass and alfalfa) [adapted from Wilman and Adesogan (2000)].*

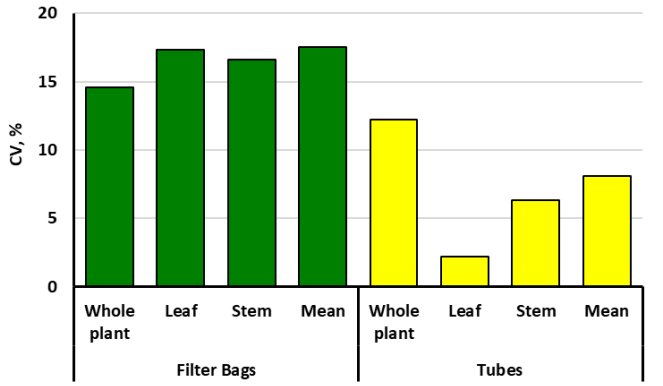


*Figure 61. Coefficients of variation (%) for 48-hour in vitro neutral detergent fiber digestibility (NDFD, % of NDF) determinations of Italian ryegrass and alfalfa forages as affected by plant part and incubation procedure. Data represents the mean of incubations of alfalfa and Italian ryegrass substrates using inoculum obtained from sheep and cattle [adapted from Wilman and Adesogan (2000)].*

between sheep and cattle fluid donors were relatively small, averaging 4.2 and 1.7 percentage units of NDF for the ANKOM and CONV procedures, respectively. Furthermore, standard errors associated with these measurements were consistently lower when the inoculum was donated from sheep (data not shown). It should be noted that the necessary procedural compromises inherent in this experiment confounded direct comparisons of rumen fluid donated from sheep and cattle. The most prominent procedural compromise was the inconsistency of basal diets, which contrasted a standardized hay diet offered to sheep against an uncontrolled pasture-based basal diet for cattle.

Throughout the study, variability was greater for NDFD determinations using ANKOM procedures, where the mean coefficient of variation (CV, %) was more than twice that of corresponding assessments using CONV procedures (17.5 vs. 8.1%; Figure 61). Concluding statements from the research were that the potential escape of particulate matter from F57 filter bags was not a major problem, and that the use of ANKOM (filter bag) procedures can give acceptable results when the emphasis is placed on saving labor costs, rather than obtaining the most precise results. It also should be noted that the ANKOM method used in this study was somewhat modified relative to the approach used most commonly. The final extraction of undigested residues in neutral detergent was conducted in individual 600-mL beakers with condensers, rather than using the specialized ANKOM fiber analysis/instrumentation procedures used typically in determinations of NDFD. The effects of this procedural variation cannot be assessed and remain unknown within the context of this study.

**Effects of Sample Size on In Vitro NDF Digestibility Using ANKOM Procedures.** In the preceding discussion, it was noted that NDFD values obtained from ANKOM procedures were consistently less than those obtained through CONV methods in which unrestrained forage samples were incubated in dedicated incubation vessels (Figure 58). While an initial concern was that largely undigested particulate matter might migrate from the F57 filter bags during in vitro incubation, the potential effect of restriction of substrate particles within filter bags was somewhat overlooked. Currently, ANKOM procedures reported on the manufacturer's website[2] state that a 0.25-gram sample should be used, but for 48-hour incubations, a 0.5-gram sample is acceptable. However, within a historical context, many ANKOM assessments of NDFD, such as those reported by Wilman and Adesogan (2000), have reported they solely used 0.5-gram samples sealed within F57 filter bags.

One study that directly compared NDFD values obtained from ANKOM methods using both 0.25- and 0.50-g sample sizes against values obtained

[2] *See: https://www.ankom.com/sites/default/files/2024-08/Method_3_InVitro_D200_D200I.pdf.*

using CONV methods was reported recently for 48 triticale forages of wide-ranging maturities that were grown in central Wisconsin (Coblentz et al., 2019). The relationships between ANKOM procedures and CONV methodologies were determined by linear regression, and they are summarized in Figure 62 with regression statistics provided in Table 15. With a 30-hour incubation, the linear relationship between ANKOM (0.25-g sample size) and CONV procedures yielded a desirable lack of bias between methods, where neither the slope (1.040) nor the intercept (- 1.8) differed ($P \geq 0.521$) from unity and zero, respectively. However, a considerable bias was detected when the larger sample size (0.50 grams) was sealed with ANKOM filter bags. In this case, the slope (0.824) differed sharply ($P = 0.002$) from unity, indicating ANKOM values of NDFD lagged behind those determined with CONV procedures, which is consistent with the observations made by Wilman and Adesogan (2000) that are summarized in Figure 58.

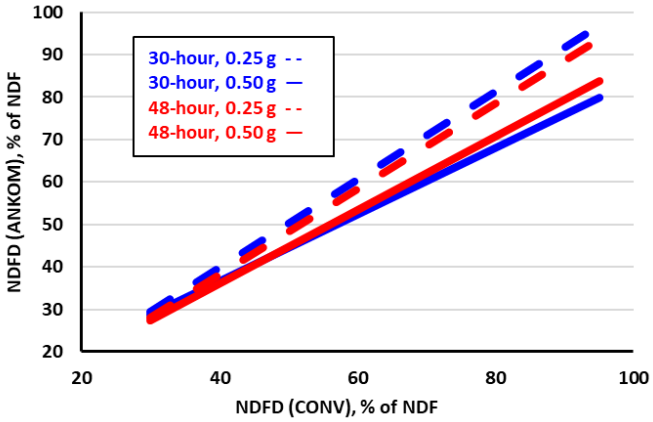


*Figure 62. Linear regressions comparing the in vitro neutral detergent fiber digestibility (NDFD, % of NDF) for 48 triticale forages as determined by ANKOM instrumentation/procedures (y-axis; dependent variable) and conventional (CONV) procedures (x-axis; independent variable) in which samples were incubated in individually dedicated vessels. Samples were sealed in F57 filter bags for ANKOM incubations at either 0.25 or 0.50 grams per bag. Regression statistics for these data are summarized in Table 15 [adapted from Coblentz et al. (2019)].*

When the incubation time was extended to 48 hours, linear regressions for both sample sizes exhibited slopes and intercepts that did not differ ($P \geq 0.305$) from unity and zero, respectively. This demonstrates better agreement with CONV values when the larger 0.50-gram sample size was used within ANKOM fiber bags at longer incubation intervals, although the overall dependent (ANKOM) mean was still less with the larger sample size (61.3 vs. 69.2% of NDF). These results beg an additional question. At what incubation interval does the 0.25- and 0.50-gram sample size become equivalent when using ANKOM procedures? Are they equivalent at any incubation interval, regardless of length?

