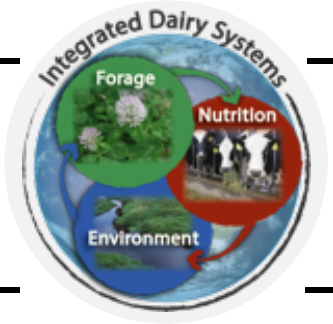
USDA Agricultural Research Service
U.S. DEPARTMENT OF AGRICULTURE

Forage Fiber:
Definitions, Descriptions, and Determining Factors



U.S. Dairy Forage Research Center

Forage Fiber Components – What Are They?

Introduction

Carbohydrates are essential compounds within forage plants, performing critical roles that are both metabolic and structural in nature. From a structural perspective, the cell walls of forage plants provide rigidity, a framework for the general architecture of the plant, and support for various plant parts, perhaps most notably the inflorescence and/or filling seed head. Generally, plant cell walls are comprised of primary and secondary layers with cellulose, hemicellulose, lignin, and pectin the principal organic components. It is important to emphasize that cellulose, hemicellulose, and pectin are polysaccharides that are widely variable with respect to composition and interactive linkages, and do not necessarily represent pure chemical entities. Forage cellulose is primarily a polymer of β-glucose molecules linked through 1-4 glycosidic bonds. Hemicellulose is rich in xylose with significant branching, often with arabinose, and it is usually closely associated with lignin. Together, hemicellulose and lignin comprise much of the secondary cell wall as it thickens during plant development. Pectin is rich in galacturonic acid and is found in the middle lamella, serving as sort of an intercellular glue.

To begin this discussion, it is important to distinguish between the terms 'cell wall' and 'fiber'. Although these terms are sometimes used interchangeably, the latter term is more nutritional in nature, generally reflecting the portion of the forage plant that demonstrates some resistance to ruminal digestion, and therefore is digested incompletely. Technically, pectin is part of the 'cell wall', but is not considered to be forage 'fiber' because it is soluble in neutral detergent and completely digestible. These characteristics of pectin are important with respect to any nutritional evaluation, and especially important when assessing whole-plant digestibility of dicots because concentrations of pectin in dicots are much greater than what is typically observed for grasses. Van Soest (1982) summarized the pectin concentrations of various forages and reported a range of 5 to 10% for alfalfa (dicot) compared to only 1 to 2% for temperate and tropical grasses.

The incomplete nature of forage fiber digestion can be visualized by comparing Lucas tests for 19 forages (11 grasses, 8 legumes) as summarized by Van Soest (1967, 1982; Figure 1). For a Lucas test, the goal is to discern or identify feed fractions that demonstrate a constant true digestibility over a wide range of feedstuffs. Potentially, this may include a wide range of forages, or a single forage harvested over a wide range of maturities. In this analysis, the digestible amount of the component is regressed against the concentration of that same component in the forage or feedstuff, where an 'ideal' fraction should exhibit a slope = 1, and a negative or 0 intercept. A (negative) intercept is an estimate of metabolic or endogenous excretion of the feed fraction, but positive intercepts have no biological meaning. In addition, the standard deviation of the

Note: *There is only peripheral mention/discussion of near infrared reflectance spectroscopy (NIRS) throughout this outreach publication. It is acknowledged that many producer and research forage/feedstuff samples are analyzed for nutritive value using this well-established technique. However, it remains a secondary method of analysis that is reliant on calibration sets generated by using the traditional laboratory methods of analysis that are described herein. As such, detailed discussions of NIRS methods and analysis are omitted here, but are clearly worthy of a separate, stand-alone document.*

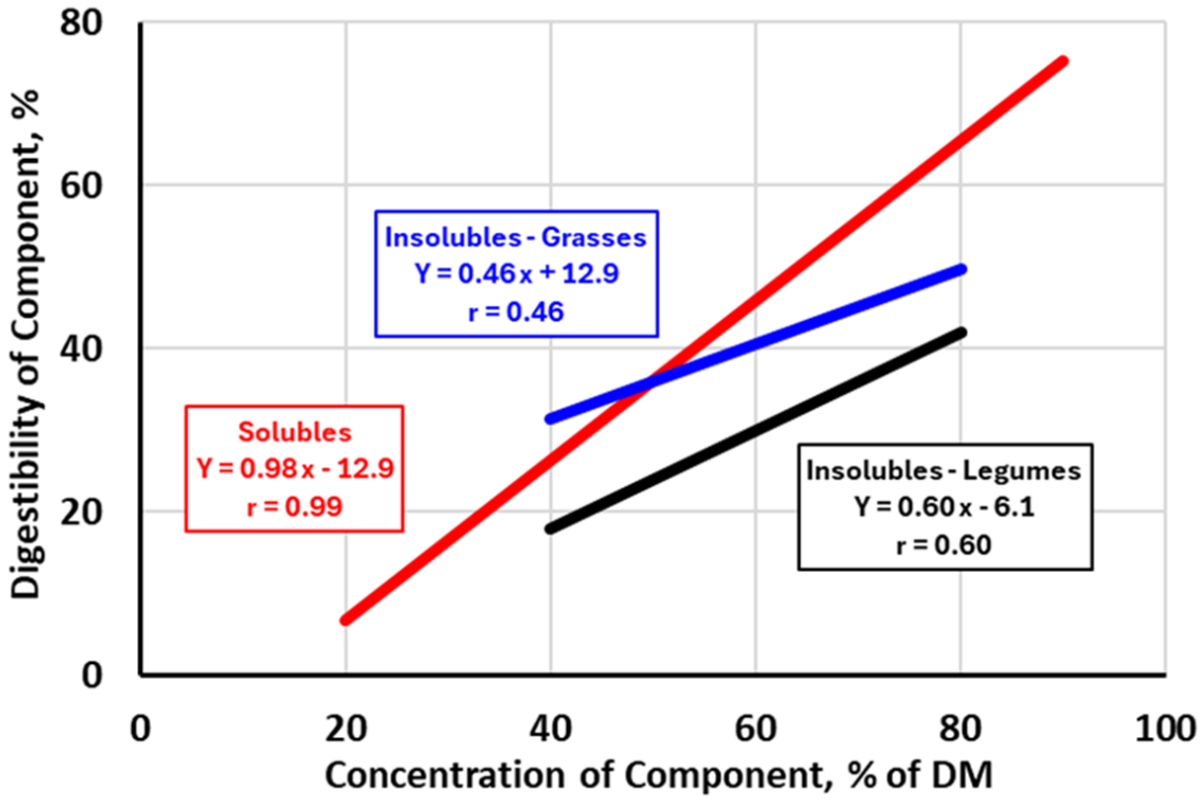


Figure 1. Lucas tests for neutral detergent solubles (—), neutral detergent insolubles of grasses (—), and neutral detergent insolubles of legumes (—), where insolubles generally represent forage fiber [adapted from Van Soest (1967, 1982)].

regression slope should be low, indicating consistency and limited variability associated with the regression relationship.

Extraction of forages in neutral detergent separates (removes) the cell solubles from the residual fibrous portion of the forage. The aforementioned Lucas test specific to neutral detergent solubles yielded a slope of 0.98, thereby indicating it is an ideal or uniform fraction that is completely digestible, regardless of the concentration of solubles within the forage (Figure 1). In contrast, Lucas tests for the insoluble residual forage fiber resulted in regression slopes of 0.46 (grasses) and 0.60 (legumes), indicating non-uniformity or incomplete availability to the ruminant animal. It also suggests the forage fiber associated with grasses is often more digestible than that of legumes, which is a concept to be developed further throughout this outreach publication.

Although not shown in Figure 1, it is possible that some forage components can be considered ideal or uniform because they demonstrate no true digestibility via Lucas test. Van Soest (1967, 1982) described such a relationship for acid detergent lignin (**ADL**), where the resulting Lucas regression equation was Y = (- 0.02 x) + 0.1. Generally, lignin is a class of phenolic compounds that are bound to structural polysaccharides; they perform important functions in plant structure and rigidity, but also are a major impediment to ruminant digestion. Lignin affects only the digestibility of forage fiber; it does not affect the digestibility of all forage organic matter (**OM**), and specifically not the digestibility of cell or neutral detergent solubles.

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**); therefore, great research effort, including continuous refinement of techniques, have been prioritized to properly quantify and assess the concentrations of forage fiber, as well as their associated digestibilities. This is critical for a wide variety of reasons, including regulation of voluntary intake, proper rumen function, and quantifying energy availability that supports production of meat and milk.

Proximate and Detergent Systems of Analysis

Throughout much of the early 20th century, the proximate analysis system was used routinely for forage and feedstuff analysis. However, this system was unsatisfactory in many respects, including its role in erroneous estimation of energy density (expressed as total digestible nutrients; **TDN**), which subsequently impaired the appropriate formulation of ruminant diets. As described by Van Soest (1982), the proximate system required analysis for: i) absolute DM (determined by drying at 100°C); ii) ether extract; iii) crude fiber; iv) nitrogen (**N**); and v) ash. Subsequently, nitrogen-free extract (**NFE**) was calculated by difference as: 100% - [ether extract + crude fiber + ash + (N × 6.25)], and was assumed to primarily represent highly digestible carbohydrates, such as sugars. There were numerous problematic assumptions associated with this system and errors varied considerably on a situational basis.

One frequently noted issue involved the determination of crude fiber, which was assumed to represent the least digestible portions of the plant, namely structural plant fiber. The crude fiber assay did not quantitatively recover hemicellulose and lignin, and to a lesser extent, cellulose. Obviously, this led to an underestimation of what was assumed to be structural plant fiber. Furthermore, since NFE was determined by difference, its calculation included the cumulative errors associated with all other measurements. However, these errors often inflated concentrations of NFE, specifically through the solubilization of lignin, hemicellulose, and cellulose. As such, the apparent digestibility of crude fiber will exceed that of NFE in about 30% of all feedstuffs, and particularly for grasses that contain greater concentrations hemicellulose (Van Soest, 1982).

As a result of these problems, the detergent analysis system was developed, primarily by Dr. Peter Van Soest (USDA-ARS) at Beltsville, MD, and quickly replaced the proximate analysis system during the 1960's. While the detergent system and its procedures have been refined during the intervening years, in part to improve standardization across laboratories, it remains a foundational bulwark in the nutritional evaluation of forages and feedstuffs today. A very simplified schematic diagram of the detergent system is illustrated in Figure 2. More detailed descriptions of the system, its procedures, and various nuances associated with determination of specific forage components are included in subsequent discussion sections.

In the detergent system, an extraction in neutral detergent is used to remove cell solubles and pectin, leaving a residue of forage fiber comprised of cellulose, hemicellulose, lignin, some associated N or crude protein (**CP**; N × 6.25), and residual ash. As noted previously, cell solubles are considered to be an ideal feed fraction that are completely digestible (Figure 1). Therefore, a high priority is placed on the quantification and incomplete digestibility of forage fiber. This analytical priority is essential in properly determining a forage's energy density but also serves other functions. From a practical perspective, forage fiber is an important contributor to the regulation of voluntary intake and helps to maintain proper rumen function and health.

In the stepwise approach of the detergent system of analysis, the first step is to extract cell solubles and pectin from the forage (or other feedstuff) using

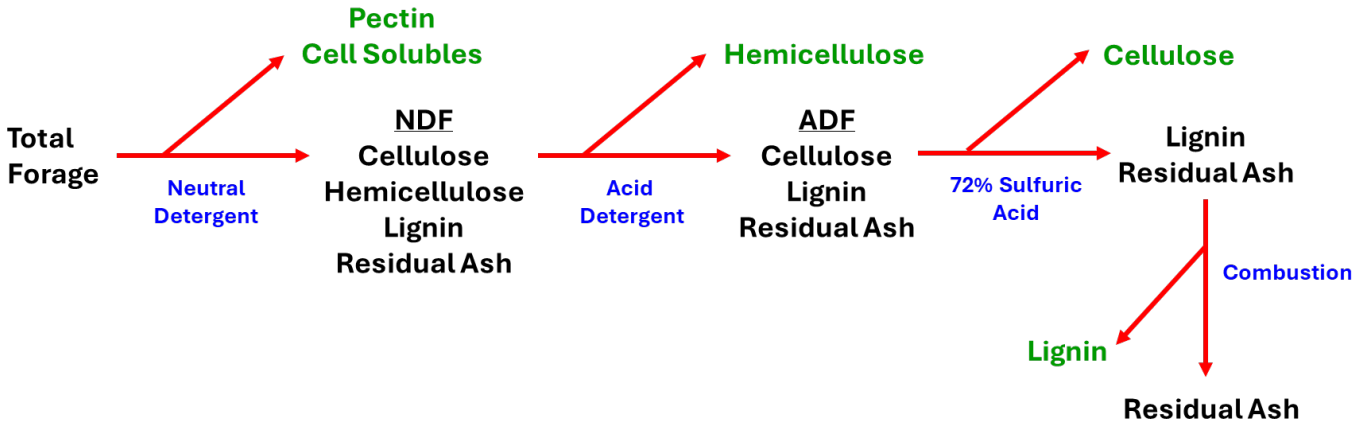


Figure 2. Simplified schematic diagram of the detergent system of analysis of forages and other feedstuffs.

neutral detergent. The residual fiber from this procedure is known as neutral detergent fiber (**NDF**). Subsequently, acid detergent is used to solubilize and extract hemicellulose. The residual fiber from this step is defined as acid detergent fiber (**ADF**). It should be noted that extractions in neutral and/or acid detergent can be conducted sequentially or independently, depending on analytical goals. Many commercial laboratories routinely conduct neutral and acid detergent extractions independently. Concentrations of hemicellulose are often estimated by difference (NDF – ADF) following independent determinations or are calculated directly by weight loss following sequential extractions. While both sequential and independent extractions are generally acceptable, particularly for routine applications, there are specific situations when one analytical approach may be preferred or recommended (Van Soest et al., 1991).

Irrespective of whether NDF and ADF are determined sequentially or independently, cellulose can then be removed from the remaining ADF residue with a 72% sulfuric acid solution. After extracting cellulose, the remaining residue contains minerals and lignin. Acid detergent lignin (**ADL**) is determined by combustion and subsequently calculated as the weight loss associated with the combustion procedure.

In some cases, the NDF and ADF residues may be referred to as 'NDR' and 'ADR', respectively, indicating they represent *residual* insoluble fibers. However, those designations are somewhat dated; furthermore, NDR often implies use of α-amylase to improve removal of starch without inclusion of sodium sulfite (Mertens, 2015). Each component in the detergent system is quantified gravimetrically, based on the weight changes associated with each step in the evaluation sequence. A summary of fiber analyses for a variety of hays is provided in Table 1, which were compiled from several commercial analytical laboratories that evaluate huge numbers of forage samples annually (NASEM, 2021).

Based on the summary provided in Table 1, several trends among forage types should become obvious, and these will be developed further throughout this publication:

- Concentrations of all fiber components increase as forages mature.

Table 1. Examples of fiber concentrations for various hays determined in analytical laboratories based on detergent procedures as summarized by NASEM (2021). Based on the analyses provided, the term 'grass' implies that these grasses possess the cool-season (C3) photosynthetic pathway.

Forage[1]	Maturity[2]	*N*[3]	NDF[4]	ADF[4]	HEMI[4]	Lignin	NDFD[4]
		#	---------- % of DM ----------				% of NDF
Legume	Immature	86,443	37.7	30.7	7.0	6.59	51.4
	Mid-Maturity	101,963	41.1	32.1	9.0	6.64	52.4
	Mature	17,405	46.6	37.2	9.4	8.12	43.4
Legume-Grass	Immature	4,877	43.9	32.9	11.0	6.92	49.9
	Mature	2,636	51.2	38.7	12.5	8.27	20.1[5]
Grass-Legume	Mid-Maturity	4,654	54.7	33.8	20.9	4.28	67.5
	Mature	36,398	62.0	40.0	22.0	6.08	47.5
Cool-Season Grass	Mid-Maturity	6,049	58.0	35.5	22.5	4.17	67.8
	Mature	28,429	66.7	41.4	25.3	5.97	55.8
Grass-Legume	Unspecified	36,240	58.2	39.3	18.9	6.70	54.5
Bermudagrass[6]	Unspecified	10,064	65.4	34.9	30.5	5.41	54.2

[1] *Unless otherwise indicated, the first listing for mixtures is the predominant forage type.*
[2] *Maturity designations are as provided by NASEM (2021).*
[3] *N = number of observations in the NDF mean, but this does not apply to other determinations.*
[4] *NDF, neutral detergent fiber; ADF, acid detergent fiber; HEMI, hemicellulose (estimated as NDF – ADF); and NDFD, 48-hour in-vitro NDF digestibility.*
[5] *Mean is based on only one observation.*
[6] *Bermudagrass is a perennial warm-season (C4) grass.*

- Grasses generally exhibit greater concentrations of NDF than legumes, and much of this difference is attributable to sharply greater concentrations of hemicellulose.
- Although more applicable for beef-related applications, perennial warm-season (C4) grasses, such as the bermudagrass shown in Table 1, typically exhibit greater concentrations of NDF than cool-season grasses or legumes. In part, this characteristic also can be linked to greater concentrations of hemicellulose compared to both cool-season grasses and legumes.
- Specific quantification of cellulose is conducted infrequently; however, cellulose concentrations tend to be less variable across forage types than hemicellulose or total NDF.
- Despite their lower NDF concentrations, legumes exhibit greater concentrations of lignin than grasses at roughly comparable stages of development.
- As a result of greater lignification, digestibility of NDF for legumes is often poorer relative to grasses, provided the maturity status of the forage types are reasonably comparable.

Detergent Analysis System

Brief Overview

It is not the objective of this publication to address all specific details concerning techniques, equipment, reagent solutions, or calculations related to quantification of fiber components in forages or concentrate feeds. Those issues are addressed in detail in various published formats that are widely available, perhaps most prominently in Goering and Van Soest (1970) and Van Soest et al. (1991). More recently, an AOAC approved methodology for determining NDF has been established (AOAC Official Method 2002.04; Mertens, 2002) that includes use of heat-stable α-amylase and sodium sulfite within the neutral detergent extraction procedures. However, the use of sodium sulfite may be appropriate or inappropriate, depending on analytical objectives. For the Mertens (2002) method, NDF also can be determined and reported on an ash-free basis.

It should be noted further that there are two common laboratory approaches to detergent analysis of forages and feedstuffs. In the original procedures, such as those described by Goering and Van Soest (1970) and Van Soest et al. (1991), individual forage samples are boiled under reflux, usually in dedicated Berzelius beakers. With this approach, ground samples are freely suspended within the detergent solutions, but are then filtered through Gooch-type crucibles, glass fiber filters, or filter paper to isolate the residual fibers. The AOAC approved method for conducting NDF analyses (Mertens, 2002) uses this general approach. Alternatively, methods pioneered primarily by ANKOM Technology (Macedon, NY) use a batch approach, where approximately 24 samples are sealed within individual filter bags and the extractions are performed simultaneously as a group under heat and pressure. Both systems are used widely in analytical laboratories, and as one might expect, each has distinct advantages, as well as some disadvantages that will be discussed further in some detail.

Neutral Detergent Fiber

Principle and Goal. As stated previously, the goal of forage and feedstuff extraction in neutral detergent is to solubilize (remove) cell solubles and pectin, which are generally assumed to be completely digestible by ruminants. This extraction leaves a fibrous residue that is incompletely digested as shown in Figure 1. The extraction is accomplished by boiling ground forage or concentrate samples in a near-saturated solution of sodium laurel sulfate at neutral pH (6.95 to 7.05). The pH is tightly controlled because alkaline or acid conditions can solubilize fiber (Mertens, 2002). Other reagents are included in the neutral detergent solution for specific purposes. As summarized by Mertens (2002), these include:

- sodium sulfite (solubilizes N)
- EDTA (chelates calcium and enhances removal of pectin)
- triethylene glycol [removes nonfibrous matter (starch), particularly from concentrate feeds]
- α-amylase (solubilizes starch and aids filtering)
- disodium phosphate and sodium borate (regulates pH)

Issues with Starch Removal. Originally, the NDF method was designed primarily to evaluate

forages, but the expanding needs to evaluate concentrate feeds or high-starch forages, such as corn silage, have required subsequent refinements to the general method. Primarily, these refinements have been necessary to facilitate filtering, which is compromised by the presence of insoluble starch, thereby slowing procedural time requirements, and potentially inflating values for structural plant fiber. Originally, starch removal was enhanced by inclusion of 2-ethoxyethanol within the neutral detergent solution, but this has now been replaced effectively by triethylene glycol due to toxicity (potential mutagenic) issues with the 2-ethoxyethanol (Van Soest et al., 1991; Mertens, 2002). Currently, NDF analyses routinely use a heat-stable, α-amylase to facilitate starch removal, as specified within the recently approved methodology standard (Mertens, 2002).

Early attempts to use α-amylases to improve starch removal sometimes proved unsatisfactory because they were often crude extracts that also exhibited other activities, such as removing some hemicellulose, which subsequently reduced recovery of insoluble fiber (Van Soest et al., 1991). The Mertens (2002) procedure specifically identifies Termamyl 120 by Novozyme Corp. (Cat. No. A3403, Sigma-Aldrich, St. Louis, MO), Spezyme FRED by Genecor International (Cat. No. FAA, ANKOM Technology Corp., Macedon, NY), or any equivalent product as acceptable for this purpose. Typically, α-amylases are heat stable. This characteristic is important because high temperatures deactivate any fiber degrading enzymes within (crude) amylase solutions (Mertens, 2002). A prefix 'a' is sometimes affixed to NDF (**aNDF**) to indicate the use of α-amylase in any NDF analysis. However, use of this 'aNDF' designation in association with the Mertens (2002) approved method also specifically implies use of sodium sulfite, which may or may not be true when used elsewhere. Great care should be used when evaluating analytical results to ensure the proper interpretation of shorthand acronyms.

Use of Sodium Sulfite/Removal of N from NDF Residues. Sodium sulfite used during extraction in neutral detergent cleaves disulfide bonds and dissolves cross-linked proteins; it also removes keratinaceous residues of animal origin (Van Soest et al., 1991). As such, it is frequently included with the neutral detergent procedures to remove proteins from fibrous residues, which otherwise may inflate concentrations of residual fiber. Its use may be indicated with the prefix 's' affixed to NDF, such as in '**asNDF**' or '**sNDF**', but these conventions are not used universally.

While removal of N or CP from residual fiber with sodium sulfite may give a more accurate determination of actual forage fiber, it also (historically) precludes any subsequent effort to quantify the N or CP associated with those fibers (neutral detergent-insoluble CP; **NDICP**). Acid detergent lignin (ADL) also contains N; therefore, sodium sulfite should be omitted in any sequential analysis where concentrations of ADL are to be quantified. Van Soest (1982) has more bluntly suggested that sodium sulfite must not be used if the cell wall preparation is to be used for any other purpose than quantifying NDF. More recently, this strict paradigm has softened, particularly within commercial labs quantifying NDF concentrations for routine uses. In such circumstances, NDF determined with sodium sulfite is largely assumed to be corrected for NDICP, and any further (requested) quantification of NDICP is often conducted on a sodium-sulfite-included basis. This procedural compromise acknowledges two generally minor errors: i) sodium sulfite used in the NDF extraction procedure does not remove all NDICP; and ii) any further quantification of NDICP will be discounted due to its partial removal by sodium sulfite. In practice, concentrations of NDICP are usually low in most dairy-quality forages, and these compromises are logical for most routine applications. However, there also are specific situations (subsequently discussed) when this procedural compromise may be problematic.

It should be emphasized that quantifying both NDICP and ADL may be analytical priorities in some situations. NDICP is viewed as a slowly degraded protein fraction that can contribute to ruminal bypass requirements. It is an important component of many models characterizing ruminant protein degradation and subsequent supplies of metabolizable protein to support production of milk and meat. Within this specific context, a discounted assessment of NDICP because of the use of sodium sulfite during NDF extraction procedures may be problematic, particularly when applied to certain heated forages and/or concentrate feeds. As stated previously, ADL is a critical component of forage fiber that provides support and rigidity to the plant, which may (for

example) limit lodging of cereal grains carrying disproportionately large head weights. Unfortunately, it also impedes ruminant digestion and is considered indigestible. As a practical example, recent studies developing low-lignin alfalfa varieties have required accurate determinations of ADL. On a practical basis, the use of sodium sulfite within NDF extraction procedures is less troublesome for determinations of ADL, largely because routine procedures for determining ADL often bypass the initial (sequential) NDF extraction and are conducted on fiber residues obtained directly (non-sequentially) from extractions in acid detergent only.

Further Discussion on NDICP. Concentrations of NDICP (% of CP) can vary sharply with forage type, where rankings are usually perennial warm-season grasses > cool-season grasses > legumes. Normally, when expressed as a percentage of the total CP pool (% of CP), NDICP for each forage type increases with plant maturity. Table 2 summarizes concentrations of NDICP expressed as percentages of both DM and CP from various hays as compiled from analytical laboratories by NASEM (2021). The NDICP fraction is highly significant within perennial warm-season grasses, often accounting for > 50% of the total CP pool. One example of this characteristic can be found in research conducted by Coblentz et al. (2010a) with eastern gamagrass in central Wisconsin. Eastern gamagrass is a tall-growing, perennial warm season (C4) grass that was fertilized with 0, 60, 120, or 180 lbs N/acre and harvested on 15-day intervals starting June 1st and ending on August 15th. The effects of maturity on concentrations of NDICP are shown in Figure 3, and by mid-August, NDICP accounted for > 57% of the total CP pool. In this specific study, the effects of N fertilization were less clear. Pools of total CP, neutral detergent-soluble CP, and NDICP all increased with N fertilization rate, but the percentage of the total CP pool partitioned into NDICP was largely unaffected by N fertilization.

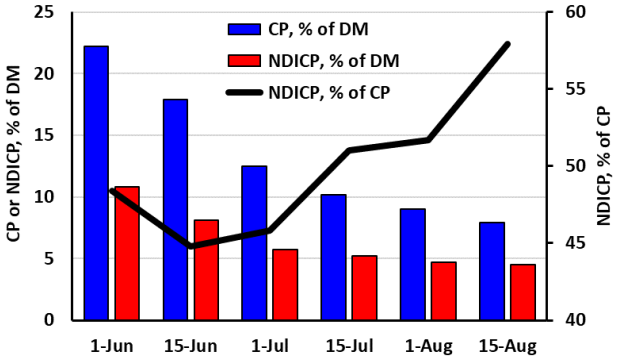


Figure 3. Effects of maturity on concentrations of crude protein (CP) and neutral detergent-insoluble crude protein (NDICP, % of CP) for eastern gamagrass, a perennial warm-season (C4) grass. Grasses were harvested in central Wisconsin, and results are adapted from Coblentz et al. (2010a).

Table 2. Examples of concentrations of neutral detergent-insoluble crude protein (NDICP) for various hays determined in analytical laboratories as summarized by NASEM (2021). Based on the analyses provided, the generic term 'grass' implies cool-season (C3) grasses.

Forage[1]	Maturity[2]	NDICP[3]	CP[3]	NDICP[3]
		---------- % of DM ----------		% of CP
Legume	Immature	2.80	21.5	13.0
	Mid-Maturity	1.85	20.7	8.9
	Mature	3.89	18.1	21.5
Legume-Grass	Immature	4.25	20.3	20.9
	Mature	4.38	17.4	25.2
Grass-Legume	Mid-Maturity	1.33	15.6	8.5
	Mature	3.57	10.9	32.8
Cool-Season Grass	Mid-Maturity	4.85	13.3	36.5
	Mature	3.79	9.2	41.2
Grass-Legume	Unspecified	4.12	12.1	34.0
Bermudagrass[4]	Unspecified	4.16	11.0	37.8

[1] *Unless otherwise indicated, the first listing for mixtures is the predominant forage type.*
[2] *Maturity designations are as provided by NASEM (2021).*
[3] *NDICP, neutral detergent insoluble crude protein; CP, crude protein.*
[4] *Bermudagrass is a perennial warm-season (C4) grass.*

Specific responses to N fertilization likely depend on whether proportionate or disproportionate portions of the additional N uptake are partitioned into the cell-soluble fraction. In truth, assessing NDICP within perennial warm-season grasses is of limited utility for dairy cows since these forages are not common components of lactating-cow diets. Furthermore, concentrations of CP are usually low in these forages, also potentially limiting their relevance within a dairy context. However, it also must be noted that this is not universally true. For example, concentrations of CP in bermudagrasses that have been heavily fertilized with commercial N sources or poultry litter can exceed 20% of DM, which is comparable to alfalfa.

Another situation where quantifying NDICP may be important occurs when hays and silages heat spontaneously during storage. Normally, heat-damaged CP is quantified by determining the residual CP within fiber residues following extraction in acid detergent (**ADICP**). However, spontaneous heating of forages also can sharply increase NDICP, which is a broader, more inclusive fiber-associated CP entity that includes all ADICP. An example of this situation was reported by Coblentz et al. (2010b) in which alfalfa-orchardgrass hays were packaged in large-round bales with different bale diameters and moisture concentrations, thereby resulting in different intensities of spontaneous heating. The relationship between the change in NDICP during bale storage (**ΔNDICP** = $NDICP_{post\text{-}storage}$ – $NDICP_{pre\text{-}storage}$) and maximum internal bale temperature is shown in Figure 4. In extremely heated hays, NDICP increased by > 25 percentage units of CP, which was roughly equivalent to 4.5 additional units of DM.

A reasonable question to ask at this point might be why these issues related to quantification of NDICP are potentially important. The NASEM (2001) summative equation for determining energy density (TDN) contains truly digestible subunits for nonfiber carbohydrates (**tdNFC**), crude protein (**tdCP**), fatty acids (**tdFA**), fiber (**tdFiber**), and a term for metabolic fecal TDN (7 percentage units), resulting in an equation where TDN (%) = tdNFC + tdCP + (tdFA × 2.25) + tdFiber – 7 (NASEM, 2001; Weiss et al., 1992). In the NASEM (2001) publication, two approaches were described for measuring the truly digestible fiber subunit (tdFiber). One method is to quantify tdFiber by formula as: $0.75 \times (NDF_{corr} - ADL) \times [1 - (ADL/NDF_{corr})^{0.667}]$, where **NDFcorr** has been corrected for associated

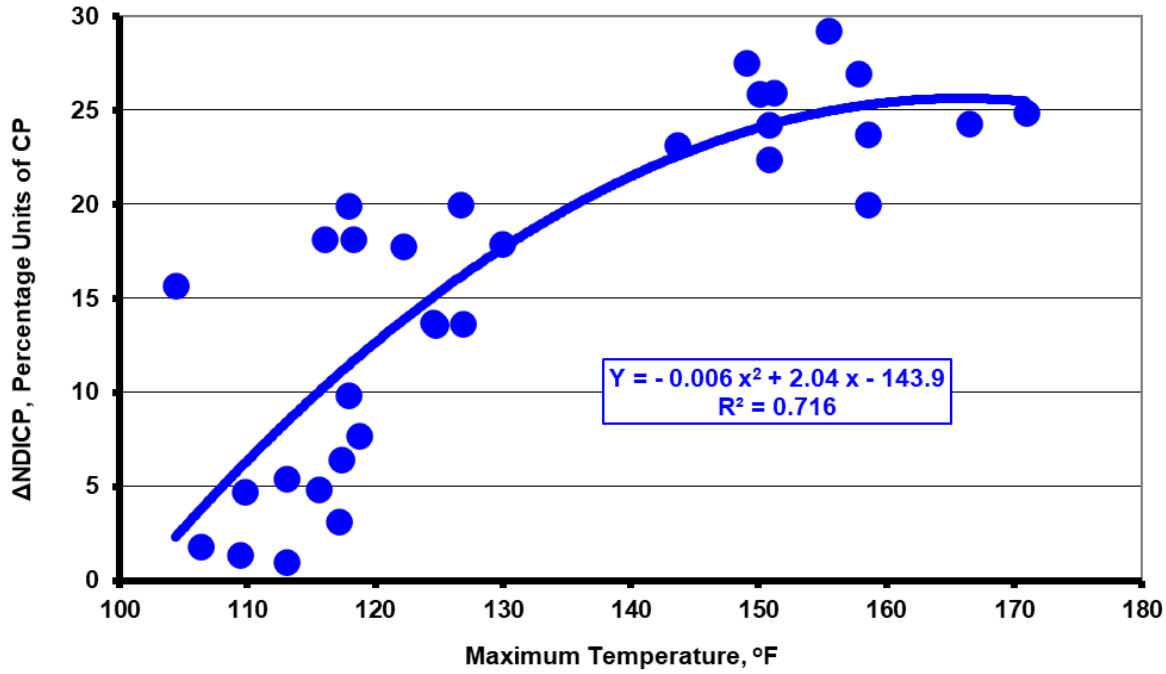


Figure 4. Changes in concentrations of neutral detergent-insoluble crude protein (NDICP; ΔNDICP = $NDICP_{post\text{-}storage}$ – $NDICP_{pre\text{-}storage}$) as affected by maximum internal bale temperature for alfalfa-orchardgrass hay produced at Marshfield, WI. The mean initial concentration of crude protein (CP) was 18.3%, and NDICP initially comprised 20.3% of CP (3.67% of DM), which generally corresponds to ΔNDICP = 0 on the y-axis [adapted from Coblentz et al. (2010b)].

NDICP. This approach requires inputs for both NDICP and ADL that theoretically should be quantified directly (not estimated) via laboratory procedures. However, NDICP values are typically low in most dairy-quality forages, and these values were often simply estimated by commercial laboratories. The alternate method for determining tdFiber was by using 48-hour in-vitro disappearance procedures of NDF (**NDFD**), where tdFiber = NDF × NDFD/100. In the NASEM (2001) publication, correction of NDF for NDICP was not specified, and it is frequently ignored when tdFiber was determined by in-vitro digestion methods. Theoretically, this is an error, since all CP has already been accounted for within the tdCP subunit of the summative TDN equation. The approaches outlined above also normally introduce minor errors whenever NDF is determined with sodium sulfite and is then substituted for (equated with) a CP-free NDF residue (NDFcorr).

In the current (newly updated) NASEM (2021) publication, the requirement for correction of NDF residues for NDICP has been dropped for a variety of reasons; however, this change should be adopted with some caution. Coblentz and Hoffman (2010) examined the effects of spontaneous heating on TDN estimates when tdFiber was calculated by: i) formula with NDF correction for NDICP (NDFcorr); and ii) by in vitro disappearance procedures, both with and without the same correction. These effects of spontaneous heating on the resulting calculated energy density are illustrated in Figure 5. Regardless of the method used to determine tdFiber, estimates of TDN predictably declined with spontaneous heating. The change in TDN (ΔTDN) became more critical (negative) when tdFiber was calculated with formula inputs of NDICP and ADL, and this occurred at about twice the negative rate (-0.21 TDN units/°F) compared to in-vitro procedures when NDF was not corrected for NDICP (-0.11 TDN units/°F). When in-vitro procedures were used to determine tdFiber, and included correction for NDICP, responses to spontaneous heating were intermediate between the other two methods. In heavily heated hays, the differential across methods could account for > 6 percentage units of TDN (> 0.12 Mcal/lb of NE_L). As

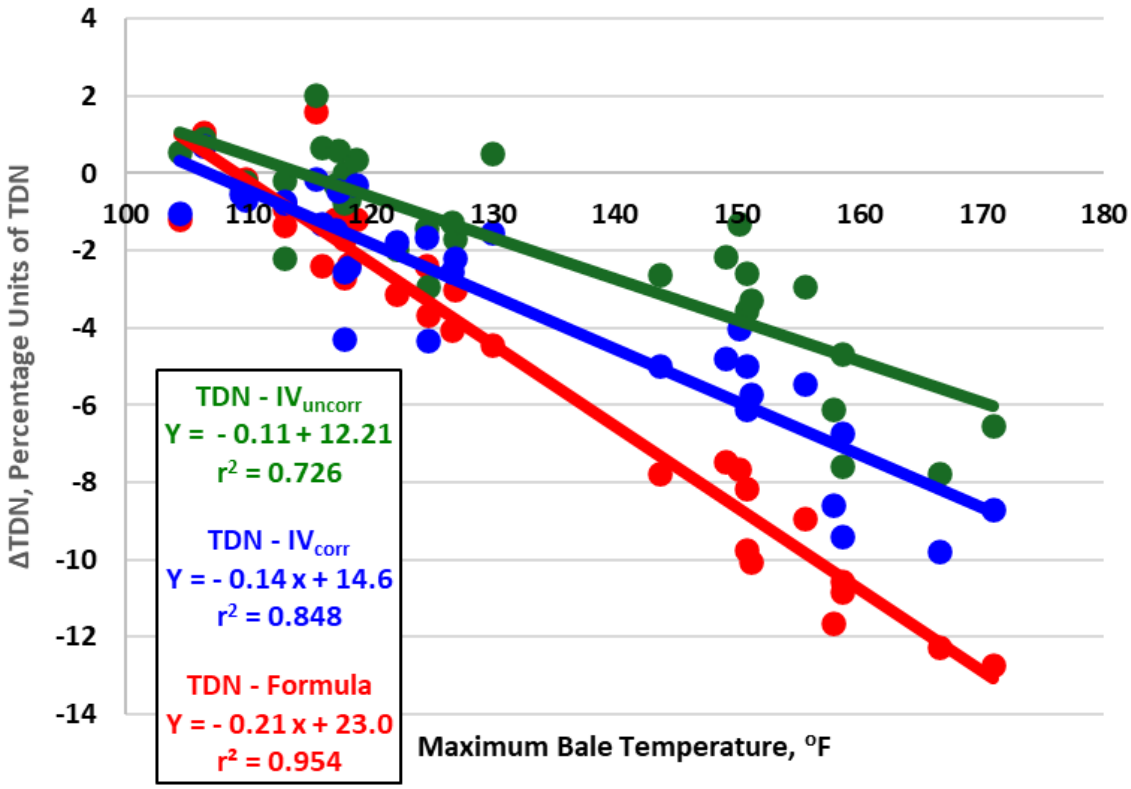


Figure 5. Changes in total digestible nutrients (TDN) (ΔTDN = TDN_{final} – $TDN_{initial}$) for heated alfalfa-orchardgrass hay as linear functions of maximum internal bale temperature during storage. Values for the truly digestible fiber (tdFiber) subunit were determined by (summative) formula (TDN-Formula) with measured inputs of acid detergent lignin (ADL) and neutral detergent-insoluble crude protein (NDICP), or by in-vitro procedures with or without correction for NDICP (TDN-IV_{corr} or TDN-IV_{uncorr}, respectively). Data are adapted from Coblentz and Hoffman (2010).

such, it seems reasonable to be cautious of the consequences of ignoring NDICP contamination within residual forage fiber when determining energy density, and this is especially relevant for hays or silages that have heated significantly during storage.

Contamination of NDF by NDICP also is quite relevant when evaluating certain concentrate feedstuffs. Hintz et al. (1996) compiled a comparative listing of the effects of sodium sulfite on neutral detergent extraction procedures for various feedstuffs (Table 3), and several points become clear:

- Large differentials in concentrations of NDF and NDICP can be observed for some feeds based on the inclusion or exclusion of sodium sulfite within the extraction in neutral detergent. For example, inclusion of sodium sulfite reduced concentrations of NDF by about 11 percentage units of DM for brewer's and distiller's grains, and even more (24.1 percentage units) for fish meal.
- In general and excepting the specific previous discussions concerning heat-damaged hays summarized in Figure 4, contamination of fiber residues by CP is relatively minor in most forages. As such, the use of sodium sulfite has relatively limited effects on corrected NDF concentrations in forages compared to some other feeds.
- The authors of this study concluded that the additional CP removed from residual NDF fibers by the use of sodium sulfite for heated (but not heat-damaged) feeds was digestible and should not be included as part of residual fiber.

Table 3. Effects of using sodium sulfite on crude protein contamination of neutral detergent fiber (NDF) residues for some forages and concentrate feeds [adapted from compilation by Hintz et al. (1996)].

Feed	CP[1]	aNDF[1] without sulfite	NDICP[1] without sulfite	asNDF[1] with sulfite	NDICP[1] with sulfite	aNDF Difference[2]
		% of DM				
Fish Meal	53.9	30.4	10.4	6.3	1.3	24.1
Brewer's Grains	30.4	52.3	12.2	40.9	4.7	11.4
Distiller's Grains	25.6	38.6	11.0	27.9	3.7	10.7
Soybean Meal	46.2	18.5	3.6	12.4	0.5	6.1
Sunflower Meal	31.9	38.5	2.4	35.2	1.1	3.3
Canola Meal	40.8	23.7	4.3	20.9	2.1	2.8
Citrus Pulp	6.5	21.3	2.1	20.2	1.6	1.1
Shelled Corn	10.7	11.4	0.7	10.1	0.1	1.3
High-Moisture Corn	8.7	15.1	0.3	14.7	0.1	0.4
Barley Grain	10.7	20.3	1.1	20.1	0.1	0.2
Whole-Grain Oat	12.0	26.3	0.4	26.2	0.2	0.1
Soybean Hulls	10.7	62.2	2.2	61.1	0.6	1.1
Wheat Middlings	18.4	34.2	2.1	33.9	0.4	0.3
Bermudagrass	10.4	67.3	2.8	64.8	0.7	2.5
Smooth Bromegrass	10.1	66.6	2.0	64.2	0.5	2.4
Barley Hay	8.3	55.8	1.5	55.3	0.1	0.5
Alfalfa Hay	16.4	45.5	1.7	44.3	0.9	1.2
Alfalfa Haylage	17.1	43.6	1.5	42.2	1.1	1.4
Corn Silage	7.7	36.1	0.5	34.7	0.2	1.4

[1] *Abbreviations: CP, crude protein; aNDF, NDF determined with a-amylase; NDICP, neutral detergent-insoluble crude protein; and asNDF, NDF determined with a-amylase and sodium sulfite.*
[2] *Difference in NDF based on use of sodium sulfite (aNDF – asNDF).*

- Sodium sulfite removes much, but not all, of the CP from fiber residues. Therefore, concentrations of NDICP determined appropriately (without inclusion of sodium sulfite) should not be used subsequently to correct NDF determined with sodium sulfite for contaminant CP. This would result in an overcorrection, and depending on the feedstuff being evaluated, potentially a serious or gross overcorrection (see Table 3).

Pectin Problem. As noted by Van Soest et al. (1991), the NDF method of analysis has frequently been criticized for not recovering pectin, which can be considered a botanical part of the cell wall. As such, the residual fibers retained after extraction in neutral detergent cannot properly be called 'cell wall' because the associated pectin has been solubilized. It should be emphasized that this argument is not nutritionally relevant. Pectin is known to be completely and rapidly fermentable by ruminants, and therefore constitutes an ideal fraction compared to other parts of the cell wall, such as hemicellulose and cellulose. Van Soest et al. (1991) concluded that pectin should be quantified as a separate entity when its presence was relevant to discussion of any results.

One potential example where the concentrations of pectin can be especially relevant occurs when digestibility coefficients for NDF and ADF residual fibers are determined experimentally in feeding trials with livestock. Concentrations of pectin in alfalfa can range from 5 to 10% (Van Soest, 1982), but solubilization does not occur when they are extracted directly in acid detergent. As a result, digestibility coefficients for ADF can be inflated in cases where there is a high concentration of pectin, and ADF is determined directly, without a preliminary extraction in neutral detergent that solubilizes pectin.

Correction of NDF for Residual Ash. The AOAC-approved methodology for determining NDF (Mertens, 2002) includes procedural/calculation options for NDF that exclude residual ash. This approach is sometimes designated with an 'om' suffix indicating a correction has been made for residual ash (**NDFom**). The Mertens (2002) procedure also includes the use of heat-stable, α-amylase and sodium sulfite, both of which may be further indicated within various acronym forms or styles. It is important to emphasize that the interpretation/use of NDF concentrations, and particularly those obtained from various research studies, should be examined closely to determine the procedural variations used and their subsequent effects on results.

Most biogenic silica or natural mineral components of the cell wall are soluble in neutral detergent, but not in acid detergent (Van Soest, 1982; Van Soest et al., 1991); therefore, errors associated with any direct (nonsequential) determination of ADF potentially exhibit the same type of error effect as described for pectin. In contrast, ash derived from soil contamination is generally insoluble in neutral detergent and can serve as an indicator of soil contamination within the forage sample (Van Soest, 1982). Soil contamination can occur in forages harvested with poor technique, such as improper rake adjustment, as well as other factors potentially including irrigation, flooding, wind/dust, or manure application. As a strictly procedural matter, ash correction also is a terminal step, which precludes any additional analyses of fibrous residues, and may complicate and/or delay laboratory flow depending on analytical goals.

The justification for correction of NDF residual fibers for this type of (soil-derived) contaminant ash is that soil-derived ash is not part of the cell wall, does not contribute in any way to energy availability within the ruminant animal, and introduces errors into various fiber and energy models, particularly the summative approach to calculating energy density. The previous NASEM (2001) publication quantified non-fiber carbohydrate (**NFC**; sugars and other highly digestible components) by difference as: 100% - [(NDF - NDICP) + CP + ether extract + ash]; therefore, any contamination of residual NDF by ash (or NDICP) potentially causes two problems. It directly elevates concentrations of NDF, but also results in double-counting, as both NDICP and ash are accounted for (in full) elsewhere. As such, NFC concentrations are mathematically depressed, which is especially relevant to calculation of energy as NFC carries a digestibility coefficient of 0.98, indicating it is an ideal fraction based on the Lucas concept.

In the revised NASEM (2021) publication, there has been some refinement of the earlier summative approach by: i) direct determination of starch; ii) renaming this fraction determined by difference as residual organic matter or '**ROM**'; and iii) revising the associated digestibility coefficient to 0.96.

However, the previous concerns about potential errors remain the same. It is essential to note that the current NASEM (2021) publication does not require ash correction of NDF residues for a variety of reasons, concluding that both NDICP and ash correction are not quantitatively important for estimation of energy for most dairy diets; however, this premise should not be applied blindly without considering the possibility of complicating factors.

Acid Detergent Fiber

Introduction and Background. As illustrated in Figure 2, the primary purpose of extracting forage samples in acid detergent is to solubilize and remove hemicellulose, thereby serving as a preparatory step for the subsequent determination of cellulose and ADL. This is accomplished with a solution of cetyl trimethyl-ammonium bromide and 1 *N* H_2SO_4 (sulfuric acid). The procedure can be performed following extraction in neutral detergent (sequentially), or directly on forage/feedstuff samples.

Historically, ADF has been used widely within regression-based empirical equations for predicting digestibility or energy density in forages and feedstuffs. One such widely used example, often referred to as the 'Western States Equation', calculates TDN for alfalfa as: TDN (%) = 82.38 – (0.7515 × ADF) (Bath and Marble, 1989). This method of estimating energy density remains attractive in its simplicity, ostensibly because ADF is easy to analyze and commonly evaluated; however, the approach is not theoretically valid (Van Soest et al., 1991). Other state or regionally based empirical equations frequently have included other routinely evaluated components, such as NDF or CP. Over time, empirical equations of this type have been replaced, albeit inconsistently, by mechanistically valid summative models.

While ADF procedures can be part of a sequential evaluation, there are some entities that are best determined directly without the preliminary extraction in neutral detergent; within this context, Van Soest et al. (1991) specifically mentions biogenic silica, acid detergent-insoluble ash, and ADICP. Of these, ADICP is determined most frequently, primarily as a sensitive indicator of heat damage to proteins. Acid detergent-insoluble ash is often used as an internal marker to determine rates of ruminal particulate passage and is quantified by combusting ADF residual fibers in a muffle furnace at high temperature (usually, 500 to 600°C). When used for this purpose, care must be extended to prevent experimental animals from ingesting ash from other sources, such as dirt or inorganic bedding materials, which potentially introduce significant errors to passage calculations.

Historically, some laboratories have filtered ADF residues through filter paper (e.g., Whatman 54; Van Soest et al., 1991). With this approach, a final rinse in acetone prior to oven-drying at 105°C is critical to prevent significant charring of the filter paper. If the analytical goal is to determine ADICP, it also is important to note that some filter papers that may be quite acceptable for filtering residual fibers also contain significant and/or variable amounts of N. Nitrogen-free filter papers should be used when final quantification of residual N includes a Kjeldahl-type digestion or combustion (Dumas-type method) of the entire filter paper containing the residual ADF fiber.

Procedural Variations. Commonly used procedural options affect the recovery of residual ADF fiber. Hintz et al. (1996) compared recoveries of ADF from 22 different forages and concentrates, as well as meat scraps and sheep feces. Samples were analyzed with three procedural options:

- directly (non-sequentially; no sodium sulfite),
- sequentially, without sodium sulfite in the preliminary NDF extraction step,
- sequentially, with inclusion of sodium sulfite.

Overall, mean concentrations of ADF for the 24 test samples using these procedural options were 23.9, 21.9, and 21.1%, respectively, indicating recovery was reduced by sequential analysis, and then further by including sodium sulfite in the preliminary NDF extraction step. Within this context, procedural comparisons for specific feeds and forages are summarized in Figure 6. Presumably, differences between the direct (non-sequential) and sequential ADF procedures are mostly related to interfering substances, such as pectin and biogenic silica, that are insoluble in acid detergent but are removed with a preliminary extraction in neutral detergent. The inclusion of sodium sulfite in sequential procedures affected final ADF values by removal of N or CP (N × 6.25).

In a companion experiment also reported by Hintz et al. (1996), ADF concentrations for 180

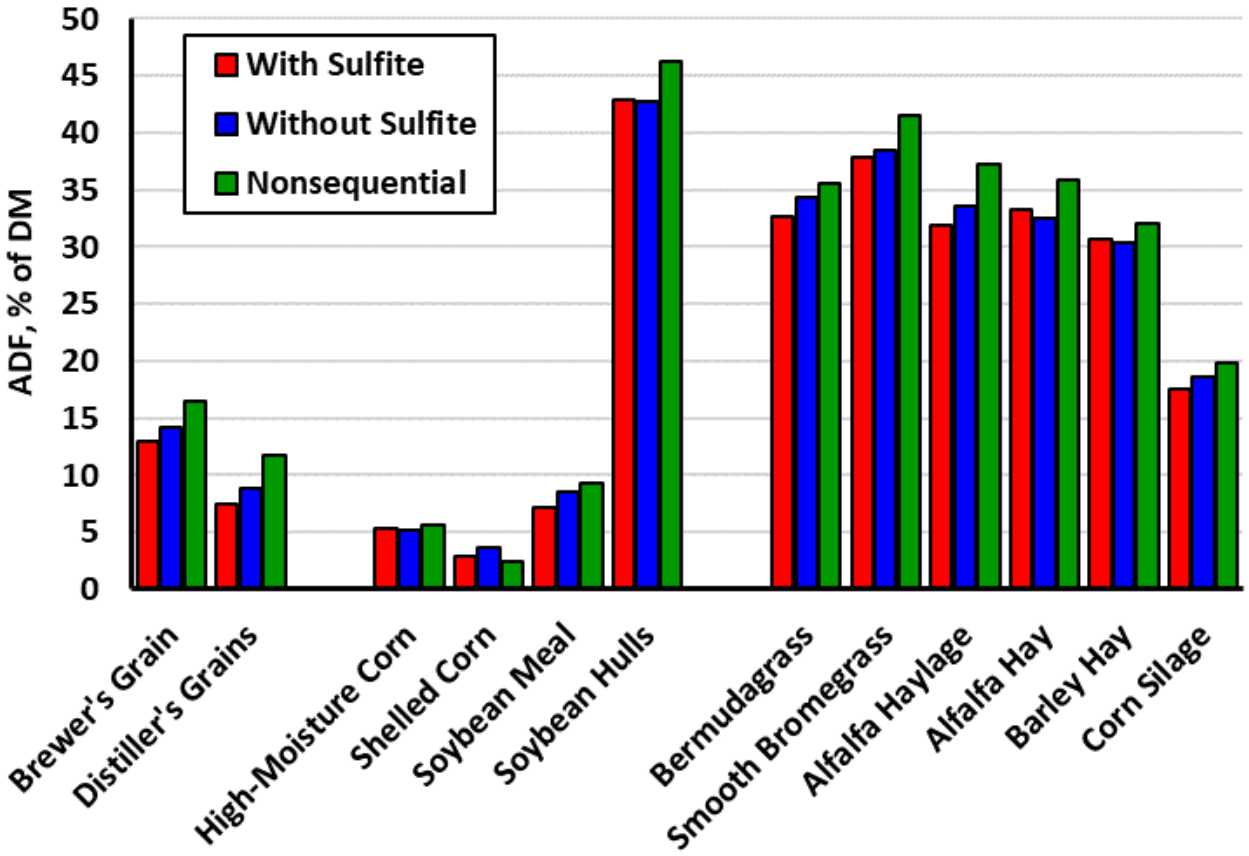


Figure 6. Comparisons of concentrations of acid detergent fiber (ADF) for selected feeds and forages determined with three procedural variations. These included a nonsequential evaluation, as well as sequential analysis with and without sodium sulfite included in the preliminary extraction in neutral detergent [adapted from Hintz et al. (1996)].

initial (first cutting) and regrowth alfalfa samples were compared based on inclusion of sodium sulfite in the preliminary neutral detergent extraction step. Inclusion of sodium sulfite reduced ADF values by 1.25 and 1.47 percentage units for initial and regrowth samples, respectively. There were also small improvements in within-sample variance associated with use of sodium sulfite.