This question was addressed in the same study by incubating the identical 48 triticale forages described previously as test substrates. The NDFD values from 0.25-gram samples were regressed linearly on those using the larger 0.50-gram sample size with results summarized in Figure 63 and Table 16. It is clear from these data that the agreement between sample sizes improved progressively as the length of incubation time increased. Furthermore, the smaller error/variability often associated with longer incubation times is reflected in the associated smaller root mean square errors (RMSE) and improved coefficients of determination ($r^2$) (Table 16),

*Table 15. Linear regressions of neutral detergent fiber digestibility (NDFD, % of NDF) of 48 triticale forages as determined by ANKOM[1] (y-axis; dependent variable) and CONV[2] (x-axis, independent variable) procedures after 30 and 48-hour in vitro incubations [adapted from Coblentz et al. (2019)].*

| Incubation Time | ANKOM Sample Size | Model | $r^2$ | $H_o$: Slope = 1[3] | $H_o$: Intercept = 0[4] |
|---|---|---|---|---|---|
| *hours* | *grams* | | | *P > F* | |
| 30 | 0.25 | Y = 1.040 x – 1.8 | 0.861 | 0.521 | 0.626 |
| | 0.50 | Y = 0.824 x + 1.6 | 0.842 | 0.002 | 0.612 |
| 48 | 0.25 | Y = 1.021 x – 3.4 | 0.866 | 0.720 | 0.430 |
| | 0.50 | Y = 0.939 x - 5.5 | 0.782 | 0.412 | 0.305 |

*[1] ANKOM; NDFD measurements determined by procedures described by ANKOM Technology Corporation (Macedon, NY) using F57 filter bags.*
*[2] CONV; NDFD measurements determined by (traditional) procedures using individually dedicated incubation vessels.*
*[3] $H_o$: Slope = 1; test of significance for slope = 1, where $P < 0.05$ indicates the slope of the regression line differed from unity.*
*[4] $H_o$: Intercept = 0; test of significance for intercept = 0, where $P < 0.05$ indicates the intercept of the regression line differed from zero.*

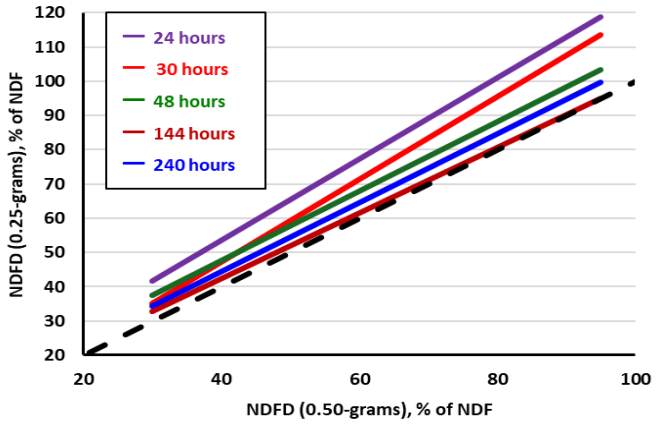


*Figure 63. Linear regressions comparing the in vitro neutral detergent fiber digestibility (NDFD, % of NDF) for 48 triticale forages as determined by ANKOM instrumentation/procedures using 0.25-gram (y-axis; dependent variable) and 0.50-gram (x-axis; independent variable) sample sizes. Regression statistics for these data are summarized in Table 16. The black hashed line (— —) represents a 1:1 relationship [adapted from Coblentz et al. (2019)].*

particularly when incubation time was extended (144 or 240 hours). Conclusions drawn from these results suggest that agreement between ANKOM and CONV determinations of NDFD are better at shorter incubation intervals (24, 30, or 48 hours) when 0.25-gram samples are sealed within F57 filter bags, which may allow better association of substrate with the buffered ruminal fluid. For the ANKOM method, discrepancies between the 0.25 and 0.50-gram sample sizes gradually disappear as incubation length increases, and may disappear entirely at longer incubation times, such as those at 144 or 240 hours that are often used to establish uNDF. However, it should be emphasized that a wider array of forages should be evaluated to firmly establish any solid recommendation for using a 0.25-gram sample size when conducting 24, 30, or 48-hour evaluations of NDFD by ANKOM methods.

**Effects of Dedicated Vessel Type.** Many procedural variations also are observed when samples are incubated within dedicated flasks or vessels (CONV), resulting in concerns about the consistency and equivalency of results. Hall and Mertens (2008) evaluated vessel types in combination with common and/or modified in vitro fermentation methods. Within that context, six treatment combinations were evaluated: 1) 50-mL polyethylene centrifuge tubes with gas-release valves; 2) 50-mL polyethylene centrifuge tubes with continuous $CO_2$ gassing; 3) 50-mL polyethylene centrifuge tubes sealed and shaken continuously parallel to the axis of the tube; 4) 125-mL Erlenmeyer flasks with continuous $CO_2$ gassing; 5) 125-mL serum vials sealed with stoppers and crimp seals with manual gas release; and 6) 125-mL serum vials sealed with stoppers and crimp seals without manual gas release. Three test forages (alfalfa hay, corn silage, and ryegrass hay) were incubated singly within two replicated fermentation runs for 24, 30, or 48 hours. Soyhulls also were evaluated, but those results are not reported in this summary.

Concentrations of NDFD for the test substrates are summarized in Figure 64. For the 24, 30, and 48-hour incubation intervals, statistically significant effects ($P < 0.01$) were observed for treatment, and for the treatment × forage substrate interaction, thereby indicating these procedural variations are not necessarily equivalent, nor do procedural variants

*Table 16. Linear regressions of neutral detergent fiber digestibility (NDFD, % of NDF) relating NDFD values using 0.25-gram (y-axis; dependent variable) and 0.50-gram (x-axis; independent variable) sample sizes within the ANKOM Daisy[II] incubation system. Regressions are based on digestions of 48 triticale forages grown in central Wisconsin [adapted from Coblentz et al. (2019)].*

| Incubation Time | Model | RMSE[1] | $r^2$ | $H_o$: Slope = 1[2] | $H_o$: Intercept = 0[3] |
|---|---|---|---|---|---|
| *Hours* | | | | ---------- *P > F* ---------- | |
| 24 | Y = 1.186 x + 6.0 | 5.99 | 0.878 | 0.007 | 0.047 |
| 30 | Y = 1.206 x - 1.1 | 4.67 | 0.933 | < 0.001 | 0.661 |
| 48 | Y = 1.014 x + 7.1 | 3.01 | 0.964 | 0.631 | < 0.001 |
| 144 | Y = 0.956 x + 4.2 | 1.65 | 0.982 | 0.027 | 0.006 |
| 240 | Y = 1.005 x + 4.2 | 1.53 | 0.983 | 0.796 | 0.007 |

[1] *Root mean square error.*
[3] *$H_o$: Slope = 1; test of significance for slope = 1, where P < 0.05 indicates the slope of the regression line differed from unity.*
[4] *$H_o$: Intercept = 0; test of significance for intercept = 0, where P < 0.05 indicates the intercept of the regression line differed from zero.*

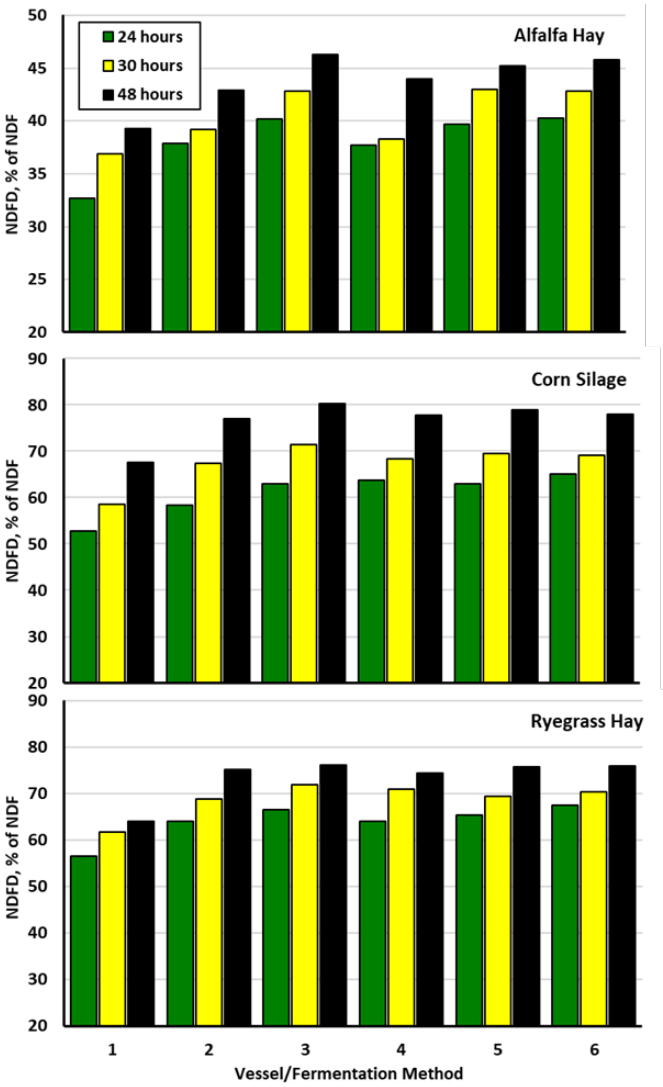


*Figure 64. Neutral detergent fiber digestibility (NDFD, % of NDF) concentrations for alfalfa hay, corn silage, and ryegrass hay as determined by six dedicated-vessel method variations after 24, 30, and 48 hours of incubation. Modified dedicated vessel methods include: 1) 50-mL polyethylene centrifuge tubes with gas-release valves; 2) 50-mL polyethylene centrifuge tubes with continuous $CO_2$ gassing; 3) 50-mL polyethylene centrifuge tubes sealed and shaken continuously parallel to the axis of the tube; 4) 125-mL Erlenmeyer flasks with continuous $CO_2$ gassing; 5) 125-mL serum vials sealed with stoppers and crimp seals with manual gas release; and 6) treatment #5 without manual gas release [adapted from Hall and Mertens (2008)].*

affect test substrates in a consistent manner. As such, it was concluded that these procedural variants used commonly in laboratories may still permit qualitative ranking of feeds and forages, but cast doubt about the suitability of comparing NDFD values across laboratories. Given the importance of NDFD in quantifying the energy density of various feeds, these variations can inject significant variation/error into projections of energy density, and subsequently, diet formulation. In particular, centrifuge tubes with gas-release values (Treatment 1) yielded NDFD values that were not comparable with the other method variations. In this study, methods that maintained increased $CO_2$ pressure and minimized the formation of floating substrate mats within the fermentation vessel produced the highest NDFD values. It was further concluded that the methods used for sealing, gassing, and/or agitating fermentation vessels may have a greater effect on NDFD values than the specific vessel type.

It should be noted and further emphasized that the error/variability measures in this study declined sharply as incubation intervals were extended from 24 to 48 hours (Figure 65). This is an observation observed consistently for both ANKOM and CONV procedures, and is an important limitation associated with all short-term incubations (< 48 hours), regardless of the specific incubation procedures/techniques used during in vitro fermentations.

**Modifications of In Vitro Procedures to Reduce Variability in Short-Term Incubations.** Interassay error has been a persistent and problematic concern with the in vitro assays necessary to determine accurate and precise estimates of NDFD. As described previously, NDFD values are often used to calculate the energy density of feedstuffs, and subsequently to properly formulate diets. As noted throughout this research synopsis, interassay error is consistently more problematic for short-term incubation intervals, especially those < 48

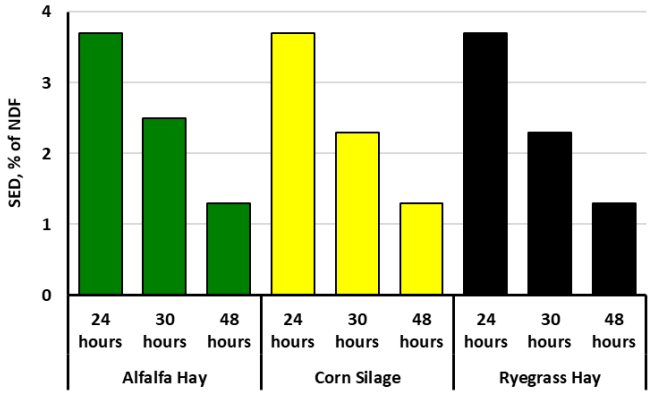


*Figure 65. The standard error of the difference (SED, % of NDF) associated with six dedicated vessel method variations for determination of neutral detergent fiber digestibility (NDFD, % of NDF). The SED are shown for three forages (alfalfa hay, corn silage, and ryegrass hay) and three incubation intervals (24, 30, and 48 hours) [adapted from Hall and Mertens (2008)].*

hours. Variable activities of the rumen inoculum, sometimes exacerbated by poor handling techniques that cause shock to rumen microbial populations, have been suspected and/or implicated as factors in the problematic variability of short-term estimates of NDFD. This issue has been addressed by investigating various priming techniques for ruminal inoculum (Goeser and Combs, 2009; Goeser et al., 2009). Procedural modifications have included priming ruminal fluid with cellulose, cellobiose, urea, and corn starch, as well as attaining a standard or threshold level of gas production prior to initiating the in vitro assay.

In an initial report, Goeser and Combs (2009) summarized two experiments. In the first experiment, an alfalfa silage sample (43.8% NDF) was incubated for 24 hours to test for improvements in interassay and intraassay error/variability; the short 24-hour incubation duration was chosen intentionally to exacerbate the potential for error/variability. The alfalfa silage samples were incubated in dedicated 125-mL Erlenmeyer flasks, but were restrained within F57 filter bags within each flask. Two procedures were used to provide rumen inoculum for the incubations: i) rumen fluid was collected from a single cow, strained through cheesecloth, and immediately used to inoculate the alfalfa samples; or ii) rumen fluid was collected from a single cow, strained, primed with cellulose, and allowed to produce a consistent amount of gas prior to inoculation. Inter- and intraassay variability were assessed by analyzing the dried silage sample 13 times/run in each of five runs. The mean concentrations of NDFD for each individual incubation run are summarized in Figure 66 and illustrate two key points. First, priming reduced ($P$ = 0.02) the sum of squares attributed to 'run' by about 90% (503 vs. 51) and indicated that interassay variability was reduced by priming the rumen inoculum. Secondly, overall 24-hour NDFD values were reduced by 2.3 percentage units of NDF (24.8 vs. 22.5% of NDF) by priming the rumen inoculum, which also was statistically significant ($P$ = 0.01). Intraassay variability was not affected by priming techniques.

In a second similar experiment, three procedures were used: i) rumen fluid was collected from two cows, strained through cheesecloth, and immediately used to inoculate the alfalfa samples; ii) rumen fluid from one cow was primed as described previously prior to inoculation; or iii) rumen fluid was collected from two cows and primed as described previously. Alfalfa samples were analyzed in five incubation runs with eight samples/run. As detected in the first experiment, priming reduced NDF digestion, with direct inoculation without priming (26.6% of NDF) exceeding ($P < 0.05$) both primed techniques (23.8 and 21.4% of NDF) (Figure 67). The primed technique using two donor cows (23.8% of NDF) also was greater ($P < 0.05$) than priming with only one donor cow. In agreement with the first experiment, interassay variability was reduced by priming with one donor cow ($P$ = 0.04), and then tended to be reduced further by adding a second donor ($P$ = 0.06). Intraassay variability was not affected by treatment. Concluding statements from the report were that priming inoculum appeared to have more consistent fermentative activity than unprimed fluid, which yielded better interassay precision compared to the traditional laboratory techniques/procedures that utilize unprimed fluid.