As discussed previously, N or CP (N × 6.25) associated with residual ADF fibers (ADICP) is an important analytical entity, particularly for evaluating the effects of heat and/or heat damage to forage or other feedstuff proteins. Concentrations of ADICP (% of DM) for selected feed and forage samples obtained from the work of Hintz et al. (1996) are illustrated in Figure 7, where ADICP was consistently reduced with the inclusion of sodium sulfite in the preliminary NDF extraction step. Averaged across the entire 24 entries, the mean difference between determinations with and without sodium sulfite was 0.30 percentage units of DM (0.58 vs. 0.88%), but the discrepancy was disproportionately larger in the heated feeds, specifically brewer's and distiller's grains. For further discussion of ADICP, see Spontaneous Heating in Hays and Silages in the Common Factors Affecting Concentrations of Fiber Components section.

Acid Detergent Lignin

Introduction and Background. The Acid Detergent Lignin (**ADL**) procedure uses 72% sulfuric acid to remove or solubilize cellulose from residual ADF fibers, leaving ADL and some residual ash. The procedure can be conducted on residual ADF fibers isolated either directly or sequentially following extraction in NDF solution, but results may not be equivalent, and procedures should be conducted consistently within any group of samples to be compared. Quantification of ADL is often important because it is generally considered indigestible and has clear implications with respect to digestibility of fiber, but also because it is structurally important to the plant, preventing undesirable responses to growing conditions, such as

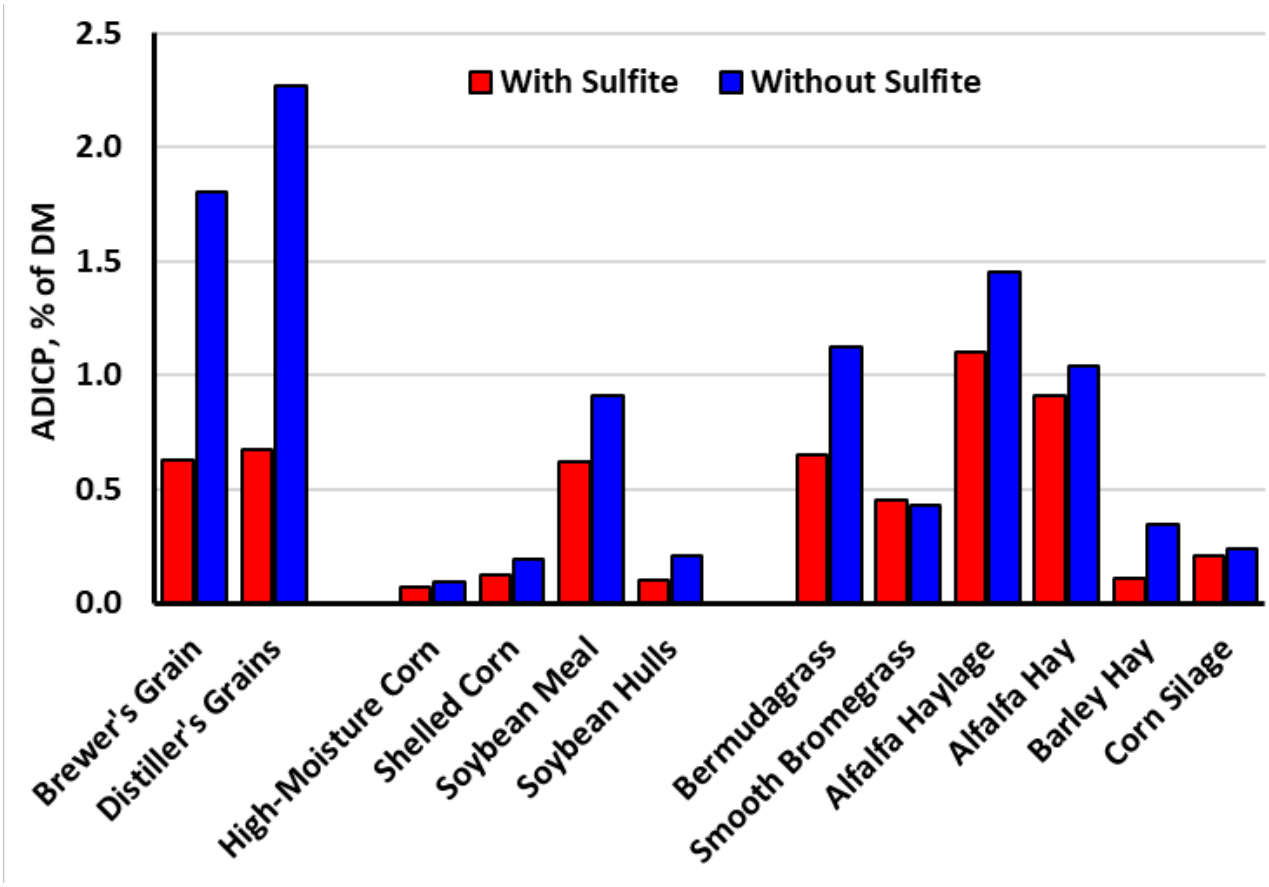


Figure 7. Comparisons of concentrations of acid detergent-insoluble crude protein (ADICP, % of DM) for selected feeds and forages determined sequentially, with and without sodium sulfite in the preliminary extraction in neutral detergent [adapted from Hintz et al. (1996)].

lodging. Van Soest (1982) has summarized concentrations of N in the lignin of grasses and legumes, indicating that with the best efforts to exclude contaminating proteins, lignin within all grasses and legumes contains about 1.5 to 2% N. As such, ADL is vulnerable to sodium sulfite whenever sequential procedures are used that include sodium sulfite in a preliminary extraction in neutral detergent.

Studies published by Hintz et al. (1996) that were conducted on both alfalfa forages and also a much wider variety of forages and concentrate feeds indicated that ADL recovery was reduced by 11.1 to 17.4% in those studies by using sodium sulfite in the preliminary neutral detergent extraction. Although it remained somewhat unclear whether these responses were exclusively due to actual losses of ADL or also had contributions from removal of other non-ADL contamination, it is clear that the use of sodium sulfite reduced concentrations of ADL, which is problematic with respect to consistent interpretation of results. Procedures including the use of sodium sulfite should always be clearly stated, and interpretation of results considered appropriately. The most common interpretation is that any analytical objective prioritizing the quantification of ADL or N bound within forage fiber components (NDICP or ADICP) precludes the use of sodium sulfite, which negatively affects the quantitative recovery of these entities. As discussed previously, this paradigm has been softened recently for determinations of NDICP, particularly for evaluations obtained from commercial laboratories (see Use of Sodium Sulfite/Removal of N from NDF Residues on page 6).

Procedural Approaches: Individual or Batch Extraction Systems

Generally, there are two analytical approaches to laboratory fiber analysis. As outlined in detail by various publications (Goering and Van Soest, 1970; Van Soest et al., 1991; Mertens, 2002) one approach (conventional; **CONV**) is to suspend individual ground forage or feedstuff particles in neutral or acid detergent that is boiled under reflux in a dedicated

vessel, most commonly a Berzelius beaker. Several options are used for filtering that include fritted Gooch crucibles, Gooch crucibles overlayed with glass fiber filters (designed to address samples with fine particles), and a Buchner funnel with glass filters (Mertens, 2002). Historically, filter papers have often been used for this purpose (e.g. Whatman 54; Van Soest et al., 1991), usually assisted by mild vacuum.

More recently, batch procedures have been developed, largely by ANKOM Technology (Macedon, NY), that can extract a group of about 24 samples simultaneously within a single heated, pressurized vessel (**ANKOM**). For the ANKOM batch procedures, individual samples are sealed within filter bags (F57 or F58) that have different sensitivities for particle retention. The F57 fiber bags have a 25-µm porosity, while the F58 bags have more restrictive porosity (6 to 9 µm) and are specifically designed to accommodate the finer grind sizes required to support analyses by near-infrared spectroscopy (**NIRS**) or for feedstuffs known for significant pulverization during grinding. Because of their formulation/construction, these reduced porosity (F58) bags cannot be used for subsequent assays of NDICP, ADICP, or ADL per recommendations of the manufacturer.

As might be expected, there are advantages and disadvantages associated with both systems. The ANKOM system requires less laboratory space and reduces the need to handle hot chemicals (Vogel et al., 1999). Due to the batch-type approach, the system is especially well suited for handling large numbers of similar samples, such as those obtained from field-plot studies. Furthermore, assuming the system is functioning properly and routinely monitored, human errors associated with analytical technique are minimized and mostly reduced to weighing or data-transcription issues. The monitoring and maintenance of ANKOM extraction units for internal temperature and pressure, as well as other factors, is critically important and should not be overlooked. The batch approach also has advantages for sequential analysis of forage fiber fractions, potentially leading to quantification of ADL, which has often been viewed as more problematic within CONV analytical systems. Major issues with the ANKOM system are:

- Although popular, it is an alternative system that differs substantially from the well-developed apparatus/techniques on which fiber analysis and the approved standard methodology for NDF are based (AOAC Official Method 2002.04; Mertens, 2002).
- Concerns have been raised relative to filtering and/or potential particle loss from fiber bags, which may (in part) be associated with preparatory sample-grinding procedures (Hall and Mertens, 2023).

The CONV approach is generally more reliant on human technique, especially during filtering and when quantifying sequential or other secondary analytes. Historically, the CONV system has been subject to numerous minor (and often unstated/unreported) procedural variations, which ultimately led (in part) to the publication of the aforementioned standard methodology for NDF (Mertens, 2002). Generally, CONV systems are better suited to laboratories routinely analyzing small numbers of widely diverse forage and feedstuff samples, especially when differing analytical endpoints are requested or required.

There have been several direct comparisons of the two analytical approaches. An early comparison was reported by Komarek et al. (1994) in which 19 samples of various feedstuffs (wheat straw, timothy and mixed hays, haylage, guineagrass, stargrass, alfalfa, corn silage, corn stover, orchardgrass, manure, soy hulls, wheat, wheat bran, gluten feed, corn, and hominy) were analyzed directly for ADF by both CONV and ANKOM procedures at Cornell University, as well as by ANKOM procedures at the ANKOM Technology laboratory. Results indicated no statistical differences between labs or filtering techniques (Table 4), and conclusions drawn from

Table 4. Comparisons of traditional single-sample/dedicated vessel (CONV) and batch (ANKOM) procedures for non-sequential ADF concentrations of 19 diverse samples [adapted from Komarek et al. (1994)].

Technique[1]	Mean	Standard Deviation
CONV (Cornell)	30.3	0.24
ANKOM (ANKOM Technology)	29.7	0.48
ANKOM (Cornell)	29.6	0.78

[1] *Sites of analysis are shown parenthetically.*

the study were that batch processing was less technically demanding, required less laboratory space, and was considerably more efficient than the established CONV methodology. However, use of CONV procedures resulted in reduced calculated standard deviations of the ADF means.

A more in-depth study was published by Vogel et al. (1999), in which NDF and ADF values determined by CONV and ANKOM methodologies were compared for 23 forages [smooth bromegrass (8), switchgrass (7), and forage sorghum (8)]. For the NDF analyses, a procedural variation of the ANKOM methodology was used that included heat-stable, α-amylase in the first 3 (of 4 total) rinses after extraction in neutral detergent. This variation was based on manufacturer recommendations for evaluating forages with significant grain content, such as corn or sorghum silages. Results for NDF are summarized by forage species in Figure 8 and indicate no significant difference between ANKOM and CONV methodologies for smooth bromegrass, forage sorghum, or all forages combined, but a difference ($P < 0.05$) of 4.0 percentage units was observed for switchgrass forages. Within this context, it must be noted that the lack of a statistically derived significant difference does not automatically indicate equivalence; rather, it only indicates that the methods compared here could not be declared statistically different at a 95% level of confidence based on the existing conditions within the experiment.

The addition of heat-stable, α-amylase reduced NDF concentrations by 7.1 percentage units across all forages compared to the CONV method and differed significantly ($P < 0.01$) within each individual forage species, as well as across all forages considered collectively. Forage NDF concentrations were ranked in the same relative order by each method. It was concluded from this study that heat-stable, α-amylase should be used only with the analysis of forages containing a grain component; however, in the intervening years, use of amylase has become commonplace, regardless of methodology, and is included in the AOAC method for NDF (Mertens, 2002), which was established after the Vogel et al. (1999) experiment was conducted.

The analysis of ADF for these forages was performed directly, without preliminary extraction in

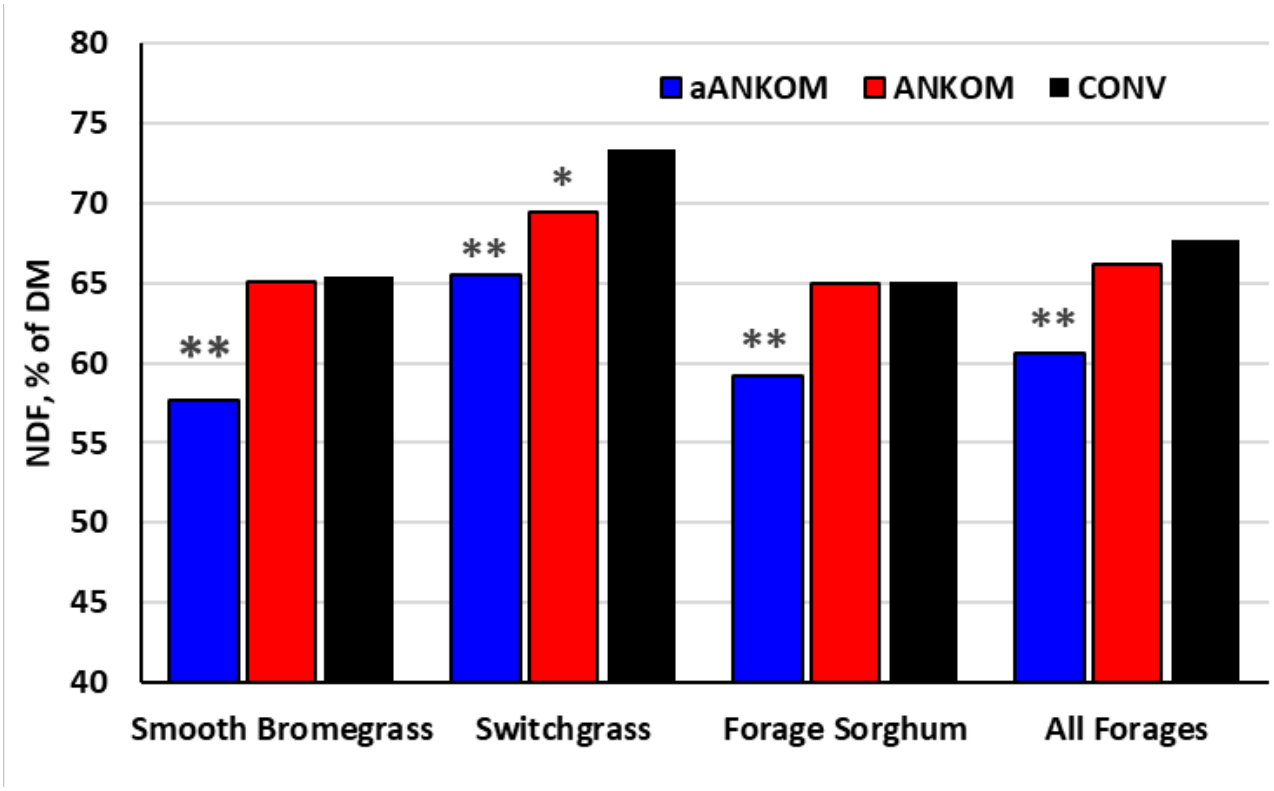


Figure 8. Comparisons of three methods for determining neutral detergent fiber (NDF) concentrations within 23 smooth bromegrass, switchgrass, and forage sorghum forages in Nebraska. Methods included conventional single-sample/dedicated flask (CONV), batch (ANKOM), and batch with heat-stable, α-amylase (aANKOM) procedures. The inclusion of asterisks (,**) above the aANKOM or ANKOM bars indicate statistical difference from CONV at the 0.05 and 0.01 levels of confidence, respectively [adapted from Vogel et al. (1999)].*

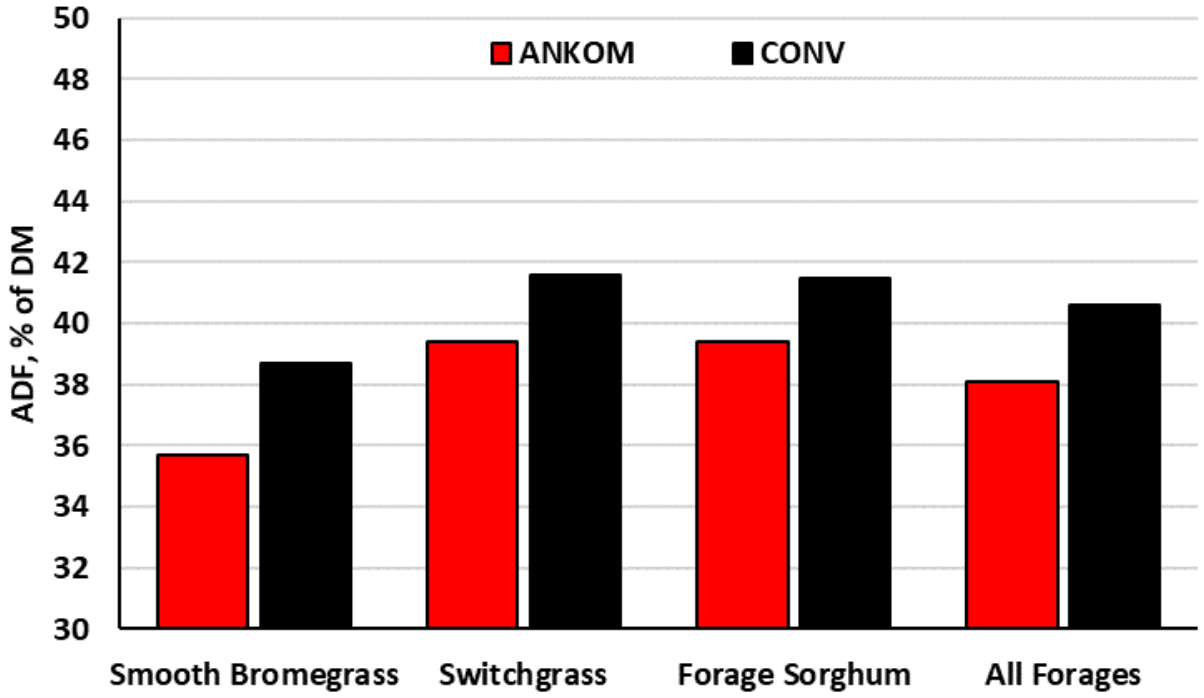


Figure 9. Comparisons of two methods for determining acid detergent fiber (ADF) concentrations for 23 smooth bromegrass, switchgrass, and forage sorghum forages in Nebraska. Methods included direct extraction by conventional single-sample/dedicated flask (CONV) and batch (ANKOM) procedures. Although the overall mean for ANKOM was 2.5 percentage units less than observed for CONV (38.1 vs. 40.6%), method comparisons did not differ statistically ($P > 0.05$) within each forage species, or across all species combined [adapted from Vogel et al. (1999)].

neutral detergent (Figure 9). The mean concentration of ADF across all forages when determined by the ANKOM method was 2.5 percentage units less (38.1 vs. 40.6%) than by the CONV method. Despite this consistent numerical difference, there was no statistical difference ($P > 0.05$) between methods for any individual forage species, or for all species combined, which is consistent with the observations made by Komarek et al. (1994; Table 4). Unlike the results summarized in Table 4, the standard deviation of the means when averaged across the 23 test forages was reduced by about half by using the ANKOM method. For ADF, Pearson and Spearman correlation coefficients (r) relating the ANKOM method with CONV were 0.94 ad 0.93, respectively, and both were highly statistically significant ($P < 0.01$). Concluding statements by Vogel et al. (1999) raised questions about the possible escape of minute particles from F57 fiber bags but acknowledged that results were similar and consistent between the ANKOM and CONV methods.

A more recent comparison of ANKOM and CONV methods for determining NDF was conducted by Schlau et al. (2021). Six samples each of grass, corn silage, and alfalfa were ground to pass through a 1-mm screen affixed within an abrasion mill (Udy Corp., Fort Collins, CO), which frequently produces a finer grind than cutting mills, and is suitable for NIRS analysis. The 18 forage samples were analyzed for NDF by CONV methods that were consistent with Mertens (2002) approved procedures, and by ANKOM methods using either F57 or F58 filter bags that have 25- and 6-µm pore sizes, respectively. Sodium sulfite and heat-stable, α-amylase were included in all method variations, and samples were analyzed inclusive and exclusive of residual ash. Results are summarized in Figures 10 and 11 and suggest that the specific type of filter bag may affect NDF values; specifically, the F58 bags may overestimate NDF values for some forages. Averaged over all forages, NDF concentrations were reduced by 2.0 to 2.2 percentage units by correcting for residual ash, implying the effects of ash-correction were relatively consistent across methods.

It is widely accepted that NDF is the most utilized metric in dairy nutrition (Hall and Mertens, 2023). Because of the pervasive use of both CONV and ANKOM procedures for NDF analysis, as well

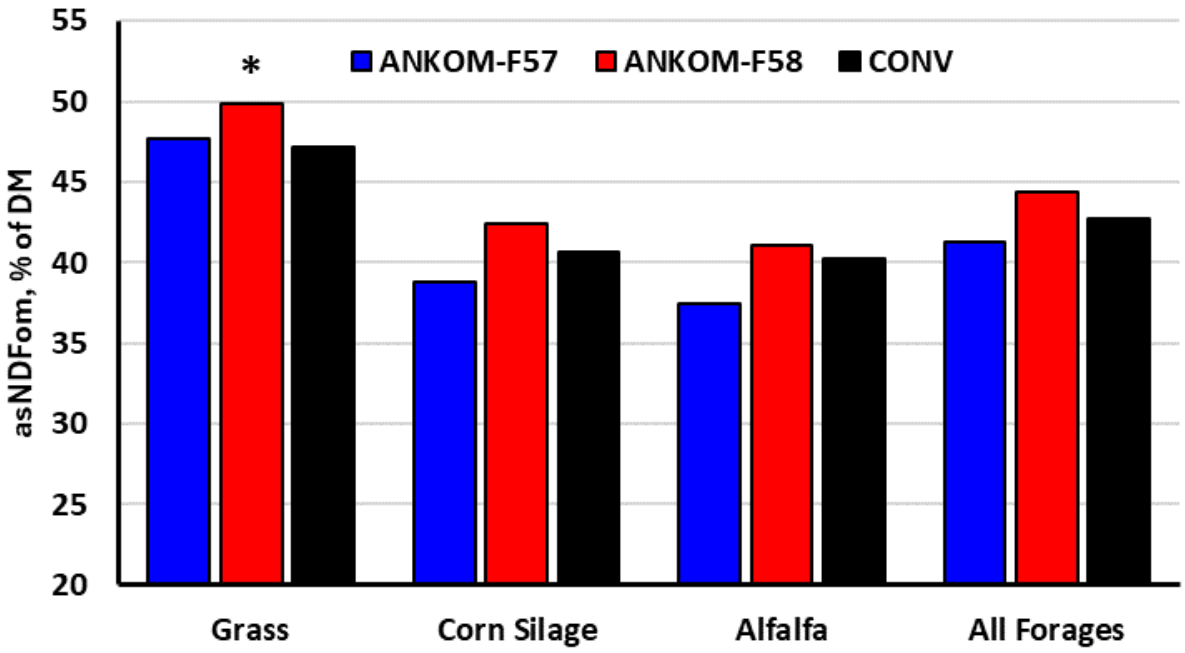


Figure 10. Comparisons of NDF concentrations from 18 forages (grass, corn silage, and alfalfa) using conventional (CONV) methods consistent with the standard method of Mertens (2002), as well as ANKOM methodologies with F57 (ANKOM-F57) and F58 (ANKOM-F58) filter bags. All methods included sodium sulfite and heat-stable, a-amylase, but were not corrected for residual ash (asNDF). An asterisk (*) denotes an ANKOM method differing from CONV at P ≤ 0.05 [adapted from Schlau et al. (2021)].

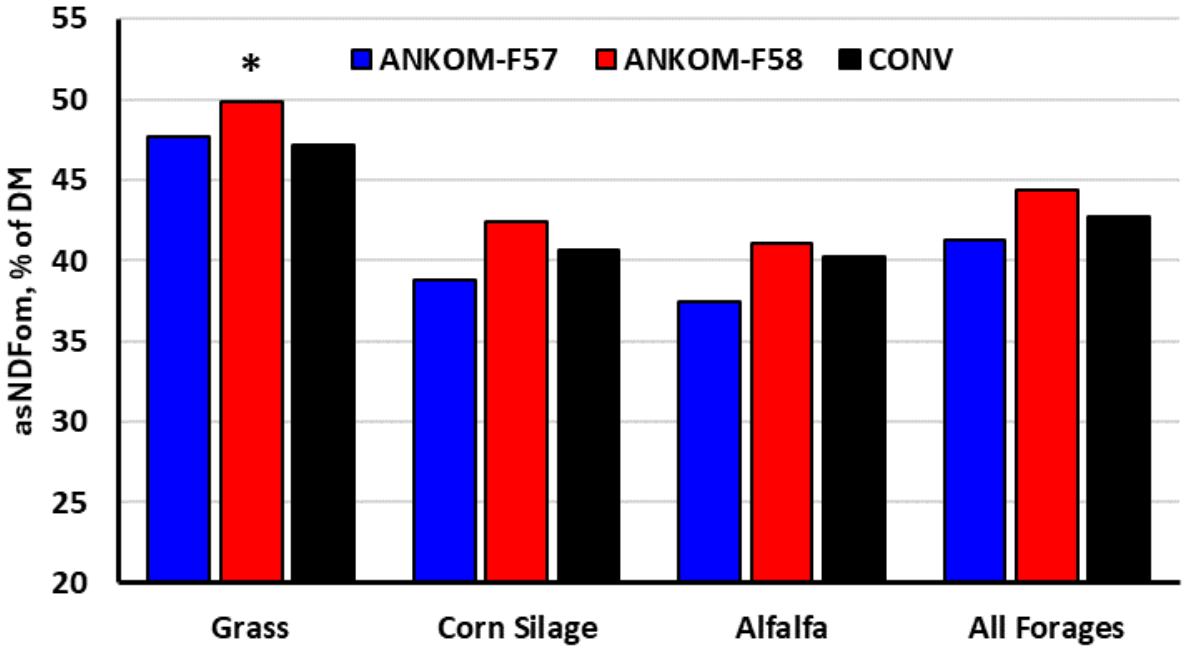


Figure 11. Comparisons of NDF concentrations from 18 forages (grass, corn silage, and alfalfa) using conventional (CONV) methods consistent with the standard method of Mertens (2002), as well as ANKOM methodologies with F57 (ANKOM-F57) and F58 (ANKOM-F58) filter bags. All methods included sodium sulfite and heat-stable a-amylase, and were corrected for residual ash (asNDFom). An asterisk (*) denotes an ANKOM method differing from CONV at P ≤ 0.05 [adapted from Schlau et al. (2021)].

as their numerous minor variations, there are recurring questions about the equivalency of results. In part, this led to the approval of a definitive method for determining NDF (AOAC Official Method 2002.04; Mertens, 2002), which is essential because it establishes an analytical standard that other methods or method variations can be compared against.

As noted previously, the AOAC standard method includes the use of sodium sulfite and heat-stable, α-amylase, where samples are extracted in dedicated vessels under reflux, and then filtered through Gooch crucibles. The procedure specifies preparatory grinding of dried samples through a 1-mm screen in a cutting (Wiley) mill or a 2-mm screen in a cyclone or centrifugal (abrasion) mill. Cyclone or centrifugal mills typically present particles to the screen at an angle, thereby resulting in a finer grind that may require a larger screen aperture to maintain relative consistency with a 1-mm screen in a typical cutting mill. Commercial laboratories often grind samples through a 1-mm screen in an abrasion mill to accommodate requirements for NIRS analysis; however, this also typically reduces particle size, which may affect filtering or the retention of small fibrous particles when using traditional laboratory-based techniques.

A recent experiment (Hall and Mertens, 2023) was conducted to evaluate the effects of grinding procedures (1-mm screen in cutting or abrasion mills) in conjunction with several methods and variants for determining NDF. Methods evaluated included:

- AOAC Official Method 2002.04
- AOAC method with a glass fiber filtration aid
- AOAC method except filtering was conducted through a glass fiber filter placed within a Buchner funnel
- ANKOM system using F57 fiber bags (25-µm particle retention)
- ANKOM system using F58 fiber bags (6 to 9-µm particle retention)

Forages and other feedstuffs analyzed were soyhulls, corn grain (dry), corn grain (high moisture), corn silage (conventional), corn silage (*bmr*), alfalfa silage (conventional), alfalfa silage (low lignin), mixed grass hay, ryegrass silage, calf starter, and sugar beet pulp. Means for analytical methods and their associated standard deviations are summarized in Figures 12 and 13, respectively. In this study, the authors compared methods within each test forage or feedstuff independently, and it is important to understand that the effects of preliminary milling technique did not affect final concentrations of NDF consistently.

For instance, milling technique did not produce significant statistical differences among methods for corn silage (conventional), alfalfa silage (conventional), ryegrass silage, or calf starter, but did for all other tested feeds. The effect of analytical method was highly statistically significant ($P < 0.01$) for all tested feeds, but the interaction of analytical method with milling techniques was observed inconsistently. Ignoring these statistical inconsistencies across individually tested feeds, overall NDF values were reduced by 0.7 percentage units (35.7 vs. 36.4%) when feeds were milled through the abrasion mill compared to the cutting mill. Similarly, NDF concentrations obtained by ANKOM methodology with F57 filter bags were 2.1 percentage units less (34.2 vs. 36.3%) than those obtained by the definitive AOAC method without filtering aids (inclusive of both grinding techniques). The mean within-sample standard deviations for ANKOM methodology with F57 filter bags was noticeably greater than observed for other methods, especially when coupled with the abrasion-type mill; however, this variability also was reduced with F58 bags that have more restrictive particle retention (Figure 13).

Another method of analyzing the collective results of this experiment was to regress results from alternative NDF methods against those obtained from the reference method described by Mertens (2002). Using this approach, relating NDF concentrations obtained from any alternative method against the standard method should ideally yield a slope = 1 and an intercept = 0, which would indicate agreement between methods. A detailed summary of this analysis is found in Table 5, which includes statistically based tests to determine if slopes and intercepts differed from 1 and 0, respectively. Most notably, the slopes obtained by comparing ANKOM methods using F57 filter bags against the reference method were significantly less than 1, indicating concentrations of NDF obtained with F57 filter bags were generally less than those obtained from the standard method. This response for the F57 filter bags occurred regardless of preliminary grinding

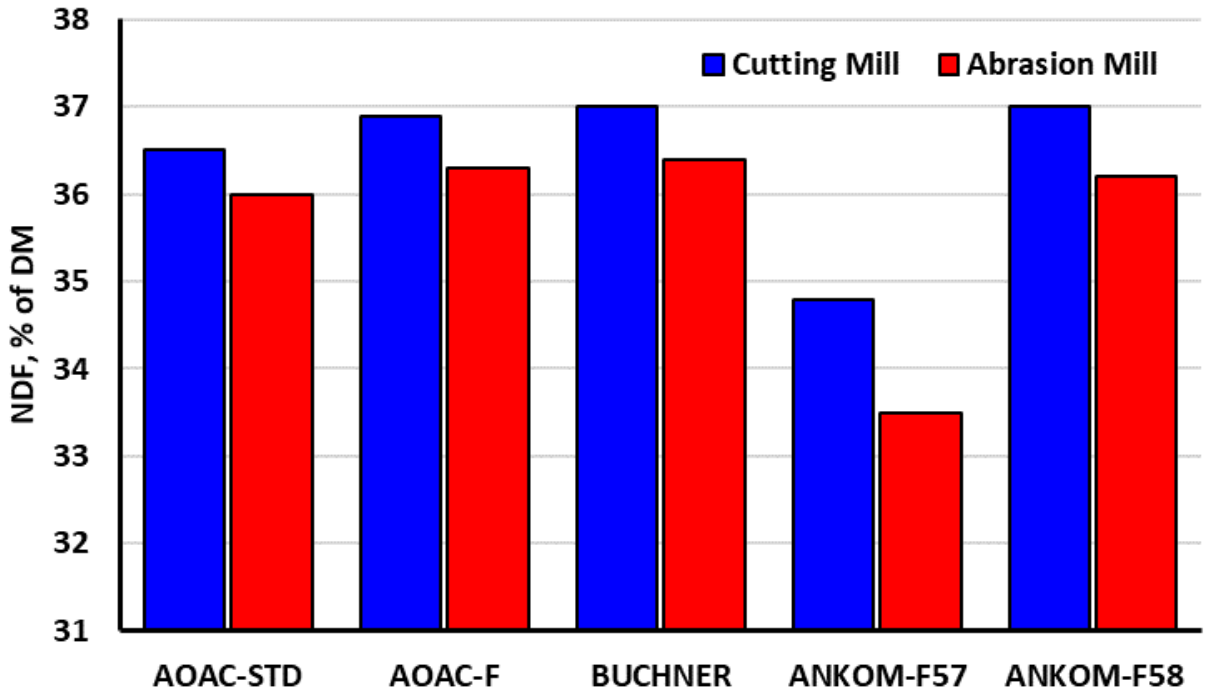


Figure 12. Mean concentrations of neutral detergent fiber (NDF) for 11 forages and other feedstuffs as affected by preliminary milling and NDF analytical methods. Cutting and abrasion mills were both fitted with 1-mm screens. Methods of NDF analysis were: i) the AOAC Official Method 2002.04 (AOAC-STD); ii) AOAC method with a glass fiber filtration aid (AOAC-F); iii) AOAC method except filtering was through a glass fiber filter placed within a Buchner funnel (BUCHNER); iv) ANKOM system using F57 fiber bags (ANKOM-F57); and v) ANKOM system using F58 fiber bags (ANKOM-F58)[adapted from Hall and Mertens (2023)].

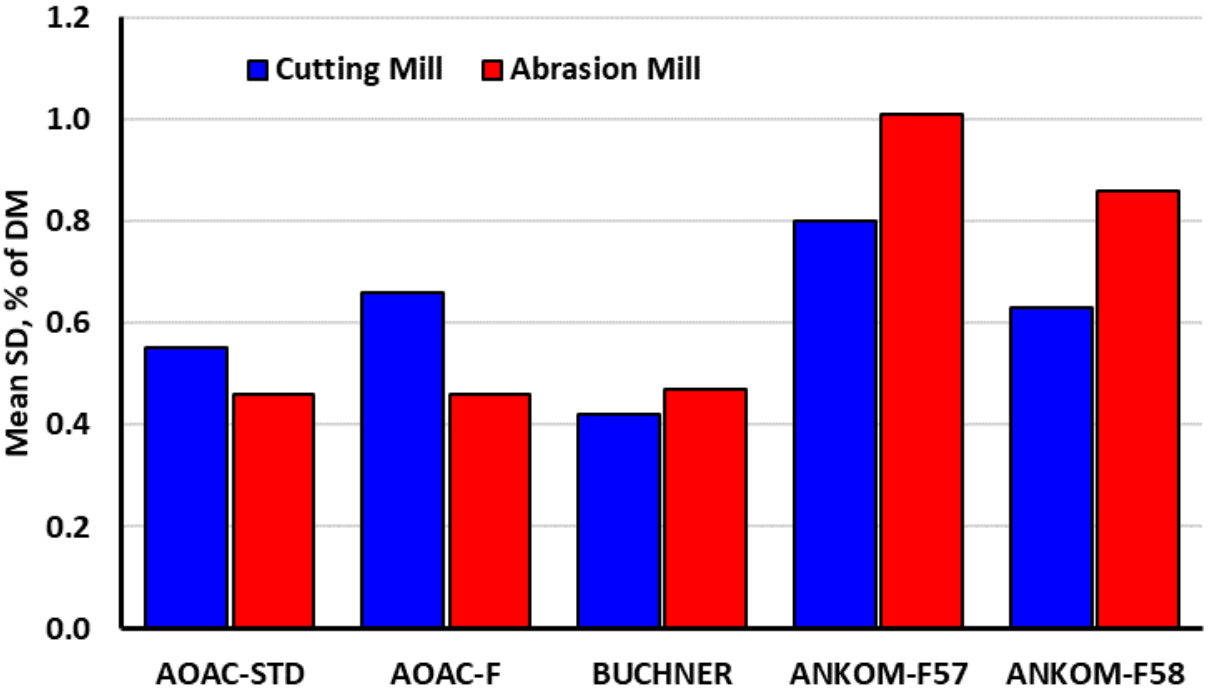


Figure 13. Mean standard deviation of neutral detergent fiber (NDF) concentrations for 11 forages and other feedstuffs as affected by preliminary milling and NDF analytical methods. Cutting and abrasion mills were both fitted with 1-mm screens. Methods of NDF analysis were: i) the AOAC Official Method 2002.04 (AOAC-STD); ii) AOAC method with a glass fiber filtration aid (AOAC-F); iii) AOAC method except filtering was through a glass fiber filter placed within a Buchner funnel (BUCHNER); iv) ANKOM system using F57 fiber bags (ANKOM-F57); and v) ANKOM system using F58 fiber bags (ANKOM-F58)[adapted from Hall and Mertens (2023)].

Table 5. Regression analysis relating alternative methods for determining neutral detergent fiber (NDF) against the approved AOAC standard method described by Mertens (2002), which specifies use of a cutting mill equipped with a 1-mm screen. Eleven feedstuffs were included in the analysis [adapted from Hall and Mertens (2023)].

	---------- Cutting Mill (1-mm) ----------			
Item	**AOAC-F**	**BUCHNER**	**ANKOM-F57**	**ANKOM-F58**
N[1]	44	44	88	81
Slope	0.990	1.003	0.947**	0.996
Intercept	0.667**	0.304	0.207	0.569**
Adjusted R^2	0.998	0.999	0.994	0.998

	---------- Abrasion Mill (1-mm) ----------				
	AOAC-STD	**AOAC-F**	**BUCHNER**	**ANKOM-F57**	**ANKOM-F58**
N[1]	44	44	44	44	42
Slope	0.998	0.983*	0.999	0.946*	1.005
Intercept	- 0.452	0.362	- 0.120	- 1.012	- 0.535
Adjusted R^2	0.997	0.997	0.997	0.978	0.992

**, ** Denotes a statistical difference from slope = 1 or intercept = 0 at the P ≤ 0.05 and P ≤ 0.01 levels of confidence, respectively.*
[1] N = number of points in the regression.

method, but slopes did not differ from unity when the F58 filter bags with restricted porosity were substituted.

Conclusions drawn from the study were that F57 filter bags were most likely to produce results that differed statistically from the standard NDF method, regardless of grinding protocol. However, the study included only a limited number of test feeds, and a greater number of more diverse feeds need to be evaluated before agreement of any alternative method with the reference method can be completely assessed. Furthermore, use of an abrasion mill with a 1-mm screen generally produced NDF values that were lower and more likely to differ from the reference method compared to those generated with preliminary milling through a cutting mill equipped with a 1-mm screen. This trend was somewhat mediated with filtering aids or procedures that provided better particle retention. More research and testing are needed to best reconcile NDF values obtained following milling through an abrasion mill equipped with a 1-mm screen with those obtained from the definitive reference method.

Common Factors Affecting Concentrations of Fiber Components

Introduction

The concentrations of various fiber components in forages can be affected by many variables, including plant maturity, fertilization, climate, and harvest management. A simplified (+ or -) summary of the effects of some environmental factors was compiled years ago by Van Soest et al. (1978). An adapted version of this summary is provided in Table 6 and serves as an interesting framework or introduction to parts of the subsequent discussion. In addition to factors directly affecting concentrations of fiber components, it is important to note that fiber components also can increase or decrease indirectly in response to the changing contributions that other (non-fiber) portions of the plant make to the total plant DM pool. Since the total plant DM pool must sum to 100%, metabolically induced increases in plant sugars or starch may decrease or dilute concentrations of fiber components, primarily by an indirect mechanism. Conversely, improper harvest/storage management that allows for aerobic deterioration (silage) or spontaneous heating (hay or silage) may reduce concentrations of plant sugars or other compounds, thereby indirectly increasing concentrations of the more stable or largely inert fiber components. The objective of this subsection is to discuss some of the common factors affecting concentrations of various fiber components. The factors discussed are most certainly not exhaustive. Furthermore, the examples provided within each subsection should not be viewed as a complete or detailed literature review, but only as representative illustrations of each effect.

Table 6. A summary[1] of environmental influences on the composition and subsequent digestibility of forages [adapted from Van Soest et al. (1978)].

Item	Ambient Temperature	Light	Nitrogen Fertilization	Water/ Precipitation	Predation
Yield	+	+	+	+	-
WSC[2]	-	+	-	-	+
Cell Wall	+	-	±	+	-
Lignin	+	-	+	+	-
Digestibility	-	+	±	-	+

[1] Compiled from various sources. + = increases the quantity or concentration of the item; - = decreases the quantity or concentration of the item. Consult Van Soest et al. (1978) for specific citations.
[2] WSC, water-soluble carbohydrates.

Plant Maturity

It is widely understood that the nutritional value of forages declines as plants develop and mature. From a management standpoint, harvesting or utilizing forages at the most appropriate growth stage for the targeted livestock class consuming those forages is critical to any producer's success. It is also understood that any goal of optimizing nutritive value by harvesting forages at less mature stages of growth likely exists in tension or conflict with yield and also may have negative effects on the persistence of perennial plants.

For alfalfa, an excellent example of the effects of plant maturity on NDF concentrations was reported by Brink et al. (2010). In that experiment, three cultivars of alfalfa were harvested during spring, early summer, late summer, and fall in both Pennsylvania and Wisconsin. During each time period, harvests were initiated at Stage 2 (Kalu and Fick, 1981), which was defined as a stem length > 31 cm (12.2 inches) with no buds, flowers, or seed pods present. Harvests were continued every 5 days thereafter for 25 days, such that concentrations of NDF could be regressed against time, which served as a proxy for harvest delays. Results from Pennsylvania and Wisconsin are summarized in Figures 14 and 15, respectively. For Pennsylvania, seasonal regressions exhibited slopes ranging from 0.18 to 0.61 percentage units/day, with an overall mean across all seasons and years of 0.40 percentage units/day. The relationship between NDF and time delay was relatively close for all regressions; coefficients of determination (r^2) ranged from 0.61 to 0.94, indicating that days after plants reached Stage 2 explained 61 to 94% of the variation in NDF concentrations, with an overall mean of about 82%.

For Wisconsin (Figure 15), results were generally similar; slopes of regressions ranged from 0.12 to 0.59 percentage units/day, with an overall mean across all seasons and years of 0.37 percentage units/day, which was similar to the mean response in Pennsylvania. Coefficients of determination (r^2) were somewhat greater for Wisconsin results, ranging from 0.77 to 0.99 for individual seasonal regressions, with an average response of about 90%. Within these humid environments, concentrations of NDF generally increased more rapidly during spring and early summer compared to later seasonal harvests. These results suggested that spring and early summer harvests could occur earlier and/or more frequently to attain better nutritive value. Slower rates of change later in the growing season suggested a possibility of delaying harvests to improve yields of DM, which also may be beneficial with respect to plant persistence by improving the energy (growth reserve) status of the plants.

It should be emphasized that the responses illustrated in Figures 14 and 15 are partially related to changes in leaf-to-stem ratio, which declines as alfalfa plants mature. This is critically important for management of alfalfa because of the great contrast in nutritive value that exists between alfalfa leaf and stem tissues. The importance of this stark differential is compounded further by the potential loss of fragile leaves during the harvest of hay or silage, making retention of leaves an extremely high priority. To illustrate this concept, the differences in fiber components between leaf and stem fractions for alfalfa and red clover forages harvested during mid-September in Kansas are summarized in Table 7 (Coblentz et al., 1998). For alfalfa, concentrations of NDF were about 2.4 times greater in stem compared

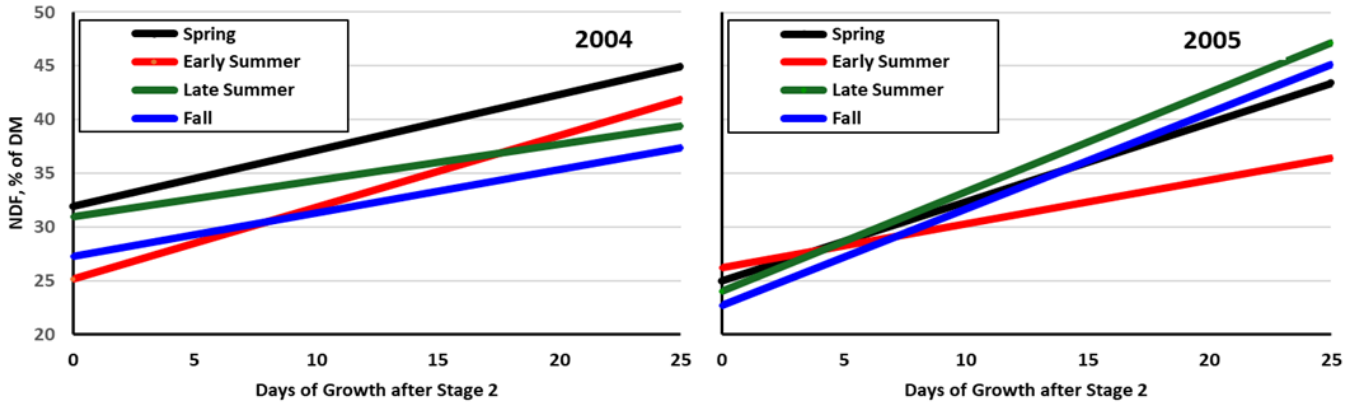


Figure 14. Linear regressions relating concentrations of NDF with days of growth after alfalfa plants reach Stage 2 maturity (Kalu and Fick, 1981) based on seasonal harvests in Pennsylvania during 2004 and 2005 [adapted from Brink et al. (2010)].

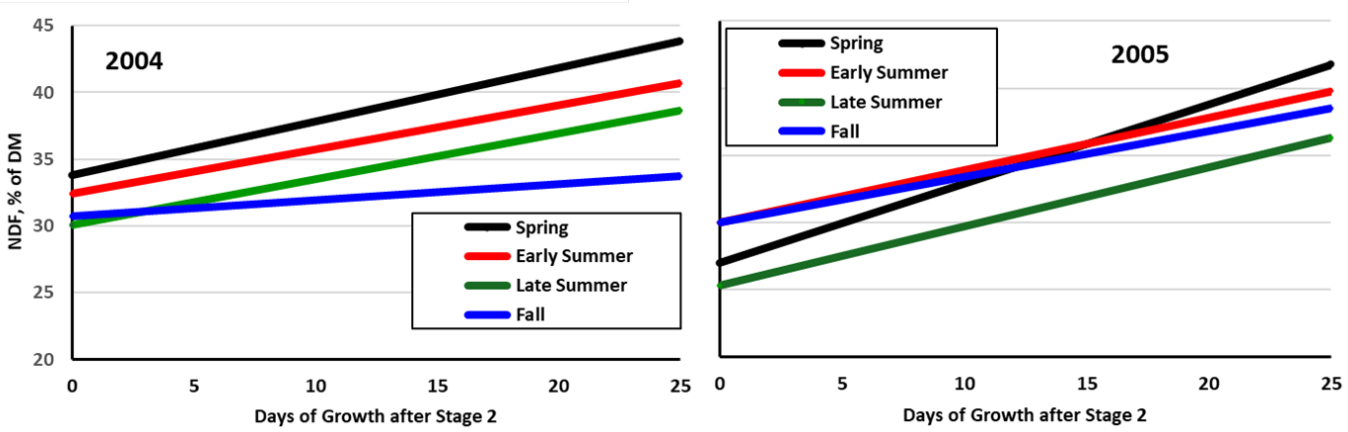


Figure 15. Linear regressions relating concentrations of NDF with days of growth after alfalfa plants reach Stage 2 maturity (Kalu and Fick, 1981) based on seasonal harvests in Wisconsin during 2004 and 2005 [adapted from Brink et al. (2010)].

to leaf tissue (59.4 vs. 25.4%). The magnitude of this differential for red clover was not as great (51.2 vs. 36.2%), but the sharp contrast between stem and leaf tissue remained consistent. For alfalfa, there also was a sharp contrast in lignification between stem and leaf tissues (11.1 vs. 3.7%), which is reflective of the disproportionate structural and metabolic functions of these tissues, respectively. Given that forage fiber is a non-ideal feed fraction that is incompletely digested by ruminants, the overall digestibility of alfalfa forages and its subsequent ability to meet the energy requirements of various livestock classes is affected negatively by maturation via:

- increasing concentrations of NDF that occur normally with plant growth and development,
- declining leaf-to-stem ratios,
- stark compositional discrepancies between leaf and stem tissues, and
- increased lignification.

From a digestibility perspective, changes associated with plant maturity are disproportionately associated with alfalfa stems. In his popular textbook, Van Soest (1982) explained that the digestibility of alfalfa leaves was relatively stable as a function of time or advancing plant maturity, but the digestibility of stem tissue declines precipitously over the same time interval (Figure 16). This work, based on research conducted in Canada (Mowat et al., 1965), further reinforces the critical importance of leaf retention whenever alfalfa is harvested as hay or silage.

For perennial cool-season grasses, the effects of plant maturity also result in increased concentrations of fiber components and ultimately reduced overall DM digestibility due to the incomplete (non-ideal) nature of forage fiber digestibility. As an example, Hoffman et al. (1993) reported NDF and lignin concentrations for five perennial cool-season grasses (smooth bromegrass, orchardgrass, perennial

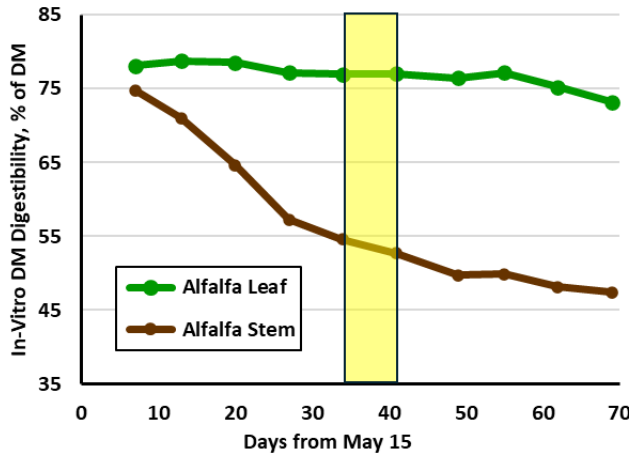


Figure 16. In vitro digestibility of dry matter (DM) for leaf and stem tissues of alfalfa as a function of harvest date. Data represent the mean of two cultivars grown over three years in Canada. The yellow shaded box represents the early flower stage of growth for the two cultivars [adapted from Mowat et al. (1965); Van Soest (1982)].

ryegrass, quackgrass, and timothy) harvested in central Wisconsin at three stages of growth (stem elongation, boot, and full flower). Both NDF and lignin increased notably with plant maturity for all species. For NDF, these increases ranged from 8.0 to 19.0 percentage units of DM between the early-stem elongation and full-flower stages of growth (Figure 17). Similarly, when averaged across the five cool-season grasses, concentrations of lignin increased by about 61% (3.88 vs. 6.24%) between these same growth stages.

While these overall trends are similar to those discussed for alfalfa, there are some notable differences between the two forage types. First, the distinct differences in fiber composition observed for alfalfa leaf and stem tissues (Table 7) are generally less definitive for grasses. This contributes to a clearly different digestibility response for leaf tissue, which also has been described for orchardgrass forages (Mowat et al., 1965; Van Soest, 1982) and summarized in Figure 18. In that graphic, DM digestibility for individual leaf and stem tissues were plotted as a function of calendar date as a proxy for maturity. In contrast to alfalfa, the digestibility of both tissue types declined substantially over time, although the rate of decline remained somewhat slower for leaf compared to the corresponding stem tissue.

Another notable difference between alfalfa and many perennial cool-season grasses is that many common erect-growing grass species (e.g., tall fescue or orchardgrass) demonstrate reproductive development only during their initial growth each spring, which includes stem elongation, flowering, and seed development. Once the initial growth is removed, any regrowth remains entirely vegetative (all leaf) until the next spring. As a result, the nutritive value of vegetative regrowth for these grasses is generally more stable over time, thereby allowing hay or silage producers greater flexibility in avoiding unstable weather during secondary harvests.

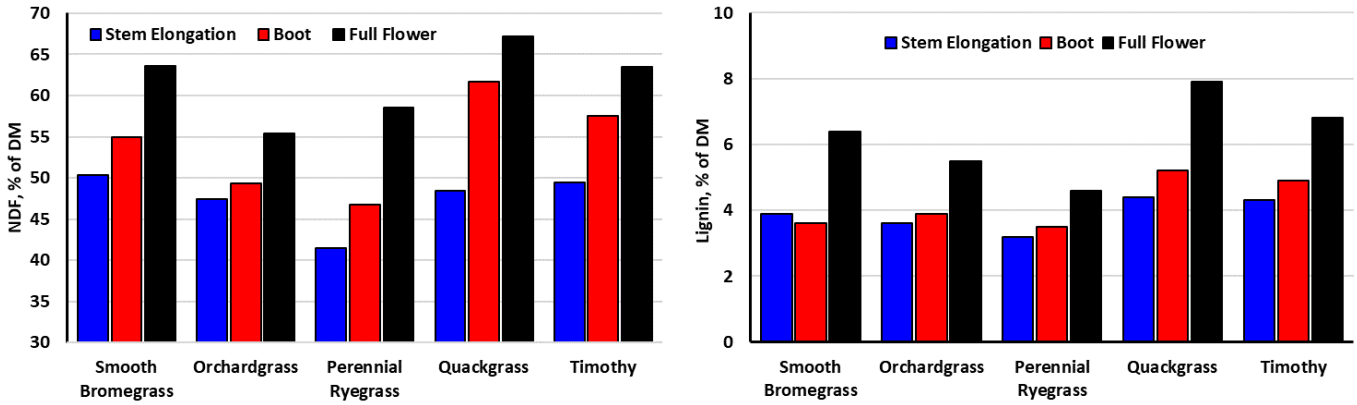


Figure 17. Concentrations of NDF and lignin for five cool-season grasses grown in central Wisconsin and harvested at three stages of growth [adapted from Hoffman et al. (1993)].