Goeser et al. (2009) further modified the priming approach by using a primer consisting of 40% cellulose, 20% urea, 20% corn starch, and 20% cellobiose. Primed rumen inoculum was allowed to produce 0.12 mL of gas/mL of inoculum before beginning in vitro incubations. In this report, three procedural treatments were compared that included

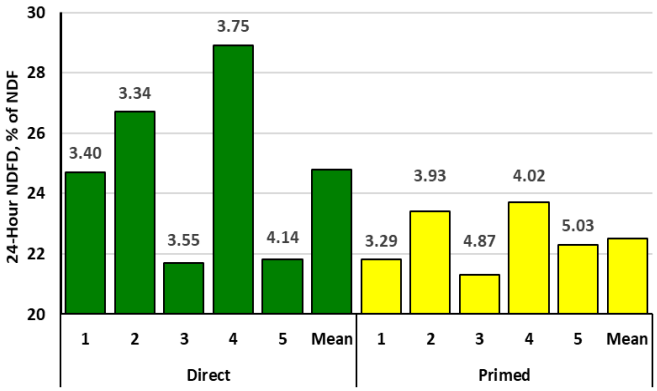


*Figure 66. Twenty-four-hour in vitro neutral detergent fiber digestibility (NDFD, % of NDF) for an alfalfa silage substrate in which rumen inoculum was used immediately after it was collected and strained through cheesecloth (Direct), or after it was strained, combined with buffer and reducing solution, primed with cellulose, and then allowed to produce a consistent amount of gas prior to inoculation (Primed). The alfalfa silage substrate (43.8% NDF) was analyzed 13 times within each of five incubation runs; data labels indicate the standard deviation (SD) within each incubation run. Method means differed at a P < 0.01 level of confidence [adapted from Goeser and Combs, 2009)].*

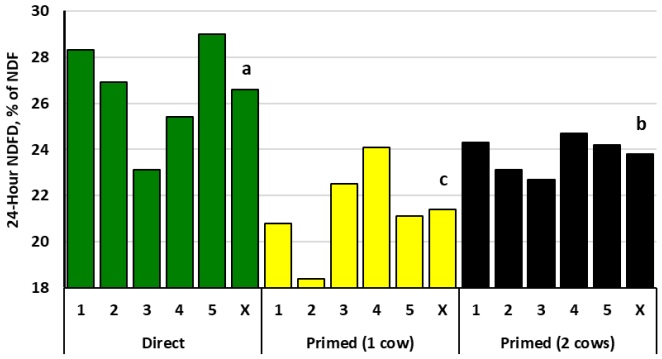


*Figure 67. Twenty-four-hour in vitro neutral detergent fiber digestibility (NDFD, % of NDF) of an alfalfa silage substrate in which rumen inoculum obtained from two cows was used immediately after it was collected and strained through cheesecloth (Direct). For primed treatments, rumen inoculum was strained, combined with buffer and reducing solution, primed with cellulose, and then allowed to produce a consistent amount of gas prior to inoculation. Primed treatments included rumen inoculum obtained from one [Primed (1 cow)] or two cows [Primed (2 cows)]. The alfalfa silage substrate (43.8% NDF) was analyzed 8 times within each of five incubation runs; overall method means differed at a P < 0.01 level of confidence, where mean separation is denoted by data labels affixed to overall method means (X) [adapted from Goeser and Combs, 2009)].*

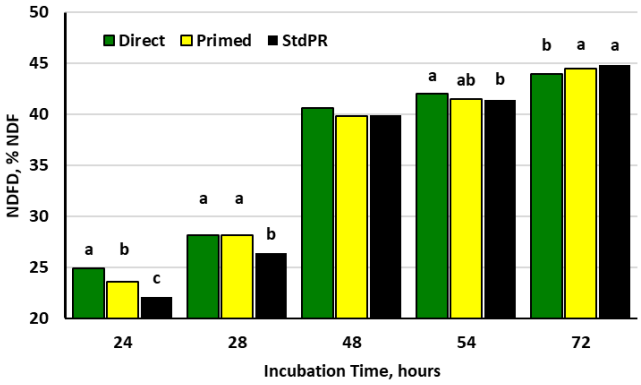


*Figure 68. In vitro neutral detergent fiber digestibility (NDFD, % of NDF) of two forages [alfalfa silage (44.9% NDF) and wheat straw (73.6% of NDF)] incubated for 24, 28, 48, 54, or 72 hours. Three incubation procedures were used: i) rumen inoculum was used immediately after it was collected and strained through cheesecloth (Direct); ii) rumen inoculum was strained, primed, and allowed to produce 0.12 mL of gas/mL of rumen fluid before forage samples were inoculated (Primed); or iii) rumen inoculum was strained and allowed to produce 0.12 mL gas/mL rumen fluid before inoculating forage samples, but the addition of priming agents was omitted (StdPR). Rumen fluid was obtained from 2 donor cows, and the primer contained 40% cellulose, 20% urea, 20% corn starch, 20% cellobiose. Means were based on five repetitions or runs, and bars within a specific incubation time affixed with different letters indicate a significant statistical difference at the P < 0.05 level of confidence [adapted from Goeser et al. (2009)].*

i) unprimed rumen fluid collected from two donor cows; ii) unprimed rumen fluid collected from two cows that was allowed to meet the standard gas threshold prior to inoculating samples, but without adding the priming compounds; and iii) primed fluid that reached the standard gas production threshold. Mean values of NDFD are summarized in Figure 68, where full or partial (gas production only) priming techniques resulted in statistically significant reductions of NDF digestion at short time intervals (24 or 28 hours), but these effects largely disappeared at longer incubation times. As observed in the previous studies (Goeser and Combs, 2009), interassay error was reduced by priming techniques, but intraassay error was not.

**Ring Test and Inter-Laboratory Variability.** It should be obvious that variability is inherent in all in vitro assays, and this is true both within and across analytical laboratories. This is realized by many field nutritionists, who often prefer to use a single, dedicated laboratory specifically to eliminate the described (across-laboratory) variability, thereby obtaining more consistent NDFD values. It is important to note that these values from any single dedicated laboratory may consistently be greater or less than other laboratories, or the mean of other laboratories, but the improved consistency from using a single laboratory allows for adjustments in diet formulation based on experience that compensate for any positive or negative biases within NDFD values.

Hall and Mertens (2012) conducted a ring test to examine within- and across-laboratory variability for 30-hour determinations of NDFD based on 14 forages [alfalfa hay or silage (4), corn silage or stover (5), or various grasses (5)]. Forage samples were ground to pass a 6-mm screen and pass a test for homogeneity; test samples were then sent to 9 laboratories three times over a 12-month period. Laboratories used different grinding, incubation, and internal replication procedures; one laboratory used two different protocols, and on that basis, was considered to be two independent laboratories within the statistical analysis. Procedural variations included those for sample size (0.25 or 0.50 grams), incubation vessels, continuous gassing, single sample or batch incubation (CONV vs. ANKOM), residual ash or ash-free calculations of NDFD, as well as the number and type of rumen fluid donors

coupled with their associated inconsistencies with respect to basal diet.

The analysis of results included laboratory, incubation run within laboratory, forage, and the laboratory × forage interaction within the statistical model, all of which were statistically significant. The presence of a detectable laboratory × forage interaction suggested that laboratory-associated bias was not simple nor consistent, but rather, was somewhat dependent on the forage substrate. Overall mean values of NDFD for the 14 test forages are summarized in Figure 69; they indicate that laboratories that used priming techniques (laboratory 3 and 10) exhibited less digestion of NDF than all other laboratories. This differential ranged from 12.7 to 23.6 percentage units of NDF, with a mean across the entire study of 18.1 percentage units (48.8 vs. 30.7% of NDF). Unlike previous studies using priming techniques (Goeser and Combs, 2009; Goeser et al., 2009), interassay variability was not improved relative to laboratories using unprimed rumen fluid.

For laboratories using unprimed rumen fluid, the within-laboratory repeatability (95% probability) of NDFD assays was 10.2 percentage units of NDF when considered across all forages. This indicates that a sample with an average NDFD value of 50% would likely yield individual results ranging from about 45 to 55%. Within this context, respective repeatabilities for alfalfa, corn silage, and grass forage types were 9.1, 11.4, 9.9 percentage units of NDF. On an across-laboratory basis, the 95% probability limit for reproducibility was somewhat larger (13.4 percentage units), with those for alfalfa, corn silage, and grass forage types being 10.2, 14.4, and 14.9 percentage units of NDF, respectively. Although the 30-hour NDFD values obtained throughout the study lacked overall precision, labs using unprimed rumen fluid were generally able to rank forages consistently, suggesting that a relative ranking system might reduce the effects of within- and across-laboratory variability and give a more accurate portrayal of the comparative values than simple numeric values.

### *Considerations for Standardization of Methods.*

It should be obvious that it would be highly desirable for all laboratories conducting in vitro analyses of NDF digestion to use the same equipment

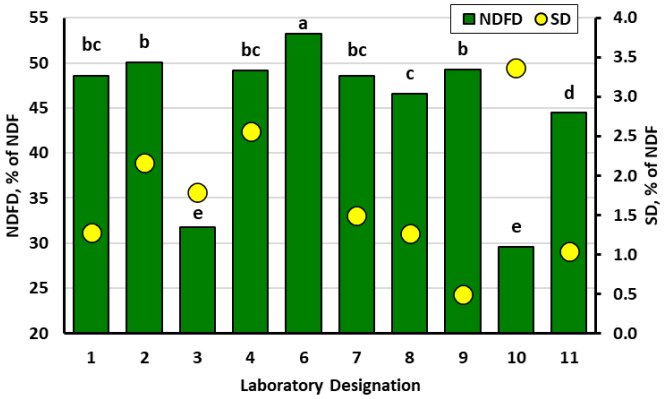


*Figure 69. In vitro neutral detergent fiber digestibility (NDFD, % of NDF; ■) of 14 forage samples [alfalfa (4), corn (5), and various cool- and warm-season grasses (5)] as determined by nine laboratories (one laboratory used two different procedures and was considered here as two separate laboratories). Yellow circles (●) indicate the standard deviation (SD) of replicates within incubation run; Laboratory #6 used only one sample replicate per incubation run, which prevented calculation of SD. Concentrations of NDFD with dissimilar data labels are different at the P < 0.05 level of confidence [adapted from Hall and Mertens (2012)].*

and procedures. Furthermore, it was correctly noted by Hall and Mertens (2012) that i) in vitro NDF digestion is an empirical assay for which there are no absolute sample values against which to compare laboratory results, and ii) proficiency tests that determine consensus values for labs running this assay are the best approach for monitoring laboratory performance. In the Hall and Mertens (2012) report, it can be noted that 5 of 8 laboratories using unprimed rumen fluid did not differ with respect to overall 30-hour NDFD values, thereby suggesting some method variation likely can be tolerated. This is desirable considering the practical realities of conducting these in vitro assays. For example, consider only the potential issues surrounding donation of rumen fluid. Within the Hall and Mertens (2012) report, 7 laboratories obtained rumen fluid from 1 to 4 lactating cows, while other laboratories drew fluid from 3 steers, 2 nonlactating cows, or 2 to 3 dry cows. Each laboratory offered donor animals different basal diets ranging from 50 to 100% forage, with forage and concentrate components varying widely in composition. It might be relatively easy for some university or government research laboratories with associated beef or dairy research herds to maintain consistency for donor animals and their basal diets. This may be considerably more problematic for private fee-for-service laboratories that may need to partner with local beef or dairy

producers to maintain accessible donor animals. Inherent in that reality is some loss of control, making consistency more difficult. In a comprehensive review, Weiss (1994) specifically mentions 1) selecting donors of the same species as the targeted livestock application and 2) collecting fluid at the same time relative to feeding as important; the latter of these implies consistency with respect to animal care and management. Many scientists prefer to synchronize the donor animal/basal diet with the targeted livestock application. For example, if the targeted livestock application was constructing diets for lactating dairy cows, the donor animal/basal diet could be synchronized to that application. Generally, maintaining donor animals on pasture is ill-advised because the basal diet cannot be controlled and will change over time with plant maturity or age. In addition, species composition will likely vary over time in mixed species pastures, as will animal selectivity and preference. It also is generally recognized that maintaining donor animals on poor quality forages with low CP concentrations is undesirable, potentially resulting in poorer accuracy and precision (Weiss, 1994).

In contrast, within-laboratory analytical procedures can (and should) be tightly controlled. Usually this can be easily accomplished. An incomplete list of relevant controllable factors could include: i) using a consistent grinding protocol, ii) standardizing sample size (0.25 or 0.50 grams); iii) consistently weighing post-assay recovery samples either hot (directly out of the drying oven) or desiccated; iv) reporting results either ash-free or with residual ash; and v) conducting the terminal extraction in neutral detergent with consistent procedures (including filtering consistency). Obviously, there are numerous other within-laboratory possibilities.

When analyzing large groups of related samples, such as those obtained from agronomic field studies, the precision (low error/variability) required to pick up small statistical differences between treatments is usually more important than the accuracy of means for which there are no standard values. Within that context, some allowance must be made to account for run-to-run variability whenever sample numbers (and their duplicates) exceed laboratory capacity for a single incubation run. This can be accomplished by digesting all samples in singlet within one incubation run, and then analyzing duplicates or triplicates in subsequent runs, or by using some statistically appropriate blocking procedure. Special considerations are advised for ANKOM incubations conducted in Daisy$^{II}$ incubators, some of which have been summarized for large research studies (Coblentz et al., 2019). For example, it may be beneficial to standardize the internal temperature of multiple incubators to an independent external thermometer, or to otherwise minimize any analytical biases caused by individual incubators whenever multiple incubators are needed.

In conclusion, single-endpoint in vitro determinations of NDFD are critical components of ruminant nutrition, affecting total DM digestibility, energy density, as well as proper diet formulation. It should be obvious that variability is inherent within and across in vitro NDFD assays, and that absolute values for standard substrates cannot be obtained. Furthermore, despite attempts to standardize some in vitro procedures, this is likely an impossible goal. However, individual laboratories can strive to maintain consistency for their procedures on a within-laboratory basis.

The 48-hour incubation remains the basis for determination of forage fiber contributions to forage energy density and subsequent diet formulation within many nutritional models. Although shorter incubation intervals, such as 30-hour incubations for lactating dairy cow applications, may make some logical sense, these shorter incubation intervals consistently exhibit greater inherent variability, thereby potentially limiting their usefulness and application. Variability associated with short-term (< 48 hours) incubations is often linked with varying activities of rumen inoculum. In part, this can be an artifact of poor or inconsistent procedures for removal and processing of rumen fluid from donor animals. Generally, these effects are mitigated at longer incubation times, particularly those (≥ 120 hours) that are often used to quantify uNDF.