Table 7. Concentrations of fiber components (% of DM) for leaf, stem, and combined (whole-plant) tissues for alfalfa and red clover forages harvested in Kansas during mid-September [adapted from Coblentz et al. (1998)].

	Alfalfa			Red Clover		
Component	**Leaf**	**Stem**	**Whole-Plant[1]**	**Leaf**	**Stem**	**Whole-Plant[1]**
NDF[2]	25.4	59.4	40.9	36.2	51.2	40.4
ADF[2]	20.4	51.2	34.7	28.5	42.7	32.3
Hemicellulose[3]	5.0	8.1	6.2	7.8	8.5	8.2
Cellulose	14.6	39.0	25.8	23.1	35.7	26.3
ADL[2]	3.7	11.1	7.3	3.7	5.6	4.2

[1] *Determined independently, and not based on the weighted mean of leaf and stem fractions.*
[2] *NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.*
[3] *Estimated as: NDF - ADF.*

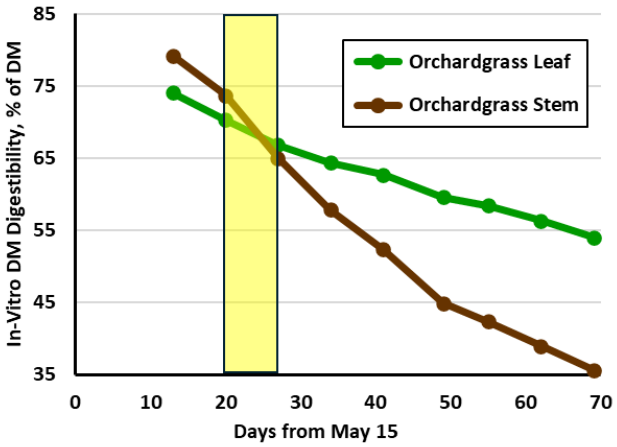


Figure 18. In vitro digestibility of dry matter (DM) for leaf and stem tissues of orchardgrass as a function of harvest date. Data represent the mean of two cultivars grown over three years in Canada. The yellow shaded box represents the early flower stage of growth for the two cultivars [adapted from Mowat et al. (1965); Van Soest (1982)].

While perennial warm-season grasses are not typical components of dairy rations, especially in the north-central or northeastern US, some of their common characteristics differ from perennial cool-season grasses and should be noted. A prominent difference is their sharply greater fiber concentrations, even at relatively immature growth stages. Table 8 summarizes concentrations of fiber components from eastern gamagrass grown in central Wisconsin over 3 years (2007 to 2009; Coblentz et al., 2010a). Eastern gamagrass is a perennial warm-season (C4) bunch grass that is a distant relative of corn and is adaptable to colder, central Wisconsin climates. Because of its warm-season nature and physiology, it is slower to initiate spring growth than perennial cool-season grasses. In central Wisconsin, spring initiation of growth often begins in late May; therefore, the June 1st harvest date noted in Table 8 coincides with a very immature (vegetative) stage of growth. Even at that immature growth stage, concentrations of NDF were exceptionally high (66.7%), which may be as much as 25 percentage units greater than the initial growth of a perennial cool-season grass harvested at a vegetative stage of growth at the same location. It is

Table 8. Concentrations of various fiber components for initial harvests of eastern gamagrass forage taken at 15-day intervals near Marshfield, WI. Eastern gamagrass is a perennial warm-season (C4) grass, and the various concentrations of individual fiber components represent the means of a 3-year study [adapted from Coblentz et al. (2010a)].

Harvest Date	**NDF[1]**	**ADF[1]**	**Hemicellulose**	**Cellulose**	**ADL[1]**
	% of DM				
June 1	66.7	28.7	38.0	26.2	1.48
June 15	67.2	32.1	35.0	29.1	2.11
July 1	72.8	37.9	34.9	34.3	2.67
July 15	74.7	39.7	35.0	35.6	3.18
August 1	76.6	41.0	35.7	36.5	3.82
August 15	77.3	41.9	35.4	36.7	4.22

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

especially noteworthy that much of the elevated concentrations of NDF observed for eastern gamagrass were related directly to large contributions from hemicellulose, which ranged from 34.9 to 38.0% of plant DM. Although concentrations of ADL predictably increased with plant maturity, ranging from 1.48 to 4.22%, they remained relatively low, regardless of harvest date. This may be explained by the cooler climate in Wisconsin relative to more southerly locations, as greater ambient temperatures typically have positive effects on forage lignification (Van Soest, 1982).

Dilution

As mentioned previously, concentrations of forage fiber components can be affected indirectly by significant changes in other (non-fibrous) entities within the forage plant. There are numerous examples of this general type of response, which often is associated with various grain crops (e.g., wheat, triticale, corn, or grain sorghum) that can exhibit disproportionately larger percentages of the total DM pool partitioned into grain or seed compared to common perennial forages. The dilution response usually becomes most evident as these grain crops begin to actively partition carbohydrates into the inflorescence or filling seeds. Various cereal grains are used as forages throughout the US, primarily within grazing systems, or as stored (ensiled) forages. Some years ago, Cherney and Marten (1982) evaluated four cereal-grain species (wheat, triticale, oat, and barley) that were harvested at various stages of growth in Minnesota. In that study, plant tissue types (leaf blade, leaf sheath, stem, and inflorescence) were separated, and the percentages by weight that each contributed to the total pool of plant DM were determined. Each tissue type also was analyzed individually for concentrations of cell wall (Figure 19).

A close inspection of the graphs in this Figure indicates several key points. First, the relative contributions that each tissue type made to the total plant DM pool changed with maturation. Predictably, the contributions of leaf blade and sheath declined, while stem increased with maturation until the aggressive partitioning of carbohydrates into the inflorescence also caused it to decline. Additionally, concentrations of cell wall increased with maturation for leaf blade, leaf sheath, and stem, but declined for the inflorescence with the onset grain fill. Thus, the overall effects of maturation on cell-wall concentrations for these cereal-grain forages were dependent on both the concentration of fiber components within individual tissue types, but also on the contribution each tissue type made to the total plant DM pool. An excellent practical example of these interacting responses to maturation is observed commonly for cereal rye, which is somewhat notorious for its sharply declining quality characteristics after reaching the boot stage of growth. In part, commonly observed increases in NDF for cereal rye can be linked to a generally taller growth habit, as well as disproportionately lower head weights compared to other cereal-grains.

Recently, evaluations of triticale at various stages of growth also have illustrated the dilution concept on a whole-plant basis. Figure 20 summarizes concentrations of NDF, hemicellulose, cellulose, and ADL for triticale forages grown during 2016 and 2017 in central Wisconsin (Coblentz et al., 2018). The most common maturities recommended for a silage harvest of cereal-grain forages are the boot and soft-dough stages of growth. Within this maturity range, concentrations of most fiber components increased with maturation, with the maximum concentrations occurring at anthesis (flowering) or very early seed development. With the onset of grain fill, concentrations of NDF declined by 9.8 and 5.7 percentage units in 2016 and 2017, respectively, primarily due to a dilution effect.

Additionally, this response is normally accompanied by an increase in energy density, but these effects remain variable from one year to the next. A similar response was observed for 14 diverse triticale cultivars grown under irrigation in Arizona (Figure 21; Coblentz et al., 2022). Mean concentrations of NDF, as well as ADL, declined with the onset of grain fill, where the mean percentage of weight partitioned within the grain head reached 53.1% at maturity. It should be emphasized that the dilution responses reported in that experiment varied sharply by cultivar. At maturity, percentages of DM partitioned into the grain head varied from 20.9 to 61.7%, and plant height varied from 38 to 54 inches. As suggested by the early work of Cherney and Marten (1982), varying proportions of specific plant parts and their respective compositions largely determine overall whole-plant concentrations of fiber components, and the effects of dilution observed commonly in grain

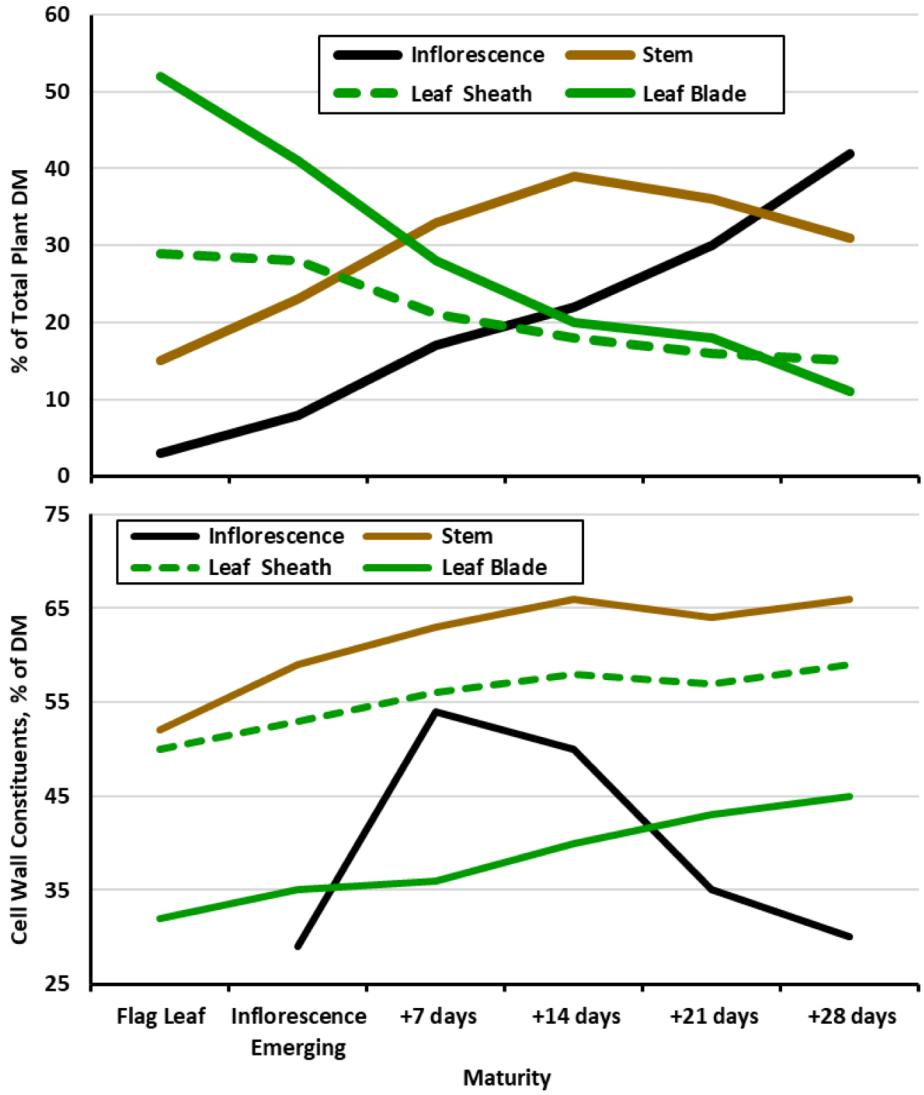


Figure 19. Contributions that various plant parts make to the total dry matter (DM) pool (top), as well as the respective concentrations of cell wall within each tissue type (bottom). Data are summarized for four cereal grain forages grown in Minnesota [adapted from Cherney and Marten (1982)].

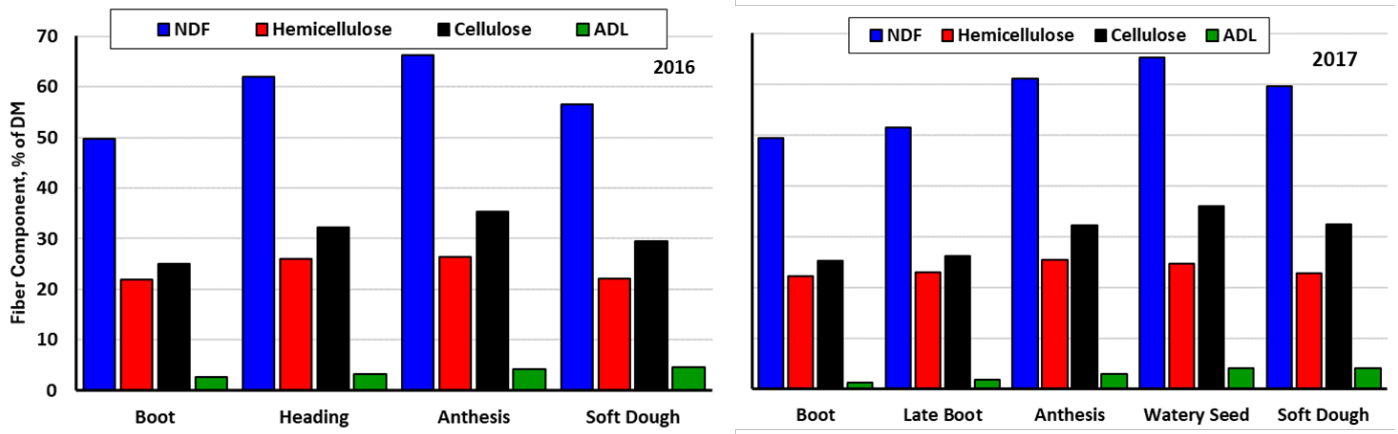


Figure 20. Concentrations of neutral detergent fiber (NDF), hemicellulose, cellulose, and acid detergent lignin (ADL) at various stages of maturity for triticale forages grown in central Wisconsin [adapted from Coblentz et al. (2018)].

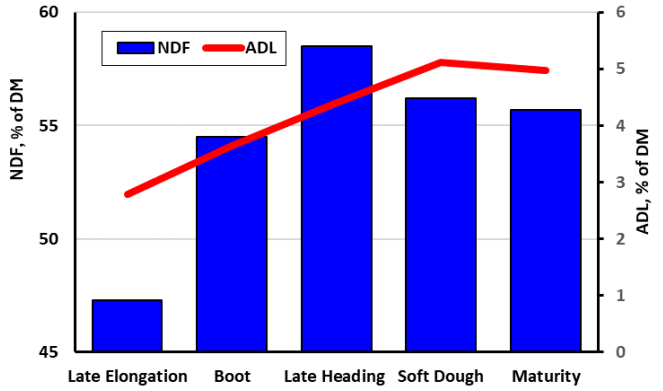


Figure 21. Mean concentrations of NDF and acid detergent lignin (ADL) for 14 diverse triticale cultivars grown under irrigation near Maricopa, Arizona [adapted from Coblentz et al. (2022)].

crops doubling as forages contribute heavily to this premise. Within that context, producers should pay particular attention to cultivars demonstrating atypical growth habits, as the resulting forages may vary sharply from typical forage quality expectations.

Without question, the most common example of a grain crop doubling as a forage crop is corn, which is utilized throughout the United States as precision-chopped silage. One illustration of forage fiber dilution by starch and non-fiber carbohydrate (NFC) was compiled by Ferreira and Mertens (2005) for 32 corn silages exhibiting diverse chemical and physical characteristics. For this study, concentrations of starch can be viewed as a proxy (albeit imperfectly) for grain content, while NFC represents a broader, cruder estimate of available carbohydrate that includes starch, sugars, pectins, soluble fibers, and organic acids. The linear regression relationships between common fiber components (NDF and ADF) and starch or NFC are summarized in Figure 22. For NDF, the slopes of regression lines were strongly negative, generally suggesting that concentrations of NDF decline by roughly 1 percentage unit for each percentage unit increase in starch or NFC. While the slopes were slightly less negative for ADF, the relationships were similar; coefficients of determination (r^2) indicated a high level of association, where starch or NFC explained 71 to 93% of the variability in NDF or ADF. Similarly, strong ($P < 0.01$) negative correlations also were observed between ADL and starch ($r = -0.65$) or NFC ($r = -0.74$). All of these relationships are consistent with dilution of forage fiber by the accumulation of starch or grain content.

Another unique example of dilution of forage fiber is captured in the photographs found in Figure 23 and further summarized in Table 9 (Coblentz et al., 2013). In this example, 'ForagePlus' oat was planted on August 1st in Wisconsin. Photos were taken subsequently on September 15th (S202) and November 3rd (S346), which were 45- and 94-days post-planting, respectively. Normal assumptions based on the effects of plant maturity, as well as the discoloration from obvious frost damage would suggest that the forage photographed in mid-September is superior in quality, and most desirable for meeting the energy requirements of high-producing livestock.

However, such an assumption would be incorrect, specifically due the effects of dilution. Although the more-mature frost-damaged forage would not survive the winter in Wisconsin, it still went through some of the physiological processes of winter hardening that includes accumulation of sugars within plant cells. Concentrations of water-soluble carbohydrates in the frost-damaged oat were 5.5 times greater (19.4 vs. 3.5%) than found in the immature forage. As a result, concentrations of NDF, ADF, and ADL for the two oat forages were nearly identical. Calculated estimates of energy density for the frost-damaged oat (69.4% TDN, 0.72 Mcal/lb NE_L) were greater than the immature oat forage, and quite comparable to corn silage. The energy density of the immature forage also was diluted by greater concentrations of ash (15.0 vs. 9.1%), which is a common observation among immature cereal-grain forages. Ash makes no contribution to energy density calculated by summative models.

Temperature

The effects of ambient temperature on concentrations of forage fiber components are difficult to assess in field research without being confounded significantly by other factors, such as soil moisture, fertilization, photoperiod, etc. For example, high ambient temperatures are often accompanied by drought, which may inject different stresses, or cause different responses with respect to plant growth and subsequent nutritive value, compared to the effects of temperature considered alone. True evaluation of temperature effects should be quantified within tightly regulated systems (i.e.,

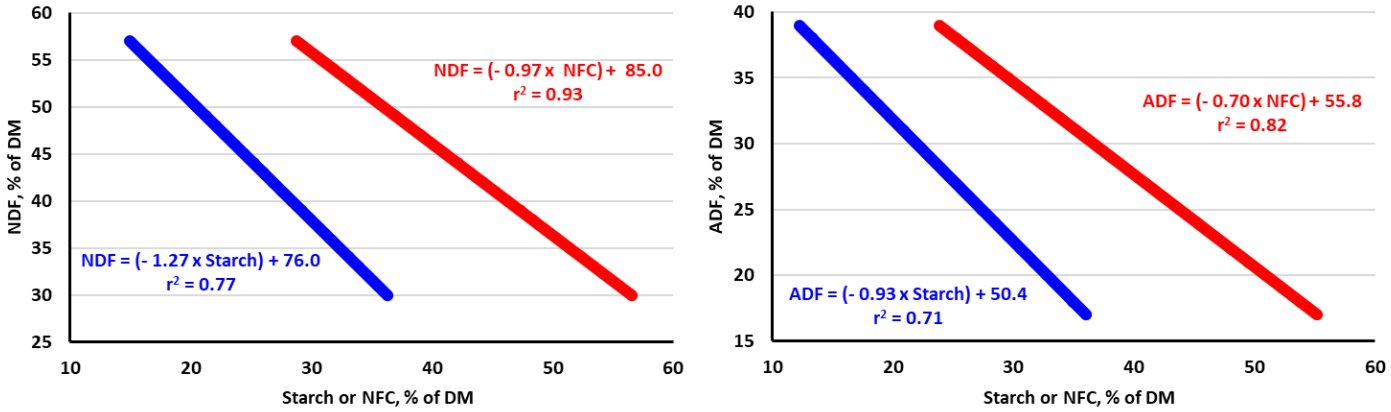


Figure 22. Linear regression relationships between concentrations of neutral detergent fiber NDF or acid detergent fiber (ADF) and starch or non-fiber carbohydrate (NFC) for 32 diverse corn silages [adapted from Ferreira and Mertens (2005)].

Figure 23. Fall-grown oat forages established in early August in central Wisconsin. Photos were taken of the same experimental plot in mid-September (S202) and early-November (S346).

Table 9. Comparative analyses for ForagePlus oat forages established in early August in Wisconsin [adapted from Coblentz et al. (2013)].

Item[1,2]	S202	S346
Planting date	August 1	August 1
Harvest (photo) date	September 15	November 3
Canopy height, inches	19	27
NDF, %	46.3	45.2
ADF, %	23.4	23.0
ADL, %	0.84	1.10
WSC, %	3.5	19.4
Ash, %	15.0	9.1
TDN, %	64.8	69.4
NE_L, Mcal/lb	0.67	0.72

[1] *Abbreviations: NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; WSC, water-soluble carbohydrates; TDN, total digestible nutrients; and NE_L, net energy of lactation.*

[2] *Concentrations expressed as % of DM unless otherwise indicated.*

growth chambers) that can either control or exclude confounding variables.

Generally, the effects of temperature affect concentrations of forage fiber components through alteration of metabolic rates, specifically via the relative activities of various enzymes. Most forage plants have optimum temperatures for various processes. As such, greater ambient temperatures generally increase metabolic rates until optimum temperatures are reached, but the opposite effect may occur when temperature optima are exceeded (Buxton and Fales, 1994). Temperature-induced enhancements of metabolic rates tend to decrease pool sizes of metabolites within cellular contents, including temporary stores of photosynthetic products, and increase the rate of conversion into structural cell wall components. Furthermore, increased temperature positively affects enzymatic activities associated with formation of lignin (Van Soest, 1982).

An excellent example of these effects was reported for vegetative tall fescue forages grown under different temperature regimes in climate-controlled growth chambers (Fales, 1986). Forages were harvested after 28 days of growth following sequential temperature regimes (day/night) of 55/50°F, 68/64°F, 86/81°F, and followed by a return to the original 55/50°F. Three harvests were made at each temperature regime in the sequence, but the first of the three was discarded to minimize any residual (carryover) effects of the previous temperature regime in the 4-treatment sequence. Results are summarized in Table 10, and indicate that concentrations of NDF, ADF, cellulose, and hemicellulose all increased with temperature, and then declined (but not completely) with the return to the original 55/50°F temperature treatment. In this study, lignin was determined by an alternative permanganate procedure, and numerical changes in response to temperature were not statistically significant.

Another somewhat circumstantial, but uniquely visual, example of the effects of temperature on both the concentration of ADL and the structural rigidity of oat plants can be observed in Figure 24, which illustrates the effects of a moderate (wet or heavy) October snowstorm on oat forages that were established in early August. These oat forages were grown in a central Wisconsin climate under cooler fall temperatures compared to most oat forages that are typically established during the spring and mature during late-spring and early-summer.

The current compendium of feedstuff analyses offered by NASEM (2021) indicates that a mid-maturity oat silage (57.4% NDF) will have a corresponding ADL concentration of about 5.4%, which equates to 9.4% of NDF. Similarly, and compiled from the same source, the mean concentration of ADL in oat hay (4.71%; no maturity

Figure 24. Lodging effects of a modest October snowfall event on fall-grown oat near Marshfield, WI.

Table 10. Concentrations of various cell-wall components in vegetative tall fescue forages as affected by four temperature regimes applied in sequence within a growth chamber. Each concentration represents the mean of two analyzed harvests [adapted from Fales (1986)].

Temperature	NDF[1]	ADF[1]	Cellulose	Hemicellulose	Lignin[2]
°F (day/night)			% of DM		
55/50	34.8	16.9	15.4	18.1	0.9
68/64	42.7	20.8	18.9	22.0	1.0
86/81	50.1	23.5	21.6	26.6	1.0
55/50	41.1	19.3	17.9	21.9	0.9

[1] *NDF, neutral detergent fiber; ADF, acid detergent fiber.*
[2] *Determined by an alternative permanganate procedure.*

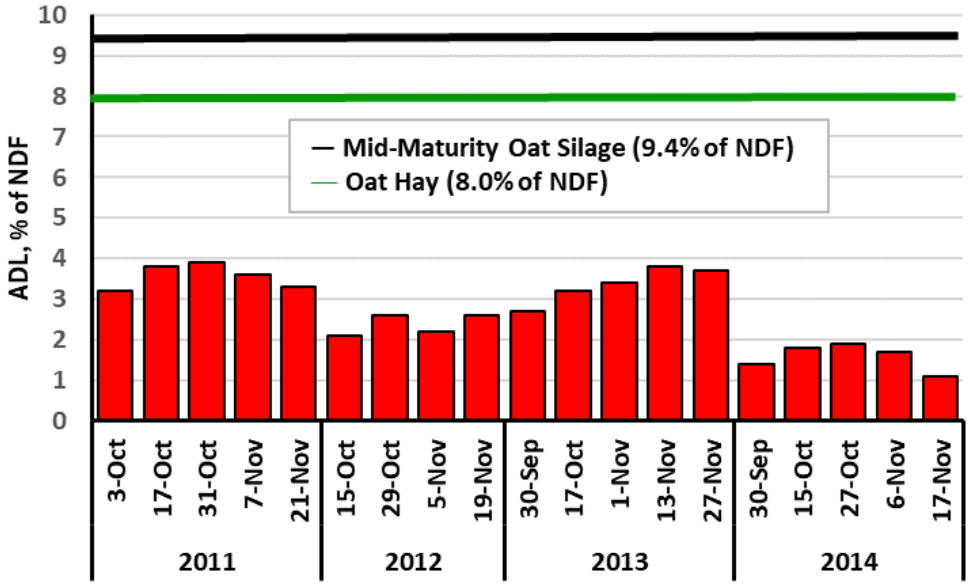


Figure 25. Concentrations of acid detergent lignin (ADL; % of NDF) for mid-maturity oat silage, oat hay, and fall-grown oat forages sampled from two grazing trials over four years [adapted from Coblentz et al. (2014); Coblentz et al. (2015a); NASEM 2021].

specified) was equivalent to 8.0% of NDF. In contrast, Figure 25 summarizes the periodic changes in ADL (% of NDF) for oat during two fall grazing studies conducted over four years at Marshfield, WI (Coblentz et al. 2014; Coblentz et al., 2015a). It should be noted that oat plants do not require vernalization in order to elongate; therefore, oat plants sampled from these grazing studies exhibited somewhat similar maturity ranges as those described within the NASEM (2021) compendium. While these data are admittedly confounded by dilution with accumulating soluble sugars (see *Dilution* section), annual climatic variability, atypical photoperiod during maturation (oat is a long-day plant), and other factors, the overall mean ADL concentration was ≤ 3.9% of NDF across all years and harvest dates, and as a little as 1.1% of NDF on one specific sampling date. This ranged from only 12 to 41% of that observed for the mid-maturity oat silage reported by NASEM (2021). The effects of this radically reduced lignification on the composition of structural plant fiber are evident in Figure 24, where oat forages were completely flattened or lodged in response to the modest (~4-inch) snowfall event. Although this response cannot be exclusively explained as a temperature-related effect on normal lignification, the effects of cooler ambient temperatures can be considered a likely contributing factor.