## References


*Allen, M.S., and D.R. Mertens. 1988. Evaluating constraints on fiber digestion by rumen microbes. J. Nutr. 118:261-270.*

*Barry, M.C, and M.B Hall. 2025. Comparison of 2-pool and 3-pool digestion kinetic model predictions of neutral detergent fiber digestibility of forages from commercially available data. J. Dairy Sci. 108:2371-2380.*

*Bender, R.W., D.E. Cook, and D.K. Combs. 2016. Comparison of in-situ versus in vitro methods of fiber digestion at 120 and 288 hours to quantify the indigestible neutral detergent fiber fraction of corn silage samples. J. Dairy Sci. 99:5394-5400.*

*Buxton, D.R. 1989. In vitro digestion kinetics of temperate perennial forage legume and grass stems. Crop Sci. 29:213-219.*

*Casler, M.D., and H.G. Jung. 1999. Selection and evaluation of smooth bromegrass clones with divergent lignin and etherified ferulic acid concentrations. Crop Sci. 39:1866-1873.*

*Casler, M.D., H.G. Jung, and W.K. Coblentz. 2008. Clonal selection for lignin and etherified ferulates in three perennial grasses. Crop Sci. 48:424-433.*

*Chandler, J.A., W.J. Jewell, J.M. Gossett, P.J. Van Soest, and J.B. Robertson. 1980. Predicting methane fermentation biodegradability. Pages 93-107 In J. Biotech. and Bioengineering Symp. 10, John Wiley and Sons.*

*Cherney, J.H., G.C. Marten, and R.G. Goodrich. 1983. Rate and extent of cell wall digestion of total forage and morphological components of oats and barley. Crop Sci. 23:213-216.*

*Coblentz, W.K., M.S. Akins, K.F. Kalscheur, G.E. Brink, and J.S. Cavadini. 2018. Effects of growth stage and growing degree day accumulations on triticale forages: 2) In vitro disappearance of neutral detergent fiber. J. Dairy Sci. 101:8986-9003.*

*Coblentz, W.K., M.S. Akins, R.K. Ogden, L.M. Bauman, and A.J. Stammer. 2019. Effects of sample size on neutral detergent fiber digestibility of triticale forages using the Ankom DaisyII Incubator system. J. Dairy Sci. 102:6987-6999.*

*Coblentz, W.K., N.M. Esser, P.C. Hoffman, and M.S. Akins. 2015. Growth performance and sorting characteristics of corn silage-alfalfa haylage diets with or without forage dilution offered to replacement Holstein dairy heifers. J. Dairy Sci. 98:8018-8034.*

*Coblentz, W.K., J.O. Fritz, W.H. Fick, R.C. Cochran, and J.E. Shirley. 1998. In situ dry matter, nitrogen, and fiber degradation of alfalfa, red clover, and eastern gamagrass at four maturities. J. Dairy Sci. 81:150-161.*

*Coblentz, W. K., and P. C. Hoffman. 2009. Effects of spontaneous heating on fiber composition, fiber digestibility, and in situ disappearance kinetics of NDF for alfalfa-orchardgrass hays. J. Dairy Sci. 92:2875-2895.*

*Coblentz, W.K., P.C. Hoffman, N.M. Esser, and M.G. Bertram. 2012. Using eastern gamagrass to construct diets that limit intake and caloric density for dairy replacement heifers. J. Dairy Sci. 95:6057-6071.*

*Coblentz, W.K., P.C. Hoffman, W.E. Jokela, and M.G. Bertram. 2010. Unique dairy applications of eastern gamagrass forages in central Wisconsin: II. Nutritive value and energy density. Agron. J. 102:1720-1730.*

*Ferreira, G., H. Galyon, A.I. Silva-Reis, A.A. Pereyra, E.S. Richardson, C.L. Teets, P. Bevins, R.R. Cockrum, and M.J. Aguerre. 2022. Ruminal fiber degradation kinetics within and among warm-season annual grasses as affected by the brown midrib mutation. Animals (Basel) 12:2536.*

*Ferreira, G. and D.R. Mertens. 2005. Chemical and physical characteristics of corn silages and their effects on in vitro disappearance. J. Dairy Sci. 88:4414-4425.*

*Ghimire, P., and G. Ferreira. 2026. Artifacts and interpretations of in-situ ruminal neutral detergent fiber degradation kinetics of forages. J. Dairy Sci. 109:5145-5153. https://doi.org/10.3168/jds.2025-27491.*

*Goering, H.K., and P.J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.*

*Goeser, J.P., and D.K. Combs. 2009. An alternative method to assess 24-h ruminal in vitro neutral detergent fiber digestibility. J. Dairy Sci. 92:3833-3841.*

*Goeser, J.P., P.C. Hoffman, and D.K. Combs. 2009. Modification of a rumen fluid priming technique for measuring in vitro neutral detergent fiber digestibility. J. Dairy Sci. 92:3842-3848.*

*Grant, R.J. 1994. Influence of corn and sorghum starch on the in vitro kinetics of forage fiber digestion. J. Dairy Sci. 77:1563-1569.*

*Grant, R.J. 2015. Making milk with forage: understanding rumen fiber dynamics. Pages 63-69 in Proc. Four-State Dairy Nutrition and Management Conference, Dubuque, IA.*

*Grant, R.J., and D.R. Mertens. 1992a. Impact of in vitro fermentation techniques upon kinetics of fiber digestion. J. Dairy Sci. 75:1263-1272.*

*Grant, R.J., and D.R. Mertens. 1992b. Development of buffer systems for pH control and evaluation of pH effects on fiber digestion in vitro. J. Dairy Sci. 75:1581-1587.*

*Grant, R.J., and D.R. Mertens. 1992c. Influence of buffer pH and raw corn starch addition on in vitro fiber digestion kinetics. J. Dairy Sci. 75:2762-2768.*

*Hall, M.B, and D.R. Mertens. 2008. In vitro fermentation vessel type and method alter fiber digestibility estimates. J. Dairy Sci. 91:301-307.*

*Hall, M.B., and D.R. Mertens. 2012. A ring of in vitro neutral detergent fiber digestibility: Analytical variability and sample ranking. J. Dairy Sci. 95:1992-2003.*

*Hall, M.B., and D.R. Mertens. 2023. Comparison of alternative neutral detergent fiber methods to the AOAC definitive method. J. Dairy Sci. 106:5364-5378.*

*Hoffman, P.C., K.M. Lundberg, L.M. Bauman, and R.D. Shaver. 2003. In vitro NDF digestibility of forages: the 30 vs. 48-hour debate. Focus on Forage. Vol. 5: No. 16. University of Wisconsin Board of Regents, Madison, WI.*

*Hoffman, P.C., S.J. Sievert, R.D. Shaver, D.A. Welch, and D.K. Combs. 1993. In situ dry matter, protein, and fiber degradation of perennial forages. J. Dairy Sci. 76:2632-2643.*

*Hoffman, P.C., K.A. Weigel, and R.M. Wernberg. 2008. Evaluation of equations to predict dry matter intake of dairy heifers. J. Dairy Sci. 91:3699-3709.*

*Hoover, W.H. 1986. Chemical factors involved in ruminal fiber digestion. J. Dairy Sci. 69:2755-2766.*

*Johnson, L.M., J.H. Harrison, D. Davidson, C. Hunt, W.C. Mahanna, and K. Shinners. 2003. Corn silage management: effects of hybrid, maturity, chop length, and mechanical processing on rate and extent of digestion. J. Dairy Sci. 86:3271-3299.*

*Jung, H.G., and M.D. Casler. 1991. Relationship of lignin esterified phenolics to fermentation of smooth bromegrass fibre. Anim. Feed Sci. Technol. 32:63-68.*

*Jung, H.G., D.R. Mertens, and R.L. Phillips. 2011. Effect of reduced ferulate-mediated lignin/arabinoxylan cross-linking in corn silage on feed intake, digestibility, and milk production. J. Dairy Sci. 94:5124-5137.*

*Lopes, F., K. Ruh, and D.K. Combs. 2015. Validation of an approach to predict total tract fiber digestibility using a standard in vitro technique for different diets fed to high-producing dairy cows. J. Dairy Sci. 98:2596-2602.*

*Mandebvu, P., J.W. West, G.M. Hill, R.N. Gates, R.D. Hatfield, B.G. Mullinix, A.H. Parks, and A.B. Caudle. 1999. Comparison of Tifton 85 and Coastal bermudagrass for yield, nutrient traits, intake, and digestion by growing beef steers. J. Anim. Sci. 77:1572-1586.*

*McBeth, L.J., K.P. Coffey, W.K. Coblentz, D.H. Hellwig, J.E. Turner, and D.A. Scarbrough. 2003. Impact of level of spontaneous heating during storage of bermudagrass hay on rumen in situ disappearance kinetics in steers. Anim. Feed Sci. Technol. 108:147-158.*

*Mertens, D.R. 1993. Kinetics of cell wall digestion and passage in ruminants. Pages 535-570 In Forage Cell Wall Structure and Digestibility. H.G. Jung, D.R. Buxton, R.D. Hatfield, and J. Ralph, ed. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.*

*Mertens, D.R. 1994. Regulation of forage intake. Pages 450-493 In Forage Quality, Evaluation, and Utilization. G.C. Fahey, Jr., M. Collins, D.R. Mertens, and L.E. Moser, ed. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.*

*Mertens, D.R., and L.O. Ely. 1979. A dynamic model of fiber digestion and passage in the ruminant for evaluating forage quality. J. Anim. Sci. 49:1085-1095.*

*Mertens, D.R., and J.R. Loften. 1980. The effects of starch on forage fiber digestion kinetics in vitro. J. Dairy Sci. 63:1437-1446.*

*Moore, K.J., and J.H. Cherney. 1986. Digestion kinetics of sequentially extracted cell wall components of forages. Crop Sci. 26:1230-1235.*

*NASEM. 2001. Nutrient Requirements of Dairy Cattle. 7th edition. The National Academies Press. Washington, D.C.*

*NASEM. 2021. Nutrient Requirements of Dairy Cattle. 8th edition. The National Academies Press. Washington, D.C.*

*Oba, M., and M.S. Allen. 2000. Effects of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber: 3. Digestibility and microbial efficiency. J. Dairy Sci. 83:1350-1358.*

*Ogden, R.K., W.K. Coblentz, K.P. Coffey, J.E. Turner, D.A. Scarbrough, J.A. Jennings, and M.D. Richardson. 2005. Ruminal in situ disappearance kinetics of dry matter and fiber in growing steers for common crabgrass sampled on seven dates in northern Arkansas. J. Anim. Sci. 83:1142-1152.*

*Raffrenato, E., C.F. Nicholson, and M.E. Van Amburgh. 2019. Development of a mathematical model to predict pool sizes and rates of digestion of 2 pools of digestible neutral detergent fiber and an undigested neutral detergent fiber fraction within various forages. J. Dairy Sci. 102:351–364.*

*Raffrenato, E., D.A. Ross, M.E. Van Amburgh. 2018. Development of an in vitro method to determine rumen undigested aNDFom for use in feed evaluation. J. Dairy Sci. 101:9888-9900.*

*Smith, L.W, H.K. Goering, and C.H. Gordon. 1972. Relationships of forage composition with rates of cell wall digestion and indigestibility of cell walls. J. Dairy Sci. 55:1140-1147.*

*Smith, L.W., H.K. Goering, D.R. Waldo, and C.H. Gordon. 1971. In vitro digestion rate of forage cell wall components. J. Dairy Sci. 54:71-76.*

*Stauss, R. 1994. Compendium of growth stage identification keys or mono- and dicotyledonous plants. Extended BBCH scale. Ciba-Geigy Basel, Switzerland.*

*Tilley, J.M.A., and R.A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Br. Grassl. Soc. 18:104-111.*

*Valentine, M.E., E. Karayilanli, J.H. Cherney, and D.J. Cherney. 2019. Comparison of in vitro long digestion methods and digestion rates for diverse forages. Crop Sci. 59:422-435.*

*Van Amburgh, M.E., and P.J. Van Soest. 2003. Corn silage neutral detergent fiber: refining a mathematical approach for in vitro rates of digestion. Proc. Cornell Nut. Conf., p. 99-108.*

*Van Soest, P.J. 1967. Development of a comprehensive system of feed analyses and its application to forages. J. Anim. Sci. 119-128.*

*Van Soest, P.J. 1982. Nutritional Ecology of the Ruminant. Comstock Publishing Associates, Cornell University Press. Ithaca, NY.*

*Van Soest, P.J., M.E. Van Amburgh, and L.O. Tedeschi. 2000. Rumen balance and rates of fiber digestion. Proc. Cornell Nut. Conf., p. 150-166.*

*Van Soest, P.J., R.H. Wine, and L.A. Moore. 1966. Estimation of the true digestibility of forages by the in vitro digestion of cell walls. Proceedings of the Xth International Grassland Congress. Finnish Grassland Association, Helsinki, pp. 438-441.*

*Vogel, K.P., J.F. Peterson, S.D. Masterson, and J.J. Toy. 1999. Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. Crop Sci. 39:276-279.*

*Waldo, D.R. 1969. Factors influencing the voluntary intake of forages. p. E1-E22. In Proc. Natl. Conf. Forage Qual. Eval. Util., Lincoln, NE.*

*Weiss, W.P. 1994. Estimation of digestibility of forages by laboratory methods. Pages 644-681 In Forage Quality, Evaluation, and Utilization. G.C. Fahey, Jr., M. Collins, D.R. Mertens, and L.E. Moser, ed. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.*

*Wilman, D., and A. Adesogan. 2000. A comparison of filter bag methods with conventional tube methods of determining the in vitro digestibility of forages. Anim. Feed Sci. Technol. 84:33-47.*

## *Supplemental Resources*

*Barry, M.C., and M.B. Hall. 2025. Comparison of 2-pool and 3-pool digestion kinetic model predictions of neutral detergent fiber digestibility of forages from commercially available data. J. Dairy Sci. 108:2371-2380.*

*Beck, P.A., S. Hutchison, S.A. Gunter, T.C. Losi, C.B. Stewart, P.K. Capps, and J.M. Phillips. 2007. Chemical composition and in situ dry matter and fiber disappearance of sorghum x sudangrass hybrids. J. Anim. Sci. 85:545-555.*

*Bender, R.W., D.E. Cook, and D.K. Combs. 2016. Comparison of in situ versus in vitro methods of fiber digestion at 120 and 288 hours to quantify the indigestible neutral detergent fiber fraction of corn silage samples. J. Dairy Sci. 99:5394-5400.*

*Coblentz, W.K., M.S. Akins, K.F. Kalscheur, G.E. Brink, and J.S. Cavadini. 2018. Effects of growth stage and growing degree day accumulations on triticale forages: 2) In vitro disappearance of neutral detergent fiber. J. Dairy Sci. 101:8986-9003.*

*Coblentz, W. K., K. P. Coffey, J. E. Turner, D. A. Scarbrough, J. S. Weyers, K. F. Harrison, Z. B. Johnson, L. B. Daniels, C.F. Rosenkrans, Jr., D. W. Kellogg, and D. S. Hubbell, III. 2000. Effect of maturity on degradation kinetics of sod-seeded cereal grains in northern Arkansas. J. Dairy Sci. 83:2499-2511.*

*Coblentz, W. K., J. O. Fritz, W. H. Fick, R. C. Cochran, and J. E. Shirley. 1998. In situ dry matter, nitrogen, and fiber degradation of alfalfa, red clover, and eastern gamagrass at four maturities. J. Dairy Sci. 81:150-161.*

*Coblentz, W. K., and P. C. Hoffman. 2009. Effects of spontaneous heating on fiber composition, fiber digestibility, and in situ disappearance kinetics of NDF for alfalfa-orchardgrass hays. J. Dairy Sci. 92:2875-2895.*