Drought or Water Deficits

The effects of drought on the quality of harvested forages is a question frequently posed by forage producers. Again, it is important to emphasize that any assessment of the specific effects of drought within field studies is a nearly impossible task because of the confounding effects of other factors. For instance, drought is often (but not always) confounded by high ambient temperatures. True evaluation of drought effects (again) requires the use of closely controlled growing environments, such as those provided by growth chambers. However, some studies have attempted to investigate forage responses to drought within a field setting.

One such report (Peterson et al., 1992) evaluated four legumes grown under drought and controlled conditions in Minnesota. Some of the results for alfalfa are summarized in Table 11. For this study, drought and control treatments were defined for a June 2nd harvest date at stem-pressure potentials of -3.2 and -0.9 MPa, respectively. For a later harvest date (July 6th), respective stem pressure potentials for drought and control treatments were -3.3 to -3.7 MPa and -0.6 to -1.4 MPa. Generally, yields were suppressed by drought (data not shown), but under these droughty conditions, alfalfa preferentially partitioned DM into leaf tissue, thereby resulting in leaf-to-stem ratios that were statistically greater than those of control plants. On a whole-plant basis, plants subjected to drought exhibited reduced

Table 11. Plant heights, leaf-to-stem ratios, and concentrations of fiber components for alfalfa grown under droughty and control conditions in Minnesota [adapted from Peterson et al. (1992)].

			NDF[1]			ADL[1]		
Date/Treatment	**Height**	**Leaf/Stem[2]**	**Leaf**	**Stem**	**Whole-plant**	**Leaf**	**Stem**	**Whole-plant**
	inches	*g/g*	*% of DM*					
2 June[3]								
Drought	18	1.0	26.6	54.3	40.5	4.3	8.7	6.5
Control	28	0.8	27.3	60.2	45.3	4.2	9.2	6.9
6 July[3]								
Drought	10	2.0	21.2	46.1	29.7	2.9	8.2	4.7
Control	20	1.2	25.8	58.2	40.7	3.2	9.8	6.3

[1] *NDF, neutral detergent fiber; ADL, acid detergent lignin.*
[2] *Expressed as a ratio (gram/gram basis)*
[3] *Respective drought and control treatments were defined as stem-pressure potentials of -3.2 and -0.9 MPa for June 2, as well as -3.3 to -3.7 and -0.6 to -1.4 MPa for July 6 sampling dates.*

concentrations of NDF. This was primarily caused by two clear factors: 1) concentrations of NDF in stem tissue were less than observed in control plants and 2) a greater proportion of the total plant DM was comprised of leaf tissue.

For the July 6th harvest date (Table 11), the differential between drought and control forages was 11.0 percentage units of NDF (29.7 vs. 40.7%). For ADL, numerical trends were similar to those observed for NDF. Based on these results for alfalfa, moderate drought will likely improve the quality of alfalfa by reducing concentrations of forage fiber components and increasing the proportion of total plant DM partitioned into leaf tissue. An obvious caveat is that drought cannot become so severe that leaves begin to senesce. As noted in Table 7, concentrations of NDF (structural fiber) are much lower in alfalfa leaf tissue than in the associated stem, which reflects the primarily metabolic and structural functions of those plant parts, respectively.

A similar question relates to the effects of drought on the fiber composition and overall nutritional value of perennial cool-season grasses. Another study was conducted in Minnesota (Sheaffer et al., 1992) that used similar field procedures to those described previously for alfalfa, and investigated drought effects on reed canarygrass, smooth bromegrass, orchardgrass, and timothy. Within this experiment, forages grown under droughty conditions exhibited leaf-water potentials of -1.3 to -4.0 MPa, while those measured for control forages were -0.1 to -1.7 MPa.

Concentrations of NDF and ADL for drought-impaired and control forages obtained from three harvests are summarized in Figure 26. As expected, drought had a negative effect on yield (data not shown), but a positive effect on the contributions of leaf blade to the total pool of forage DM. The positive effects of drought on the percentage of leaf blade were particularly evident for reed canarygrass, smooth bromegrass, and timothy, which displayed some stem elongation during regrowth harvests taken in July of 1987 and 1988. For these three species, overall mean percentages of leaf blade from regrowth harvests were 86 and 68% under droughty and controlled conditions, respectively. Drought also slowed the maturation rate for these three forage species. In contrast, orchardgrass exhibited only vegetative regrowth, such that leaf blade percentage was 100% for regrowth harvests.

Figure 26 indicates that concentrations of NDF were consistently reduced by drought, with a maximum differential between drought and controlled conditions of 14.2 percentage units of NDF for reed canarygrass harvested in July 1987. Considered across all forage species and harvest dates shown in Figure 26, the mean differential between droughty and controlled growing conditions was 5.7 percentage units of NDF (54.8 vs. 60.5%), and this was statistically significant for most comparisons of watering regimes within forage species and harvest date. Concentrations of NDF within stem tissues (data not shown) also were reduced by drought, with an overall differential of 9.0 percentage units of NDF (63.9 vs. 72.9%) for those cases where there was sufficient stem tissue to support a direct within-species comparison. Generally, within-species effects of drought on

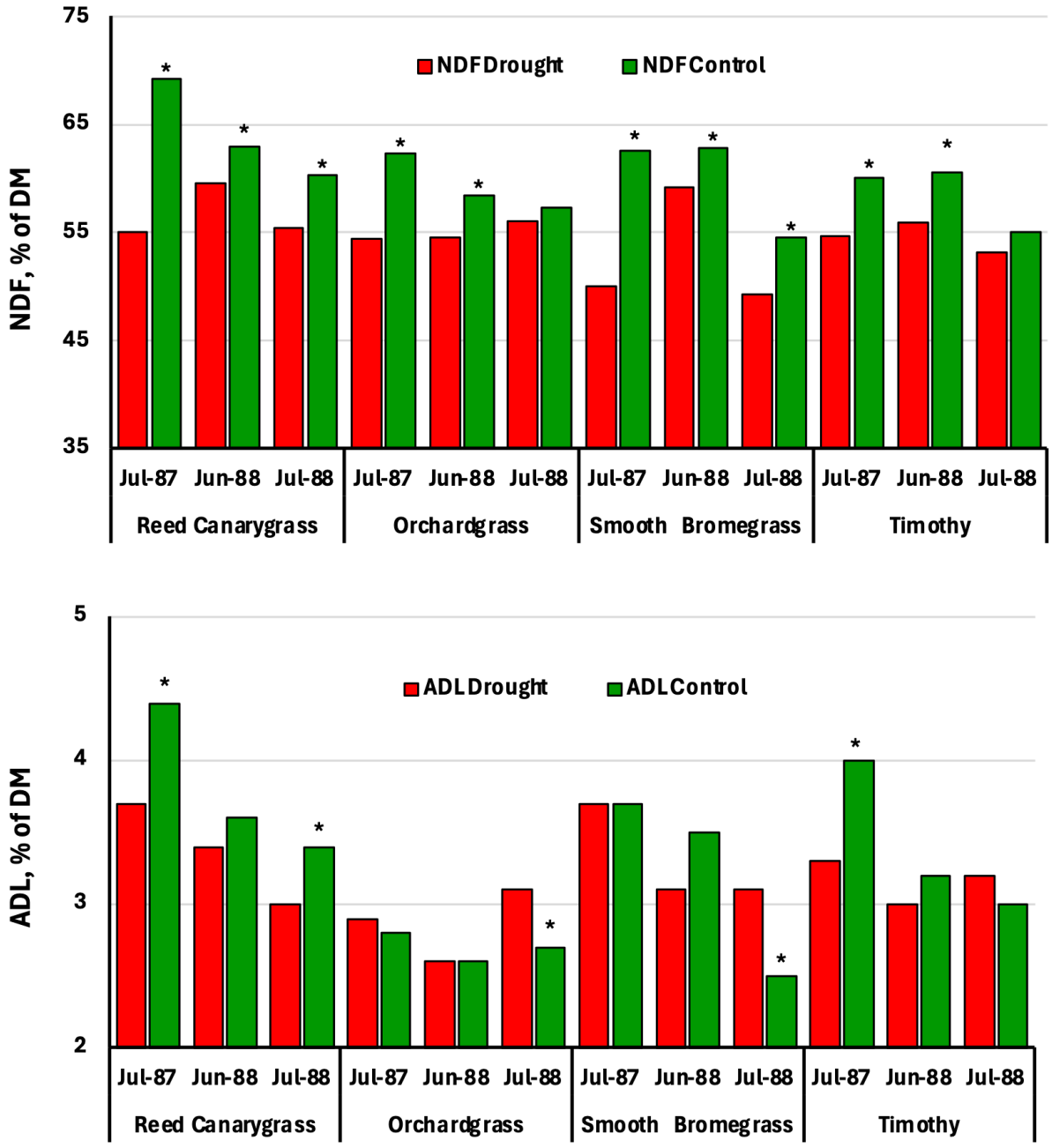


Figure 26. Effects of drought on concentrations of neutral detergent fiber (NDF) and acid detergent lignin (ADL) for four perennial cool-season grass species in Minnesota. An asterisk (*) denotes a statistically significant difference between watering regimes for a specific species/harvest date combination [adapted from Sheaffer et al. (1992)].

concentrations of ADL were observed inconsistently; however, ADL was at least numerically reduced by drought in each of the three harvests for reed canarygrass.

Nitrogen Fertilization

Nitrogen (N) fertilization of forages with either commercial or manure sources is widely documented to improve yields of DM. However, the often-held view that N fertilization improves the overall quality of forages is flawed, and can only be applied to concentrations of CP, which may or may not be beneficial based on the nutritional requirements of the targeted livestock class (Van Soest, 1982).

Fertilization with N sources often reduces concentrations of NDF within the forage; however, the magnitude of this response is usually small, and

may or may not be statistically significant. Nitrogen fertilization will increase concentrations of CP within forages, but the various nutritional components within forages still must sum to 100%. At a minimum (excluding any other physiological response), this mathematically forces the concentration of other forage components to decline in a corresponding manner.

This concept can be illustrated from the work of Johnson et al. (2001) in Florida, where bermudagrass, stargrass, and bahiagrass forages were fertilized with 0, 35, 70, 105, or 140 lbs N/acre/cutting and harvested five times at 28-day intervals beginning in early June. Concentrations of NDF declined linearly for each forage with increasing N fertilization rate (Figure 27). In reality, the magnitude of this NDF response was small, amounting to 3.0 percentage units of DM when averaged across all forages and harvest dates. A similar NDF response to N fertilization was observed in Wisconsin for eastern gamagrass forages fertilized with ammonium nitrate (34-0-0) at rates of 0, 60, 120, and 180 lbs N/acre. In that study, concentrations of NDF declined linearly with N fertilization rate, but the differential between the (negative) control and maximum rate was only 0.5 percentage units (73.1 vs. 72.6%; Coblentz et al., 2010a).

It should be noted that the limited, and often linear declines of NDF in response to N fertilization, such as those shown in Figure 27, are not observed universally and some contrasting responses can be quite complex. One example of a complex response to N fertilization can be illustrated for fall oat grown in Wisconsin (Figure 28; Coblentz et al., 2017). As documented in a previous subsection (*see Dilution*), fall-grown oat can accumulate very large concentrations of WSC (sugars), but one common response to N fertilization is a reduction in concentrations of WSC. For the fall-grown oat forages summarized in Figure 28, forages were fertilized with urea (46-0-0) at rates of 0, 18, 36, 54, 72, or 90 lbs N/acre. In response to those treatments, concentrations of CP predictably increased by about 31% from 13.2 to 17.3% of DM. However, this response was juxtaposed against a 32% decline in concentrations of WSC (21.2 to 14.4%). As a result, the net effect of N fertilization on NDF was uniquely and substantively positive, increasing from 41.5 to 46.7% of DM.

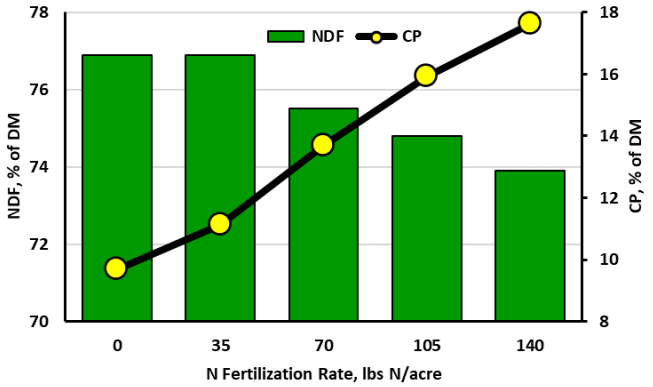


Figure 27. Effects of N fertilization on concentrations of NDF and crude protein (CP) within bermudagrass, bahiagrass, and stargrass forages harvested in Florida at 28-day intervals beginning in early June [adapted from Johnson et al. (2001)].

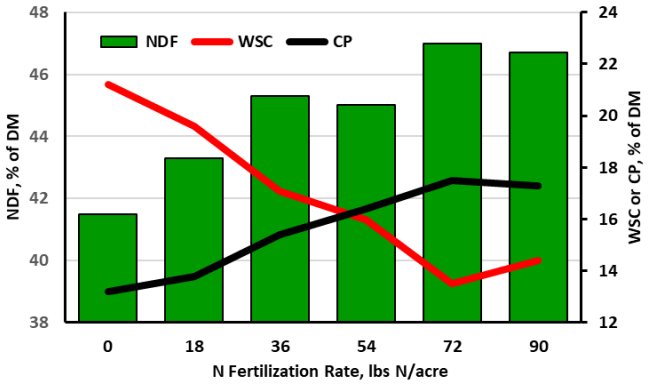


Figure 28. Effects of nitrogen (N) fertilization on concentrations of NDF for fall-grown oat in Wisconsin that includes the juxtaposing responses for crude protein (CP) and water-soluble carbohydrates (WSC) [adapted from Coblentz et al. (2017)].

One consistent response to N fertilization is an increase in concentrations of ADL. This can be clearly illustrated based on the previously described work with fall-grown oat (Figure 29). Based on a simple quadratic regression, N fertilization rate explained about 99% of the variability in concentrations of ADL, which increased by 59% across the range of fertilization rates evaluated. A similar response to N fertilization rate was observed for eastern gamagrass forages (Coblentz et al., 2010a; Figure 30).

As a result, the overall effects of N fertilization on the subsequent DM digestibility of forages are somewhat dependent on the resulting balance of various forage components, and may be positive, negative, or indistinguishable. Increased concentrations of ADL will decrease digestibility, as will any increase in NDF or decrease in WSC. In

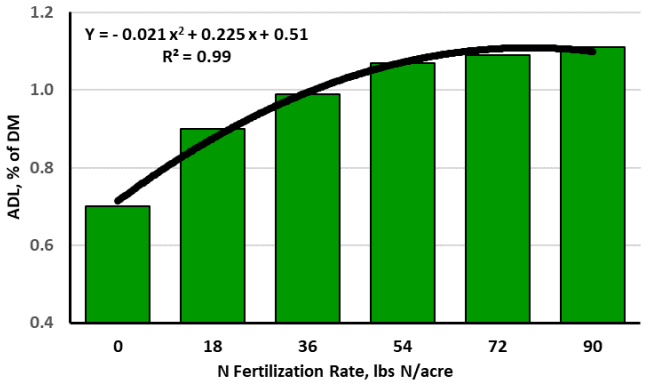


Figure 29. Relationship between concentrations of acid detergent lignin (ADL) and nitrogen (N) fertilization rate for fall-grown oat forages in Wisconsin [adapted from Coblentz et al. (2017)].

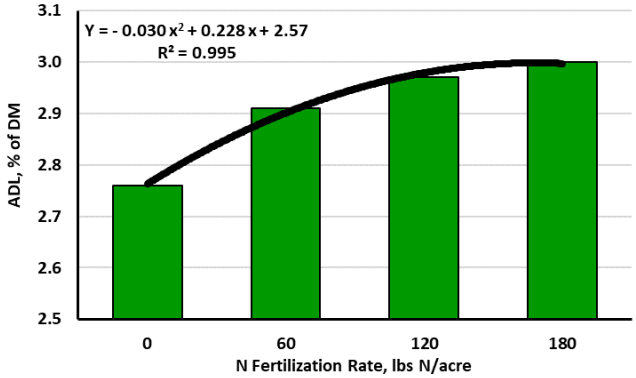


Figure 30. Relationship between concentrations of acid detergent lignin (ADL) and nitrogen (N) fertilization rate for eastern gamagrass grown in Wisconsin [adapted from Coblentz et al. (2010a)].

contrast, reduced concentrations of NDF, such as those observed in Figure 27, may yield a (small) net positive effect. Digestion of poor-quality forages with very low concentrations of CP, such as dormant native range, are improved by adding CP to the diet, but this is typically applied via external supplementation, and not by N fertilization during the growing season.

Aging of Standing Stockpiled Forages

Although the technique of stockpiling forages for fall and winter grazing, sometimes referred to as 'deferred grazing', is used infrequently within the dairy industry, it is a common practice within beef cow-calf enterprises throughout the upper South. Generally, forages are removed by haying or close grazing during late-summer, which is then followed immediately by an application of commercial N fertilizers or manure. Grazing is then deferred until fall in order to allow the forage to accumulate. One advantage of this technique throughout the upper South is the potential to utilize both perennial warm- and cool-season grasses, most commonly bermudagrass and tall fescue, respectively. Among the most important issues related to a deferred utilization of these forages is the relative stability of forage nutritive value throughout the late-fall and early-winter.

An early evaluation of stockpiled 'Greenfield' bermudagrass was conducted in northern Arkansas (Scarbrough et al., 2001) on both hay and pasture management sites. At the hay site, a second cutting was removed from the site on July 26th, and about 50 lbs N/acre was applied as ammonium nitrate (34-0-0) on August 18th. Grazing was then deferred until October 17th. Subsequently, forages were then sampled monthly through January to assess the relative stability of nutritive value over time. At the pasture management site, broiler litter (4000 lbs/acre) and commercial N fertilizer (67 lbs N/acre) were applied in April and June, respectively. Pastures were purposely understocked, such that forage accumulated throughout the growing season, and fall sampling also began on October 17th. At both sites, beef cows grazed continuously throughout the evaluation period, but ungrazed forages also were sampled on each date by using wire exclusions to restrict access by the cows. Concentrations of NDF and ADL are summarized in Figure 31, and indicate that both increased across sampling dates, regardless of previous site management. Differentials also were observed between ungrazed and grazed forages, with ungrazed forages generally exhibiting superior quality characteristics compared to grazed forages, particularly with respect to ADL. Conclusions drawn from this study were that stockpiled bermudagrass should be utilized by spring-calving beef cows during late fall but probably discontinued before winter because the nutritive value becomes very poor.

For perennial cool-season grasses, Kallenbach et al. (2003) compiled a list of reasons why tall fescue is a preferred species for autumn-stockpiling compared to other grasses with similar physiologies:

- Tall fescue produces a greater percentage of its total annual forage production in the fall compared to other perennial cool-season grasses.

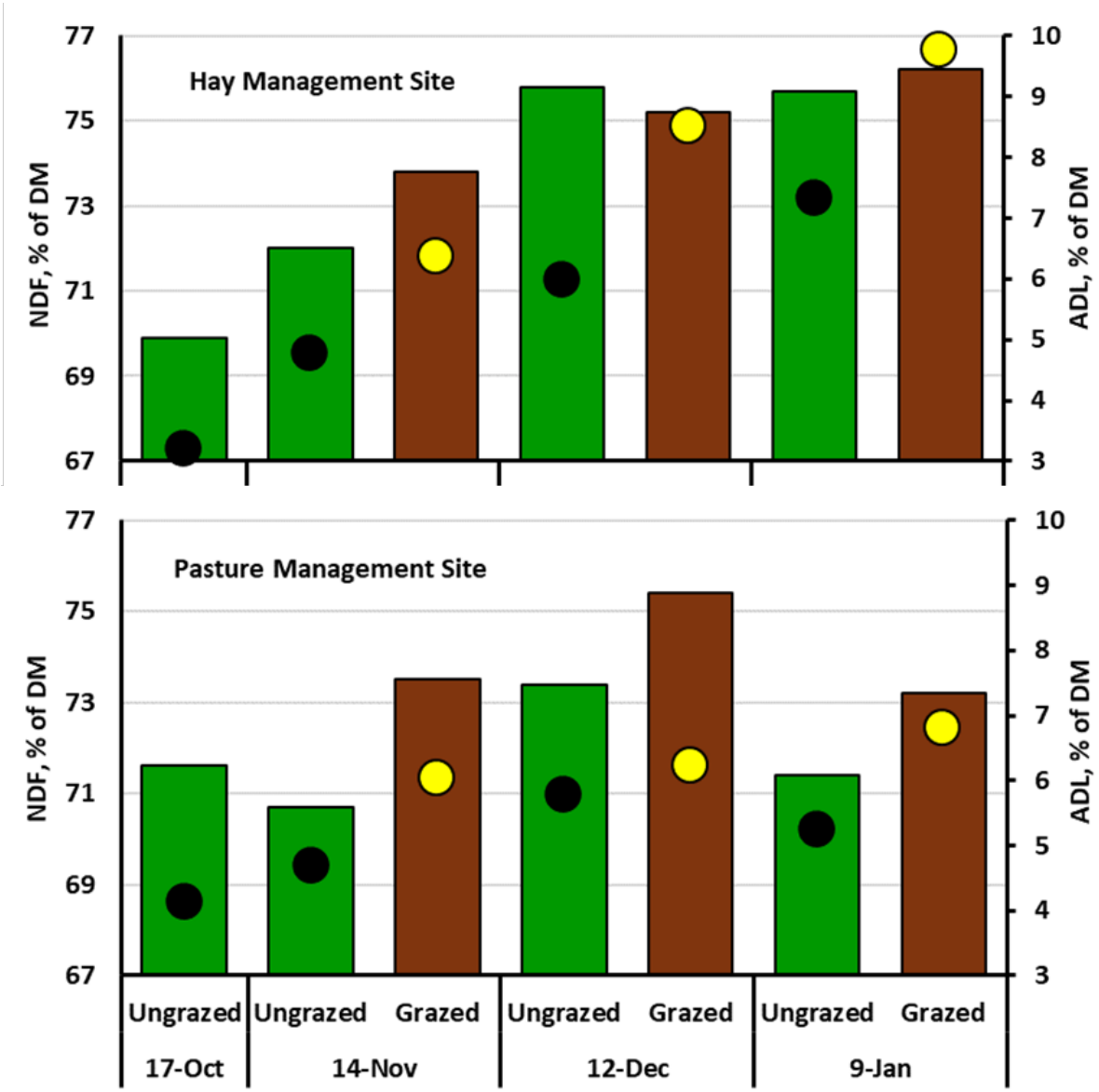


Figure 31. Concentrations of NDF and acid detergent lignin (ADL) for grazed and ungrazed stockpiled bermudagrass sampled in October, November, December, and January at two sites near Lincoln, AR. Concentrations of ADL are depicted by black (●; ungrazed) or yellow (○; grazed) dots superimposed on bars representing NDF concentrations. Hay and pasture management sites indicate management strategies during the previous spring and summer [adapted from Scarbrough et al. (2001)].

- The nutritive value of tall fescue is relatively stable throughout the winter.
- Late-fall/winter defoliation by livestock has minimal effect on forage production the following spring.

Flores et al. (2007) evaluated stockpiled tall fescue cultivars infected with either a wild-type or novel endophyte, where the wild-type endophyte produced significant concentrations of toxic ergot alkaloids, but the corresponding production of alkaloids by the novel endophyte was minimal or nil. Pasture replicates in the study were clipped on September 9th and fertilized with ammonium nitrate (34-0-0) at a rate of 50 lbs N/acre. All livestock were removed from the pastures, and forage was allowed to accumulate until December 4th (54 days). Tall fescue forages were then sampled on December 4, December 26, January 15, February 4, and February 26, and analyzed for concentrations of forage fiber components (Table 12). Fescue cultivar/endophyte status had no effect on concentrations of fiber components. Concentrations of all fiber components increased gradually through mid-January before either stabilizing or declining slightly thereafter. Modest reductions in some fiber components in late February may have been due to the initiation of spring growth in this upper South (northern

Table 12. Concentrations of fiber components for stockpiled tall fescue forages sampled through late-fall and winter in Fayetteville, AR [adapted from Flores et al. (2007)].

Harvest Date	NDF[1]	ADF[1]	Hemicellulose	Cellulose	ADL[1]	DM Digestibility[2]
	---------- % of DM ----------					
December 4	56.5	27.7	28.8	25.0	3.74	68.4
December 26	57.1	28.1	29.0	25.5	3.61	69.5
January 15	64.0	30.8	33.2	27.2	4.64	61.8
February 4	66.4	32.4	34.0	27.1	7.69	61.2
February 26	64.6	31.4	33.3	27.8	5.17	62.7

[1] *NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.*
[2] *Determined by in situ procedures in crossbred steers and calculated with an experimentally determined passage rate of 0.026/hour.*

Arkansas) environment. These changes in concentrations of fiber components were reflected closely by in situ determinations of DM digestibility, which declined through mid-January before stabilizing thereafter.

Brown Midrib Mutants

Brown midrib (***bmr***) genetic mutations (either spontaneously occurring or chemically induced) typically reduce concentrations of ADL in corn, sorghum, and millet. They have been observed and researched exhaustively, particularly by dairy nutritionists, because of the possible improvements that can be realized with respect to voluntary intake, digestibility of forages, and subsequent milk production. Generally, the presence of a *bmr* mutation can be identified by brown coloration associated with the mid veins of leaves on these forage plants. The presence of this trait results in consistently reduced concentrations of ADL, and oftentimes NDF and ADF. Many illustrations of the effects of *bmr* mutations are available within the research literature, most of which are specific to the use of *bmr* corn silage in the diets of high-producing dairy cows.

One notable example summarized from work conducted in Michigan (Oba and Allen, 2000) shows the effects of the *bmr* mutation on the nutritive characteristics of two corn silages that were nearly isogenic, except for the presence of the *bmr* mutation (Table 13). Reduced concentrations of NDF, ADF, and ADL were associated with the *bmr* mutation, which was reflected in greater digestion of fiber and DM following a 30-hour in-vitro incubation. Subsequent effects on dry matter intake (**DMI**) and milk production when these silages were offered to high-producing dairy cows in high- and low-NDF dietary formulations are further summarized in Table 14. Dry matter intakes increased by about 3 lbs DM/day and milk yields were improved by more than 7 lbs/day at both dietary NDF levels by using the *bmr* corn silage compared to the control silage. At the high dietary NDF level, DMI may have been improved by reducing ruminal fill, thereby allowing greater meal sizes. Milk fat and solids non-fat were not depressed by using the *bmr* corn silage within the

Table 13. Nutritive comparisons of Cargill 657 (bmr) and 6208FQ (Control) corn hybrids, which were nearly isogenic except for the presence of the bmr mutation and were offered subsequently to high-producing dairy cows [adapted from Oba and Allen (2000)].

Item[1]	*bmr*	Control
NDF[2]	41.4	42.9
ADF[2]	20.2	22.4
ADL[2]	1.3	2.0
Starch	38.3	35.4
30-hour NDFD[2], % of NDF	55.9	46.5
30-hour True DM Digestibility	83.3	78.2

[1] *Concentrations expressed on a % of dry matter (DM) basis, unless otherwise specified.*
[2] *Abbreviations: NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; and NDFD, NDF digestibility.*

Table 14. Diet formulation, dry matter intake (DMI), and milk production for high- and low-fiber diets containing corn silages that were essentially isogenic except for a brown midrib mutation (bmr) or no mutation (Control) and offered to high-producing dairy cows in Michigan [adapted from Oba and Allen (2000)].

	---------- Low NDF ----------		---------- High NDF ----------	
Item	***bmr***	**Control**	***bmr***	**Control**
Diet Formulation, % of DM				
Corn Silage (Control)	. . .	32.1	. . .	50.5
Corn Silage (*bmr*)	35.8	. . .	55.9	. . .
Alfalfa Silage	8.1	7.7	12.6	12.2
Ground Corn	26.2	29.2	. . .	5.4
Cottonseed	3.6	3.7	5.4	5.6
Protein Mix	23.3	23.5	23.0	23.2
Vitamins and Minerals	3.0	3.1	3.0	3.1
Diet Composition*[1]*, % of DM				
NDF, % DM	28.7	29.1	37.5	38.4
ADF, % DM	15.5	16.0	21.9	22.8
ADL, % DM	2.0	2.4	2.9	3.4
Indigestible NDF[2], % DM	10.5	11.8	13.4	15.6
Starch, % DM	37.2	37.6	26.1	26.8
Corn Silage NDF, % DM	14.8	14.9	23.1	23.5
Intake/Production[1]				
DMI, lbs/day	54.5	52.7	50.5	47.4
Digestible DMI, lbs/day	35.1	34.2	32.2	30.9
Milk Yield, lbs/day	81.4	73.9	74.3	67.0
3.5% FCM, lbs/day	78.5	75.4	78.9	71.9
Milk Fat, %	3.28	3.67	3.86	3.90
3.5% FCM/DMI	1.45	1.43	1.56	1.50

[1] Abbreviations: DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; DMI, DM intake; and FCM, 3.5% fat-corrected milk.
[2] Based on a 120-hour incubation.

high NDF diet, thereby suggesting that *bmr* corn silages may be more beneficial for cows offered high NDF diets.

An example of the effects of *bmr* mutations on the composition, voluntary DMI, and apparent digestibility of forage fiber for sorghum × sudangrass hybrids based on work conducted in Illinois was reported by Fritz et al. (1988).

Regrowth hay (boot stage to early heading) of two hybrids (Redlan × Greenleaf and Redlan × Piper), each possessing the *bmr* mutation or its normal counterpart, were compared in a digestion trial conducted with non-lactating Holstein cows using a 4 × 4 Latin square design. The fiber composition of each forage, DMI, and apparent digestibilities of fiber components are summarized in Table 15. Concentrations of NDF were reduced in the *bmr* mutants by more than 2 percentage units, which was primarily related to small reductions in hemicellulose (0.9 or 1.2 percentage units) and the commonly observed reductions in ADL. While the voluntary DMI was greater only for the *bmr* mutant of the Redlan × Piper hybrid, apparent digestibilities for fiber components were generally improved with the *bmr* mutant, irrespective of hybrid. These apparent digestibility results are consistent with expectations for the reduced ADL concentrations commonly observed within *bmr* mutants.

Rain Damage to Wilting Forages

During times of frustration with unpredictable or uncooperative weather, forage producers often wonder about the effects of rainfall events on the quality characteristics of mowed forages wilting in the field prior to conservation as dry hay or silage.

Table 15. Fiber composition, voluntary dry matter intakes (DMI), and apparent digestibilities of fiber components for two sorghum × sudangrass hybrids grown in Illinois [adapted from Fritz et al. (1988)].

	------- Redlan × Greenleaf -----		------ Redlan × Piper -------	
Item[1]	***bmr***	**Normal**	***bmr***	**Normal**
Fiber Composition, % of DM				
NDF	74.2	76.6	74.5	76.6
ADF	44.9	46.1	44.2	45.5
Cellulose	39.2	39.8	39.1	40.0
Hemicellulose	29.3	30.5	30.2	31.1
ADL	4.6	5.6	4.0	4.8
DM Intake, lbs/day	20.3	20.7	25.4	20.7
Apparent Digestibilities, %				
DM	50.7	45.3	56.6	51.8
NDF	58.7	53.2	62.8	58.3
ADF	56.3	51.2	60.3	56.2
Cellulose	68.1	63.2	71.5	66.7
Hemicellulose	62.3	56.3	66.4	61.2

[1] *Abbreviations: NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; and DM, dry matter.*

Generally, the effects of these unfortunate rainfall events result in poorer quality forages that exhibit greater concentrations of fiber components. However, the magnitude of these responses varies widely on the basis of several factors:

- forage species,
- moisture concentration of the forage at the time the rainfall event occurs,
- amount and duration of the rainfall event,
- length of additional wilting time required to reach a target moisture concentration suitable for dry hay or silage, and
- additional swath manipulation required to facilitate drying, which is more problematic for legume forages with fragile leaves.

The primary mechanism by which fiber concentrations increase is via losses of WSC and other soluble cell contents due to leaching. Sugars also can be lost by extended or re-initiated respiration within the forage swath. In contrast, forage fiber is insoluble in water, and therefore relatively inert. As a result, concentrations of forage fiber components increase indirectly through losses of cellular contents, which (unfortunately) also are the most digestible portions of the forage. A limited number of research studies have investigated rainfall effects on wilting forages, with even fewer actually documenting responses to naturally occurring rainfall. Of the studies available, most have used various forms of artificial rainfall application, ranging from garden-variety sprinkling cans to sophisticated rainfall simulation systems. There remain open questions about whether artificial application systems generate effects equivalent to natural rainfall, but they still can suggest and/or illustrate informative trends.

One such study was conducted in Arkansas with a rainfall simulator that was designed primarily to examine nutrient runoff losses from manured grass stands (Scarbrough et al., 2005). In that experiment, orchardgrass and bermudagrass forages were mowed in the field, placed in wire baskets, and subjected to simulated rainfall applied in half-inch increments at a rate of 3 inches/hour. Both forage types were evaluated at different initial moisture concentrations that ranged from wet (immediately after mowing) to suitability for baling as dry hay. Concentrations of NDF resulting from these treatments are summarized in Figure 32 and indicate several clear points.

- Bermudagrass exhibited (far) more stable concentrations of NDF in response to simulated rainfall than orchardgrass, which is likely reflective of its inherently low concentrations of WSC.
- Concentrations of NDF increased with rainfall amount.

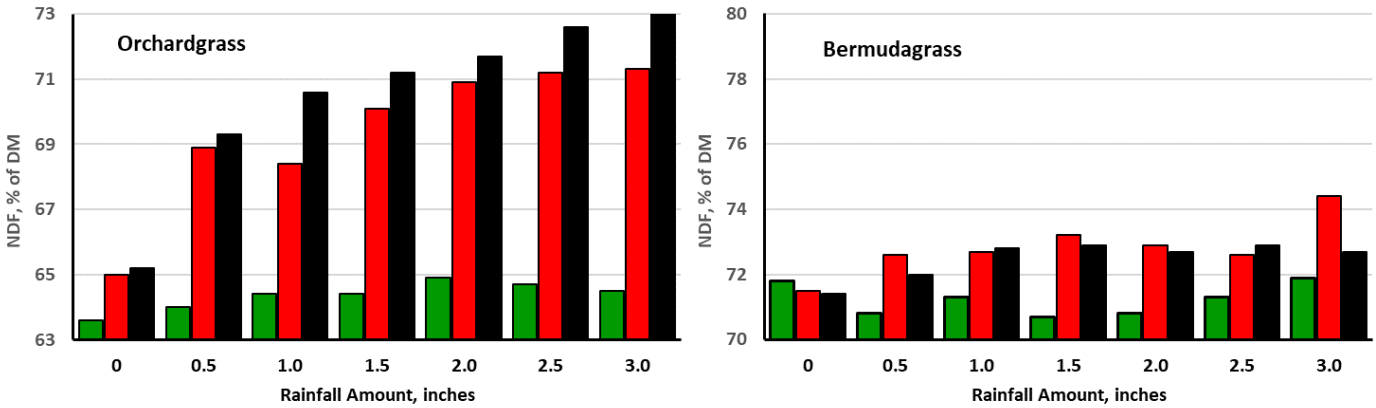


Figure 32. Concentrations of NDF in orchardgrass (left) and bermudagrass (right) following application of simulated rainfall. Moisture concentrations at the time rainfall was applied for orchardgrass were 67.4% (■), 15.3% (■), and 4.1% (■), and for bermudagrass 76.1% (■), 40.0% (■), and 13.0% (■). Rainfall was applied at a rate of 3 inches/hour in half-inch increments [adapted from Scarbrough et al. (2005)].

- Increases in NDF were notably less (even minimal) for forages subjected to simulated rainfall immediately after mowing compared to those dry enough to bale as hay.

Although data were somewhat more variable, similar trends were observed for concentrations of ADL (Table 16). This study suggests that perennial cool-season grasses, which typically contain greater concentrations of WSC and other cellular contents, are more susceptible to damage by rainfall events compared to perennial warm-season grasses that have less potential for leaching. Unfortunately, susceptibility is clearly greater when forages approach a suitable moisture concentration for baling as dry hay.

A study further investigating the effects of natural rainfall on mowed/wilting alfalfa in Wisconsin is illustrated in Figure 33 (Coblentz and Muck, 2012). For this experiment, the emphasis was on silage conservation, rather than dry hay. Four different lots of alfalfa were mowed and sampled immediately, and then after 0, 0.5, 1.1 or 1.9 inches of natural rainfall. The 1.9-inch rainfall comparison included a prolonged exposure period of about 8 days under very poor drying conditions, while the total exposure periods for the other comparisons were only 1 to 2 days. Although conditions in each comparison were different, results suggest that the large concentrations of cellular contents within alfalfa forages clearly are a prominent factor in (indirectly) increased concentrations of NDF in response to rain damage. It must be noted that each comparison was terminated at a moisture concentration suitable for ensiling (54.1 to 65.6%), which was not dry enough to completely eliminate

Table 16. Effects of simulated rainfall on concentrations of acid detergent lignin (ADL) in orchardgrass and bermudagrass forages wilted to different moisture concentrations before the rainfall was applied [adapted from Scarbrough et al. (2005)]. Simulated rainfall was applied in half-inch increments at a rate of 3 inches/hour.

	Forage Moisture Concentration When Rainfall Was Applied					
	Orchardgrass			**Bermudagrass**		
Rainfall Amount	**67.4**	**15.3**	**4.1**	**76.1**	**40.0**	**13.0**
Inches	*ADL, % of DM*					
0.0	3.36	2.85	3.91	3.62	3.03	3.32
0.5	3.04	4.31	6.20	3.08	3.70	3.49
1.0	3.24	4.62	4.87	4.43	3.50	3.85
1.5	3.87	4.55	5.91	2.72	3.84	3.72
2.0	4.03	5.39	4.09	3.15	3.45	3.44
2.5	2.70	5.43	3.87	3.76	3.49	3.71
3.0	2.71	6.59	4.28	5.77	3.59	3.44

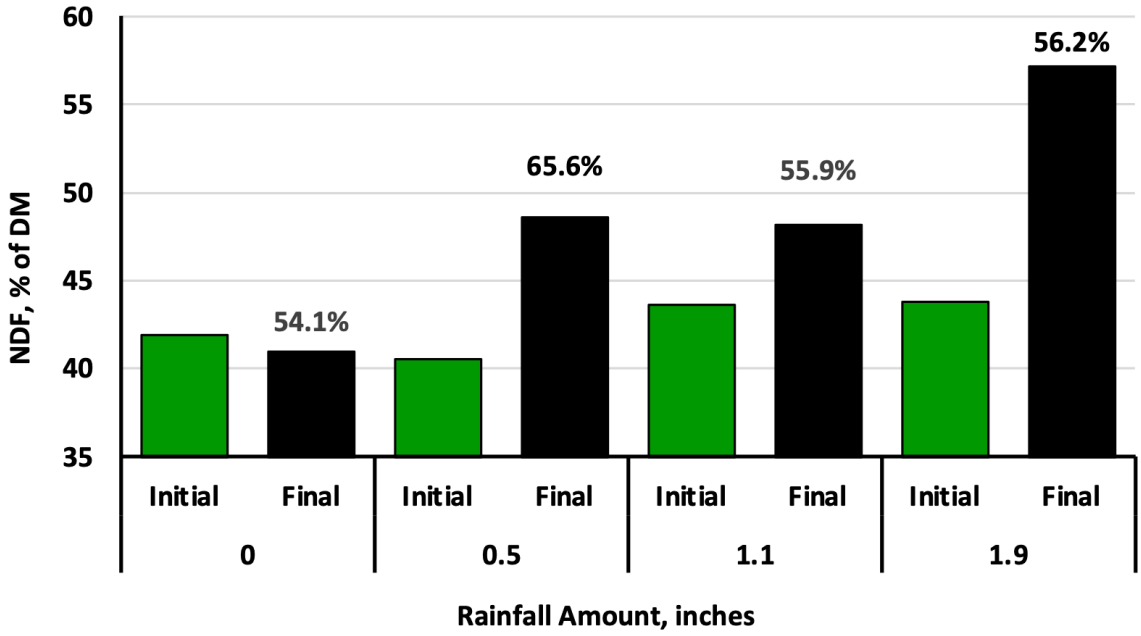


Figure 33. Comparisons of initial and final concentrations of NDF for different lots of alfalfa forages subjected to various increments of natural rainfall in Wisconsin. The 1.9-inch rainfall increment occurred over an 8-day exposure period, while the total exposure periods for the other comparisons ranged from 1 to 2 days. Experiments were conducted within a silage context, and data labels indicate the moisture concentration of the forage when the experiment was terminated [adapted from Coblentz and Muck (2012)].

respiratory losses of sugars. Furthermore, these studies were conducted with procedures that did not assess any subsequent swath manipulation that would be necessary to facilitate drying. However, any loss of leaves from the direct impact of rain droplets would be reflected in the results. The fragility of legume leaves adds a layer of complexity to evaluations of this type that is far less relevant for grasses. However, the amount of rainfall and exposure time during wilting appear to affect concentrations of NDF and other fiber components in rain-damaged alfalfa significantly.

Weathering of Hay Stored Outdoors

With the development of round balers, outdoor storage of large-round hay bales has become a common practice throughout areas of the country that are humid and/or receive significant amounts of rainfall. Numerous studies have evaluated the effects of weathering on DM losses and changes in nutritive value in response to outdoor (uncovered) weather exposure. A number of factors affecting the recovery of DM and nutrients within the hay have been identified, and include forage species, bale density, bale placement and orientation, various types of bale covers, bale binding type (usually, net wrap or sisal twine), as well as various options for disrupting hay/soil contact. Disruption of direct contact between soil and hay is widely understood to prevent wicking of moisture from the soil surface into the hay. Responses to these factors can vary widely and are likely situationally and climatically dependent. In response to outdoor storage, concentrations of fiber components typically increase, especially within the surface layer of the hay bales. Generally, wetting of the hay stored outdoors moistens the surface layer of the bales and activates microbial activity that utilizes cell contents (sugars) as a substrate. Concentrations of fiber components increase indirectly because fiber is largely inert with respect to this type of activity. However, in extreme cases when recovery of DM is very poor, fiber is likely lost as well. It is generally assumed that DM losses are greater in climates likely to incur greater rainfall and warmer ambient temperatures (Rotz and Muck, 1994). Conversely, freezing temperatures throughout the winter may restrict losses from weathering.

A 2 × 2 factorial study was conducted in Wisconsin that evaluated alfalfa-orchardgrass hays that were bound with either net wrap or sisal twine and elevated off the soil surface on wooden pallets or placed directly on the ground (Coblentz, 2009). For a comparison against an ideal storage system, a

group of positive control bales were bound with net wrap and stored under roof on wooden pallets. All bales were stored for 10 to 11 months (over winter) before a terminal sampling date the following spring. Concentrations of NDF and ADL within the 6-inch surface layer are summarized in Figure 34 for the two-year study. Generally, outdoor exposure increased concentrations of fiber components within the 6-inch surface layer but had little effect on fiber concentrations within the bale core (data not shown). While concentrations of NDF and ADL were greater within the surface layer for bales stored outdoors compared to control bales, the magnitude of these differences was small, and not always statistically significant. For this study, tying method and disruption of soil/bale contact had minimal effect on fiber components at the bale surface, demonstrating inconsistent statistical effects. While not definitive, these results are consistent with the premise that outdoor storage losses of DM or nutritive value are limited by cold winter conditions. Much greater deleterious changes have been reported when bales have been stored outdoors in wetter and warmer climates.

Spontaneous Heating in Hays and Silages

A common problem that occurs in the production of dry hay is the potential for spontaneous heating within hay bales, and it is well known that this typically occurs when forages are baled without adequate desiccation. The threshold level of desiccation that prevents spontaneous heating varies with the type of bale package and numerous other factors, but large bale packages (large-round or large-square bales) consistently require desiccation to lower (drier) moisture concentrations than small-rectangular (≤ 100 lbs) bales. Obviously, this can become problematic in humid and/or high-rainfall environments, which are most commonly observed east of the Mississippi River. When hays heat spontaneously, plant sugars are respired, primarily by microbes associated with the hay, yielding carbon dioxide and water. As carbohydrates are depleted, other plant components can be utilized, but at a slower rate (Rotz and Muck, 1994). Respiration of sugars results in increases in concentrations of fiber components, not generally through their additional formation, but indirectly via the oxidation of the most digestible plant components.

Within the heat increments that can be incurred within small-rectangular bales, changes in concentrations of NDF, ADF, hemicellulose, cellulose, and ADL are normally linear functions of heat, which can be measured as maximum internal bale temperature, average temperature, or in a heating-degree-day concept. One research study conducted in Arkansas with bermudagrass hays that illustrates these relationships is summarized in Figure 35 (Coblentz et al., 2000). Small-rectangular bales were made at forage moisture concentrations of 17.8, 20.8, 24.8, 28.7, and 32.5%, resulting in maximum internal bale temperatures ranging from 104 to 143°F. As a result, concentrations of NDF increased by > 7 percentage units in hays baled at 32.5% moisture compared to pre-storage concentrations (76.6 vs. 69.5%). A similar response was observed for ADL (5.59 vs. 2.62%). Considered across all bales, changes in NDF and ADL were strong linear functions of heating degree days

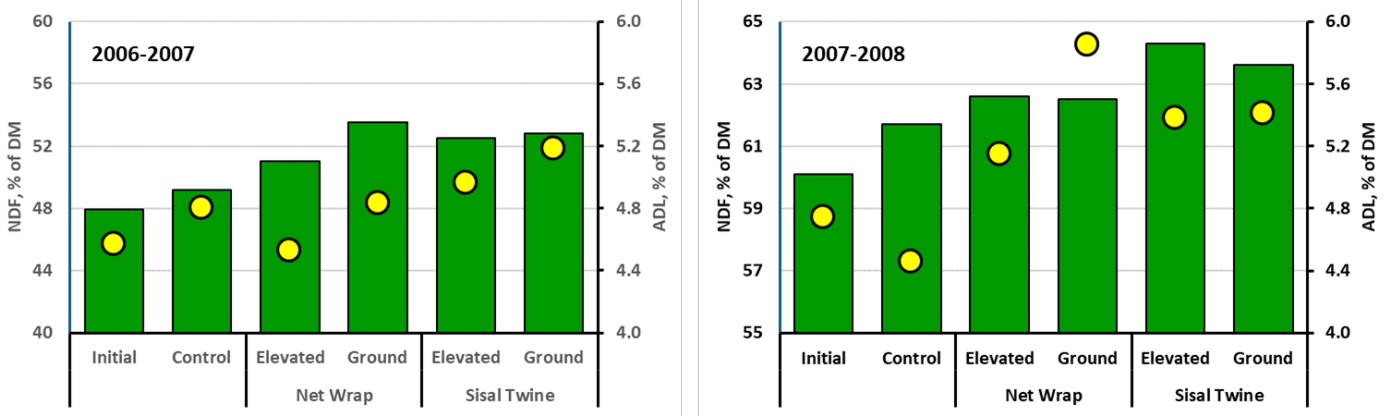


Figure 34. Concentrations of neutral detergent fiber (NDF) and acid detergent lignin (ADL) within the 6-inch surface layer of large-round bales of orchardgrass/alfalfa hay stored outdoors either directly on the ground or elevated on wooden pallets over winter in Wisconsin. Concentrations of NDF are represented by green bars (■), and ADL by yellow circles (●) [adapted from Coblentz (2009)].

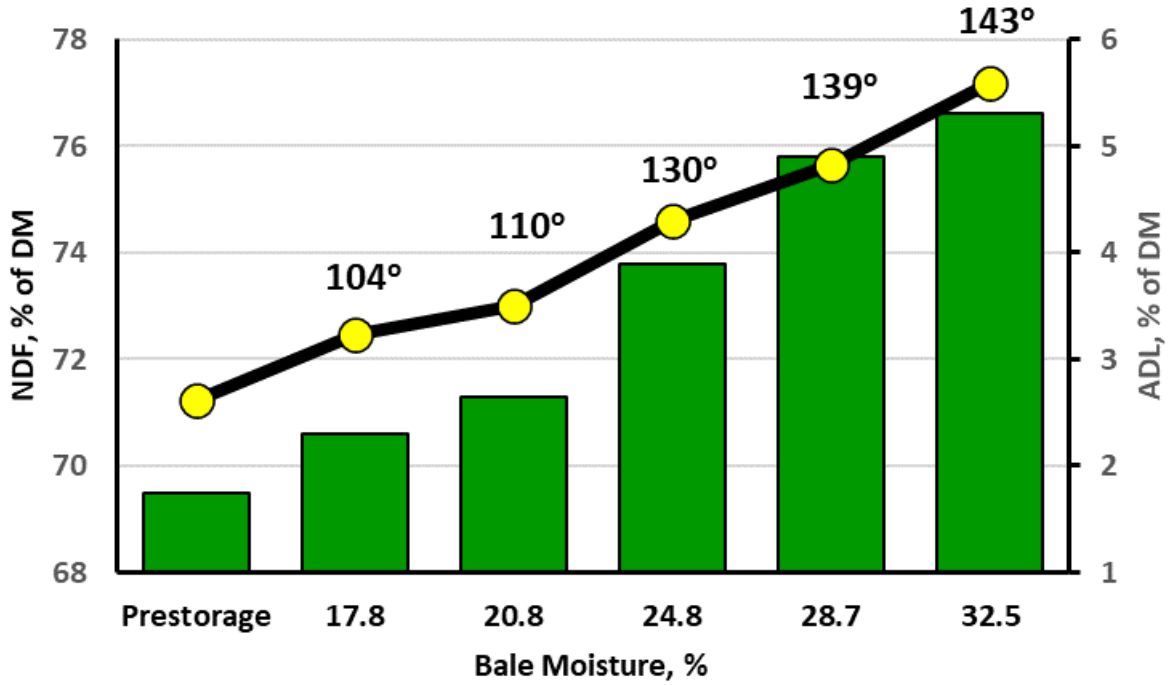


Figure 35. Concentrations of neutral detergent fiber (NDF) (■) and acid detergent lignin (ADL; ●) in small-rectangular bales of bermudagrass hay made in Arkansas as affected by the initial (pre-storage) moisture concentrations within the hay. Data labels indicate the maximum internal bale temperatures (°F) incurred during storage [adapted from Coblentz et al. (2000)].

(**HDD**), or maximum and average bale temperatures incurred during storage. For NDF, coefficients of determination (r^2) for these independent heating variables ranged from 0.940 to 0.952. Similarly, the relationships between ADL and these variables also were quite high (r^2 = 0.844 to 0.855).

Within large bale packages, spontaneous heating is often more severe, owing to greater amounts of DM packaged within each bale, and less surface area per unit of DM necessary to dissipate water and heat. Consequently, the relationships with spontaneous heating become more complex, typically losing linearity. A graphic illustration of this response is shown in Figure 36, where alfalfa-orchardgrass hays were packaged in large-round bales at differing bale diameters (3, 4, or 5 ft.) and moisture concentrations, thereby generating a wide range of spontaneous heating increments measured as HDD > 86°F (Coblentz and Hoffman, 2009). This metric can be interpreted as a single number that integrates both the intensity and duration of heating within the hay. As a practical frame of reference, 700 to 800 HDD > 86°F would be about the maximum that could be accumulated within a small-rectangular (60 to 100-lb) bale. Figure 36 illustrates that within that range (< 800 HDD) the change in NDF ($\Delta NDF = NDF_{post\text{-}storage} - NDF_{pre\text{-}storage}$) was essentially linear but reached a plateau at about 9 percentage units of NDF with greater heating increments.