*Coblentz, W.K., P.C. Hoffman, N.M. Esser, and M.G. Bertram. 2012. Using eastern gamagrass to construct diets that limit intake and caloric density for dairy heifers. J. Dairy Sci. 95:6057-6071.*

*Coblentz, W. K., and R. P. Walgenbach. 2010. In situ disappearance of dry matter and fiber from fall-grown cereal-grain forages from the north-central US. J. Anim. Sci. 88:3992-4005.*

*Ferreira, C., H. Galyon, A.I. Silva-Reis, A.A. Pereyra, E.S. Richardson, C.L. Teets, P. Bevins, R.R. Cockrum, and M.J. Aguerre. 2022. Ruminal fiber degradation kinetics within and among warm-season annual grasses as affected by the brown midrib mutation. Animals (Basel) 12:2536.*

*Ferreira, C., C.L. Teets, H. Galyon, A.L. Cappellina, M.E. Schultz, K.M. Payne, S. Stewart, and W.E. Thomason. 2025. Effect of maturity at harvest of small-grain grasses on the nutritional composition of forage and ration formulation. J. Dairy Sci. 108:4934-4945.*

*Flores, R., W.K. Coblentz, R.K. Ogden, K.P. Coffey, M.L. Looper, C.P. West, and C.F. Rosenkrans, Jr. 2007. Effects of fescue type and sampling date on the ruminal disappearance kinetics of autumn-stockpiled tall fescue. J. Dairy Sci. 90:2883-2896.*

*Fritz, J.O., K.J. Moore, and E.H. Jaster. 1988. In situ digestion kinetics and ruminal turnover rates of normal and brown midrib mutant sorghum x sudangrass hays fed to nonlactating Holstein cows. J. Dairy Sci. 71:3345-3351.*

*Galdámez-Cabrera, N.W., K.P. Coffey, W.K. Coblentz, J.E. Turner, D.A. Scarbrough, Z.B. Johnson, J.L. Gunsaulis, M.B Daniels, and D.H. Hellwig. 2003. In situ ruminal degradation of dry matter and fiber from bermudagrass fertilized with different nitrogen rates and harvested on two dates. Anim. Feed Sci. Technol. 105:185-198.*

*Galyon, H., B.A. Corl, and G. Ferreira. 2024. Ruminal passage rate and digestibility of fiber from dairy cows consuming diets containing alfalfa and orchardgrass hays with different concentrations of undegradable neutral detergent fiber (uNDF). J. Dairy Sci. 107:10751-10760.*

*Grant, R.J. 1994. Influence of corn and sorghum starch on the in vitro kinetics of forage fiber digestion. J. Dairy Sci. 77:1563-1569.*

*Grant, R.J., and D.R. Mertens. 1992. Influence of buffer pH and raw corn starch addition on in vitro fiber digestion kinetics. J. Dairy Sci. 75:2762-2768.*

*Grant, R.J., and S.J. Weidner. 1992. Digestion kinetics of fiber: influence of in vitro buffer pH varied within observed physiological range. J Dairy Sci. 75:1060-1068.*

*Hoffman, P.C., S.J. Sievert, R.D. Shaver, D.A. Welch, and D.K. Combs. 1993. In situ dry matter, protein, and fiber degradation of perennial forages. J. Dairy Sci. 76:2632-2643.*

*Johnson, L.M., J.H. Harrison, D. Davidson, C. Hunt, W.C. Mahanna, and K. Shinners. 2003. Corn silage management: effects of hybrid, maturity, chop length, and mechanical processing on rate and extent of digestion. J. Dairy Sci. 86:3271-3299.*

*Mandebvu, P., J.W. West, M.A. Froetschel, R.D. Hatfield, R.N. Gates, and G.M Hill. 1999. Effect of enzyme or microbial treatment of bermudagrass forages before ensiling on cell wall composition, end products of silage fermentation and in situ digestion kinetics. Anim. Feed Sci. Technol. 77:317-329.*

*Mandebvu, P., J.W. West, R.N. Gates, G.M. Hill. 1998. In vitro digestion kinetics of neutral detergent fiber extracted from Tifton 85 and Coastal bermudagrass. Anim. Feed Sci. Technol. 73:263-269.*

*Messman, M.A., W.P. Weiss, and D.O. Erickson. 1991. Effects of nitrogen fertilization and maturity of bromegrass on in situ ruminal digestion kinetics of fiber. J. Anim. Sci. 69:1151-1161.*

*McBeth, L.J., K.P. Coffey, W.K. Coblentz, D.H. Hellwig, J.E. Turner, and D.A. Scarbrough. 2003. Impact of level of spontaneous heating during storage of bermudagrass hay on rumen in situ disappearance kinetics in steers. Anim. Feed Sci. Technol. 108:147-158.*

*Mertens, D.R., and J.R. Loften. 1980. The effect of starch on forage fiber digestion kinetics in vitro. J. Dairy Sci. 63:1437-1446.*

*Moore. K.J., and J.H. Cherney. 1986. Digestion kinetics of sequentially extracted cell wall components of forages. Crop Sci. 26:1230-1235.*

*Ogden, R.K., W.K. Coblentz, K.P. Coffey, J.E. Turner, D.A Scarbrough, J.A. Jennings, and M.D. Richardson. 2005. Ruminal in situ disappearance kinetics of dry matter and fiber in growing steers for common crabgrass forages sampled on seven dates in northern Arkansas. J. Anim. Sci. 83:1142-1152.*

*Scarbrough, D.A., W.K. Coblentz, K.P. Coffey, J.E. Turner, G.V. Davis, D.W. Kellogg, and D.H. Hellwig. 2001. Effects of calendar date and summer management on the in situ dry matter and fiber degradation of stockpiled forage from bermudagrass pastures. J. Anim. Sci. 79:3158-3169.*

*Smith, L.W., H.K. Goering, and C.H. Gordon. 1972. Relationships of forage compositions with rates of cell wall digestion and indigestibility of cell walls. J. Dairy Sci. 55:1140-1147.*

*Smith, L.W., H.K. Goering, D.R. Waldo, and C.H. Gordon. 1971. In vitro digestion rate of forage cell wall components. J. Dairy Sci. 54:71-76.*

*Turner, J.E., W.K. Coblentz, K.P. Coffey, R.T. Rhein, B.C. McGinley, N.W. Galdámez, C.F. Rosenkrans, Z.B. Johnson, D.W. Kellogg, and J.V. Skinner. 2004. Effects of natural rainfall and spontaneous heating on voluntary intake, digestibility, and in situ disappearance kinetics, passage kinetics, and ruminal fermentation characteristics of tall fescue hay fed to growing steers. Anim. Feed Sci. Technol. 116:15-33.*

*Twidwell, E.K., K.D. Johnson, J.H. Cherney, and J.J. Volenec. 1988. Forage quality and digestion kinetics of switchgrass herbage and morphological components. Crop Sci. 28:778-782.*

*Valentine, M.E., E. Karayilanli, J.H. Cherney, and D.J. Cherney. 2019. Comparison of in vitro long digestion methods and digestion rates for diverse forages. Crop Sci. 59:422-435.*

*Van Amburgh, M.E., P.J. Van Soest, J.B. Robertson, and W.F. Knaus. 2003. Corn silage neutral detergent fiber: refining a mathematical approach for in vitro rates of digestion. Pp. 99-108. Proc. Cornell Nutr. Conf., Syracuse, NY.*

**U.S. Dairy Forage Research Center,** USDA-Agricultural Research Service
1925 Linden Drive, Madison, WI 53706 | Phone: 608-890-0050

**https://www.ars.usda.gov/midwest-area/madison-wi/us-dairy-forage-research-center/**

**Leading the world in integrated dairy forage systems research.**