In his popular textbook, Van Soest (1982) describes in some detail how forage fiber also can be involved in the formation of Maillard (heat-

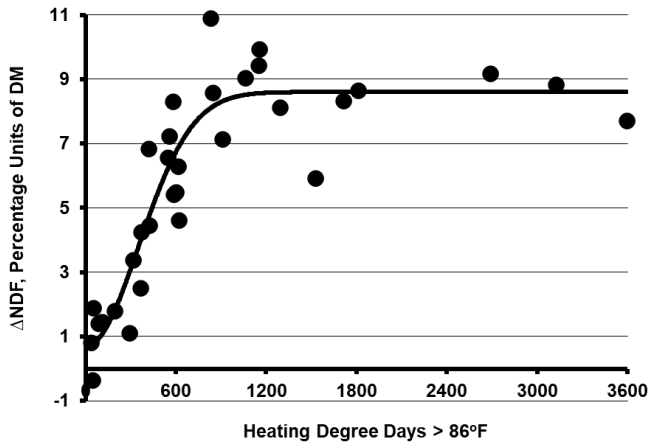


Figure 36. Changes in concentrations of neutral detergent fiber (NDF; $\Delta NDF = NDF_{post\text{-}storage} - NDF_{pre\text{-}storage}$) as affected by heating degree days > 86°F accumulated within alfalfa-orchardgrass hays packaged in Wisconsin at different moisture concentrations and at different bale diameters. The initial concentration of NDF was 46.5%, which corresponds generally to ΔNDF = 0 on the y-axis [adapted from Coblentz and Hoffman (2009)].

damaged) products. Carbohydrates degrade thermally by condensing with amino acids or amines, ultimately forming polymers possessing many of the chemical properties of lignin. Water is an important catalyst within the reaction; therefore, dry forages are less susceptible. While the carbohydrates involved in this process often are assumed to be soluble sugars, hemicellulose can be a reactive (structural) carbohydrate. Under extreme heating, concentrations of NDF can exhibit modest declines as its hemicellulose component contributes to the formation of Maillard products, which can be recovered subsequently as ADL. This response to spontaneous heating within forages (also referred to as non-enzymatic browning) renders forage proteins unavailable within the ruminant digestive system. Analytically, a quantitative assessment of heat-damaged forage protein is measured as the nitrogen (N) or protein (N × 6.25) bound within insoluble ADF residues (ADICP). All forages contain some naturally occurring or 'native' ADICP that is considered indigestible by ruminant livestock. The additional ADICP added through Maillard reactions also is largely indigestible but may retain some limited bioavailability.

Foundational work on the role of hemicellulose in the Maillard process was thoroughly investigated by Goering et al. (1973) using laboratory experiments in which ground orchardgrass forages were rehydrated to about 53% moisture, sealed in glass flasks, and then subjected to various heating regimes. In one study, rehydrated orchardgrass forage was heated to 176°F in 50 mL flasks for 0, 1, 2, 4, 8, 16, 24, 48, or 72 hours. Responses for concentrations of hemicellulose and ADICP (% of CP) as a function of time are summarized in Figure 37. Concentrations of both entities remained relatively stable through the 8-hour heating duration but changed sharply at longer time intervals. After 72 hours, hemicellulose had declined by about 19% from its initial concentration (18.2 vs. 14.8%), and ADICP had increased more than 8-fold (40.2 vs. 4.8% of CP). In a companion experiment, a similar relationship between hemicellulose and ADICP was observed when rehydrated orchardgrass forages were sealed in 10-mL flasks and heated for 24 hours at 104, 140, 176, or 212°F (Figure 38). These studies indicate that both time and temperature can have strong effects on non-enzymatic browning, which can include the reactivity of hemicellulose.

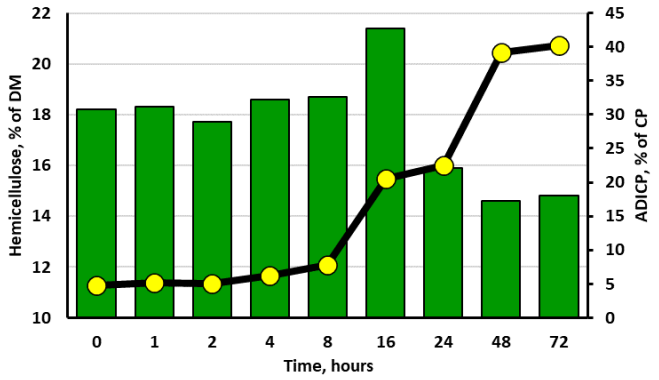


Figure 37. Effects of heating rehydrated orchardgrass forages at 176°F in 50-mL flasks for 0, 1, 2, 4, 8, 16, 24, 48, or 72 hours on concentrations of hemicellulose (■) and acid detergent-insoluble crude protein (ADICP, % of CP; ●), which illustrates the presumed reactivity of hemicellulose in Maillard (non-enzymatic browning) reactions [adapted from Goering et al. (1973)].

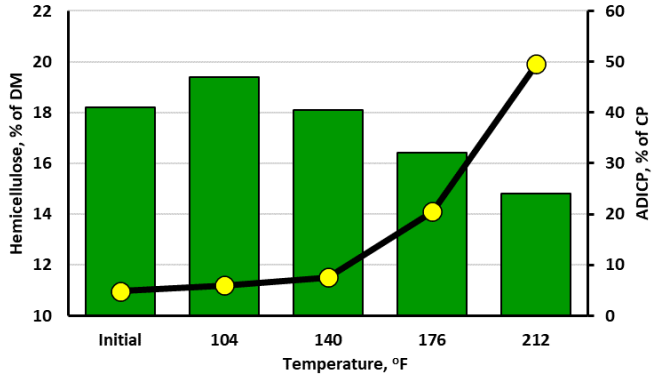


Figure 38. Effects of heating rehydrated orchardgrass forages at 104, 140, 176, and 212°F in 10-mL flasks for 24 hours on subsequent concentrations of hemicellulose (■) and acid detergent-insoluble crude protein (ADICP, % of CP; ●), thereby illustrating the presumed reactivity of hemicellulose in Maillard (non-enzymatic browning) reactions [adapted from Goering et al. (1973)].

Furthermore, these responses are relevant for hays and silages, both of which can attain internal temperatures within the ranges evaluated in these studies for extended periods of time (potentially months).

While these foundational studies formed the basis of what is known about Maillard reactions within hays and silages, the presumed reactivity of hemicellulose in these reactions is often less than obvious. For example, many studies conducted with small-rectangular hay bales indicate that concentrations of hemicellulose and ADICP often increase in parallel in response to spontaneous heating during bale storage. One such example of

this typical pseudo-parallel response can be viewed in Figure 39 for the bermudagrass hays referenced previously in association with Figure 35, where maximum internal bale temperatures ranged from 104 to 143°F. It should be noted that bermudagrass is a perennial warm-season (C4) grass that characteristically exhibits both large concentrations of hemicellulose (about 38%; Figure 39) and low concentrations of soluble sugars. Hemicellulose increased linearly over this range of heating at a rate of 0.12 percentage units per degree of maximum internal bale temperature (r^2 = 0.681). Similarly, ADICP (% of CP) increased linearly in very close association with maximum bale temperature (r^2 = 0.854). Results of this study suggested that increases in ADICP occurred mostly independent of hemicellulose throughout this temperature range, which is (potentially) inconsistent with the results of the foundational work of Goering et al. (1973) that were summarized in Figures 37 and 38.

The development and wide-spread use of large-round and large-rectangular balers results in the formation of bale packages that often exceed 1000 lbs, depending on characteristics of the forage and the specific baler design. These larger bale packages are less able to dissipate water and heat because of their reduced surface area per unit of DM. As a result, they are more susceptible to heating and can achieve greater maximum temperatures that persist for much longer time intervals than the responses for small rectangular bales summarized in Figure 39. A logical question might be whether the greater temperatures potentially achieved in large hay bales during storage might generate responses for hemicellulose, ADICP, and possibly ADL, that are consistent with the foundational work of Goering et al. (1973).

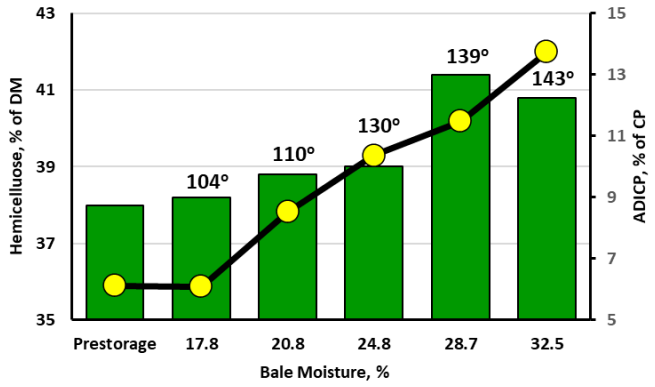


Figure 39. Concentrations of hemicellulose (■) and acid detergent-insoluble crude protein (ADICP, % of CP; ●) in bermudagrass hays baled at different moisture concentrations in small-rectangular bales in Fayetteville, AR. Data labels indicate the maximum internal bale temperature incurred during storage [adapted from Coblentz et al. (2000)].

To examine this question, return to the experiment described previously for Figure 36, in which large-round bales of alfalfa-orchardgrass hay were packaged at various moisture concentrations and at 3-, 4-, and 5-ft. bale diameters in order to generate different levels of spontaneous heating. In that experiment, maximum internal bale temperatures ranged from 104 to 171°F, and in some cases remained elevated for several months after baling. For this presentation, the 32 individual baling treatments are grouped on the basis of similar maximum temperatures for simplicity and clarity. The relationships between changes in concentrations of hemicellulose or ADICP (% of CP) during storage (Δ = post-storage – pre-storage) and maximum storage temperature are presented in Figure 40. In the foundational laboratory-based work of Goering et al. (1973), hemicellulose showed evidence of becoming reactive at temperatures > 140°F (Figure 38), which are easily attainable in poorly managed (stored) hays or silages. The experiment with large-round bales of alfalfa-orchardgrass hay exhibited a notably similar response, where Δ hemicellulose declined by nearly 5 percentage units in response to extremely high (> 144°F) internal bale temperatures. Concurrently, Δ ADICP (% of CP) increased sharply by about 16 percentage units of CP over the same heating interval. A similar relationship existed for ADL (Figure 41), which is where reactive hemicellulose can be recovered analytically (Van Soest, 1982).

Although somewhat circumstantial, these data suggest that the concentration of hemicellulose is likely to increase during storage of hays in response to relatively mild spontaneous heating. This likely occurs indirectly through preferential oxidation of other compounds, such as sugars. However, at heating increments largely unattainable in small-rectangular bales, hemicellulose becomes particularly reactive in hays, thereby contributing to rapidly elevating concentrations of both ADICP and ADL. Additional study is needed to firmly understand how the laboratory-based foundational work of Goering et al. (1973) relates to various production situations, but it would seem there is some agreement with the greater heating that can occur within large hay packages.

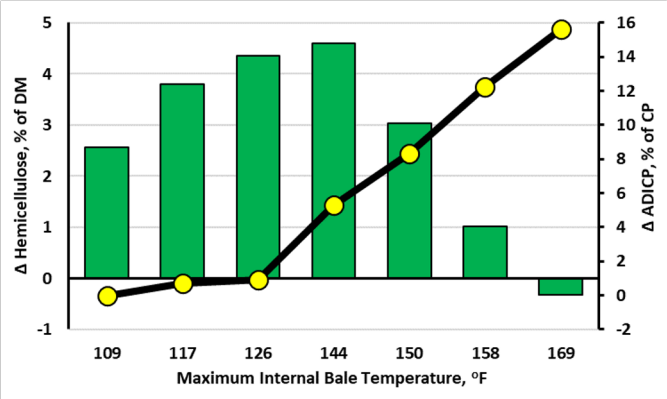


Figure 40. Changes (Δ = post-storage – prestorage) in concentrations of hemicellulose ( ) and acid detergent-insoluble crude protein (ADICP, % of CP;) as affected by maximum internal bale temperatures incurred during storage for alfalfa-orchardgrass hay packaged in large-round bales. Maximum bale temperatures for the 32 baling treatments were created by varying forage moisture and bale diameter and are grouped here on the basis of similar temperature responses for the simplicity and clarity of presentation [adapted from Coblentz and Hoffman (2009); Coblentz et al. (2010b)].

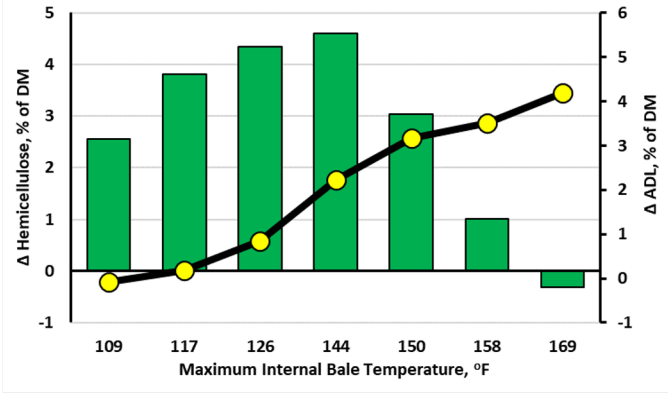


Figure 41. Changes (Δ = post-storage – prestorage) in concentrations of hemicellulose ( ) and acid detergent lignin (ADL;) as affected by maximum internal bale temperatures incurred during storage for alfalfa-orchardgrass hay packaged in large-round bales. Maximum bale temperatures for the 32 baling treatments were created by varying forage moisture and bale diameter and are grouped here on the basis of similar temperature responses for the simplicity and clarity of presentation [adapted from Coblentz and Hoffman (2009); Coblentz et al. (2010b)].

Silage-Related Issues

Much of the forage consumed by dairy cows in the United States has been fermented and stored previously as silage. Various processes associated with silage production have the potential to affect concentrations of fiber components within the final silage product offered to livestock. In most cases, even well-managed silage production can increase the concentrations of NDF, ADF, hemicellulose, cellulose, and ADL, primarily by indirect mechanisms. This occurs as a result of the DM losses sustained by other portions of the forage, such as plant sugars. Borreani et al. (2018) has provided practical estimates of these DM losses for silages produced with different levels of management (Table 17). Losses associated with rain damage, respiration during wilting, and leaf loss have been discussed previously. Once the forage has been transferred into a silo of various types (upright, bunker, pile, linear bag, or wrapped bale), DM losses occur primarily through four mechanisms:

- initial purging of oxygen from the silage mass, exacerbated by poor packing, delayed sealing, or other means by which oxygen has extended or continuing access to the silage,
- production of silage effluent,
- undesirable silage fermentations, and
- aerobic instability after the fermented silage is exposed to air.

Dry matter loss and concomitant indirect increases in concentrations of fiber components always occur with the initial purging of oxygen from the silage mass via respiration. In well-packed, quickly sealed silos, unavoidable DM losses of this

Table 17. Summary of dry matter (DM) losses (%) during different aspects of silage production as affected by rain and management (summarized and compiled from various sources[1] by Borreani et al. (2018)].

Item	Direct Ensiling	Field Curing, Respiration, Harvesting	In-Silo Respiration, Fermentation, Effluent	Top Spoilage, Feed Out
Good Management	1	4	5	3
Without Silo Cover	. . .	. . .	3	13
Sub-Optimal Management	3	11	13	18
Rain	. . .	5	. . .	. . .

[1] *Consult Borreani et al. (2018) for specific contributing citations.*

type are quite small, resulting in minimal increases in NDF, but losses can be greatly increased by poor management. An extreme illustration of these effects has been reported in the work of Bolsen et al. (1993), where alfalfa, corn, and sorghum silages were stored in covered (polyethylene) and uncovered production-scale bunker silos. Losses of DM were measured subsequently at 10-, 20-, and 30-inch depths, and were ≥ 77% at the 10-inch depth for all uncovered silages (Figure 42). Greater DM losses persisted for each uncovered silage type at deeper depths when compared against the covered bunker silos. While these studies emphasize the practical consequences of particularly poor silage management, they also clearly illustrate the effects of continuous oxygen access into the silage mass.

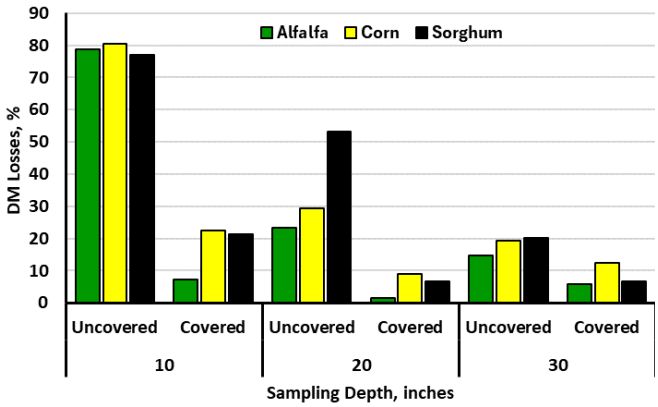


Figure 42. Losses of dry matter (DM) at three depths (10, 20, or 30 inches) for alfalfa, corn, and sorghum silages stored in bunker silos that were either covered with polyethylene film or left uncovered [adapted from Bolsen et al. (1993)].

Undesirable silage fermentations also have great potential for causing DM losses. Calculated losses associated with specific biochemical pathways and fermentation types have been compiled by McDonald et al. (1991; Table 18). Among these, the activities of clostridia, enterobacteria, and anaerobic yeasts are particularly serious, all theoretically exceeding a 41% DM loss. A practical example of the effects of (presumed) yeast-dominated fermentations is summarized in Figure 43, where fall-grown oat forages were baled and ensiled at the boot- and early-heading stages of growth on November 15th in central Wisconsin (Coblentz et al., 2015b). Results indicated that strong respective productions of ethanol (5.8 and 4.9%) were accompanied by sharp increases in NDF (≥ 6.7 percentage units), which is consistent with the expected large theoretical losses of DM for this fermentation type (Table 18).

Not all processes occurring within silage fermentation act to increase concentrations of fiber components in the final fermented silage. An early, laboratory-scale study examined the release of reducing sugars from hemicellulose using enzyme preparations isolated from perennial ryegrass, Italian ryegrass, and orchardgrass (Dewar et al., 1963). Enzyme activity was optimized in each case at pH = 6 and increased significantly over a 3-day period (24, 48, or 72 hours). Results of the study also provided some evidence of long-term release of reducing sugars, specifically by acid (rather than enzymatic) hydrolysis of hemicellulose at low (acidic) pH. Obviously, the increased concentrations of reducing sugars released from hydrolysis of hemicellulose can be beneficial, potentially providing additional

Table 18. Calculated dry matter (DM) losses based on various biochemical pathways for fermentation of sugars and organic acids as compiled by McDonald et al. (1991).

Fermentation Type	Primary Substrate(s)	Primary Product(s)	Calculated DM Losses, %
Homofermentative LAB[1]	glucose or fructose	(2) lactate	0
	(2) citrate	lactate, (3) acetate	29.7
	malate	lactate	32.8
Heterofermentative LAB[1,2]	glucose	lactate, ethanol	24.0
	(3) fructose	lactate, acetate, (2) mannitol	4.8
Clostridia	(2) lactate	butyrate	51.1
Enterobacteria	glucose	acetate, ethanol	41.1
Yeast	glucose	(2) ethanol	48.9

[1] *LAB, fermentation by lactic-acid producing bacteria.*
[2] *Heterofermentative LAB fermentations of citrate and malate are the same as calculated for homofermentative LAB.*

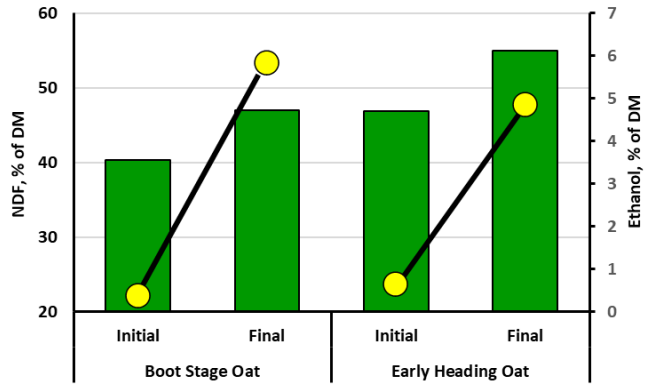


Figure 43. Initial and final concentrations of ethanol (yellow circles; ●) and NDF (green bars; ▮) obtained from baled fall-grown oat silages made at the boot- or early-heading stages of growth in central Wisconsin that depict the consequences of a yeast-dominated fermentation. Bales were ensiled on November 15th, and the terminal sampling date was on May 15th. Respective concentrations of ethanol designated as 'Initial' were obtained on December 13th and January 2nd [adapted from Coblentz et al. (2015b)].

substrate for silage fermentation. Enzymatic and/or acid hydrolysis of hemicellulose also reduces concentrations of both NDF and hemicellulose within the final fermented silage, and the effects of these processes work in conflict with the indirect effects of DM losses discussed previously.

Partially because abnormal DM losses are clearly reflective of poor silage management (see Figure 42), the effects of hydrolysis of hemicellulose on concentrations of fiber components are infrequently noted or discussed in research studies. Nevertheless, there are practical, field-scale examples of these effects. In Arkansas, Nieman et al. (2021, 2023) examined the effects of surface and sub-surface applications of poultry litter on the subsequent fermentation and quality of baled silages made from cool- and warm-season grasses. Primary forage species in the cool-season grass experiment were tall fescue, cereal rye, and annual ryegrass, while those included in the warm-season grass study were bermudagrass, barnyardgrass, and giant foxtail. Initial and post-storage concentrations of NDF, hemicellulose, and ADF for the two studies are summarized in Figure 44. In both experiments, concentrations of hemicellulose were reduced during silage fermentation; furthermore, these reductions were substantial, averaging 6.4 and 4.7 percentage units across the litter-application treatments in the cool- and warm-season experiments, respectively. It is noteworthy that concentrations of NDF also were reduced by similar amounts, but ADF was largely unaffected. Concentrations of ADF that are comprised primarily of cellulose and ADL increased only minimally (by < 1 percentage unit when averaged across both experiments), which is consistent with the expected effects of minimal DM losses in well managed silages.

A related aspect of this general concept can be initiated intentionally by using inoculants that exhibit enzyme activities that target fiber components, thereby potentially releasing fermentable substrate. A good example of this concept was conducted in Florida with bermudagrass conserved as baled silage at 47.5% moisture (Arriola et al., 2015). Bermudagrass is a perennial warm-season grass that typically exhibits very low concentrations of WSC, potentially compromising silage fermentation due to inadequate substrate. In

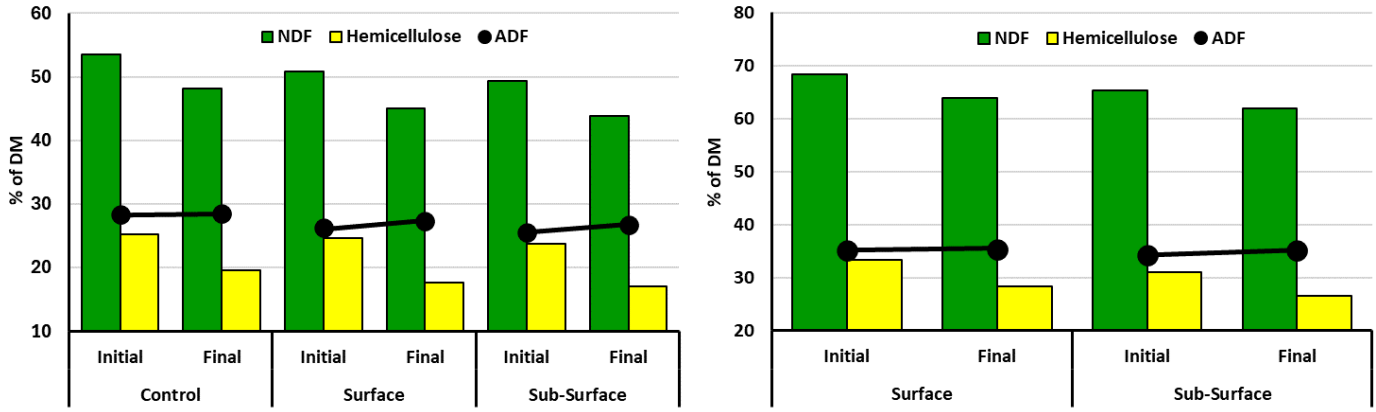


Figure 44. Concentrations of NDF, hemicellulose, and acid detergent fiber (ADF) in baled silages made from cool-season (left) and warm-season (right) grasses that depict probable enzymatic and/or acid hydrolysis of hemicellulose during silage fermentation. Treatments assessed the effects of surface and sub-surface applications of poultry litter during forage growth [adapted from Nieman et al. (2021, 2023)].

this study, bales were made without an inoculant (control) or with one of four commercial microbial inoculants, each of which also included various enzymes (cellulases, xylanases, etc.) designed to improve substrate adequacy. For clarity and simplicity of presentation, results for the inoculated silages are presented collectively for comparison against control silages (Table 19). Some evidence of release of substrate units from cell walls is suggested by the reduced concentrations of NDF and hemicellulose in inoculated silages, but the magnitude of differences between control and inoculated silages were not always great enough to draw clear statistical inference. However, use of these inoculants generally improved production of fermentation acids, resulting in a lower (more acidic) final silage pH.

Table 19. Effects of commercial inoculants on bermudagrass baled silages made at 47.5% moisture in Florida [adapted from Arriola et al. (2015)].

Characteristic	Control	Inoculated[1]
NDF, %	69.1	65.5
Hemicellulose, %	35.2	33.5
Lactic Acid, %	1.04	2.63
Acetic Acid, %	0.28	0.44
Lactic:Acetic Ratio	3.9	6.2
pH	5.37	4.77

[1] Collective means of bermudagrass baled silages inoculated with four different commercial inoculants. Detailed descriptions of the inoculants can be viewed in the original publication.

List of Abbreviations

ADF; *acid detergent fiber*. The fibrous forage or feedstuff residues remaining after extraction in acid detergent, typically including cellulose, lignin, residual ash, and some nitrogen (N). The assay can be performed directly on the ground sample or sequentially following a preliminary extraction in neutral detergent.

ADICP, *acid detergent insoluble crude protein*. Measurement of the nitrogen N associated with residual (ADF) fibers following extraction in acid detergent. It is the most commonly used indicator of heat damage to proteins in forages and other feedstuffs. This metric can be measured as N or crude protein (CP; N × 6.25) and subsequently expressed as either a percentage of dry matter (DM) or total CP in the sample.

ADL, *acid detergent lignin*. A measure of the lignin concentration within feed samples. The ADL concentration is determined by weight-change after removing cellulose from ADF residues with 72% H_2SO_4 and then burning off the lignin in a muffle furnace leaving only residual ash.

ANKOM. A system for batch fiber analysis of feeds using instrumentation and procedures developed by ANKOM Technology (Macedon, NY).

bmr, *brown midrib mutation***.** Genetic mutations (either naturally occurring or induced) within annual warm-season forages, such as corn or sorghum, that typically result in reduced concentrations of lignin, and associated benefits with respect to fiber digestibility.

CONV, *conventional fiber analysis procedures*. Traditional procedures for determining concentrations of various fiber components that feature extractions in neutral or acid detergent within dedicated vessels as described by Goering and Van Soest (1970) or Van Soest et al. (1991). This methodology is in contrast to the batch procedures of ANKOM Technology.

CP, *crude protein*. Determined by multiplying the N concentration of a sample by 6.25. This is a crude metric, as the total N pool of various feedstuffs include N-containing compounds that are not actually protein.

DM, *dry matter*. In most cases, concentrations of various nutritional metrics are (or can be) reported on a DM basis, implying all water has been removed from the reported concentration.

DMI, *dry matter intake*. Daily intake of DM by various livestock classes, which is often measured on a voluntary (ad-libitum) basis and analyzed as a response variable in research trials.

HDD, *heating degree days*. A single calculated number that represents the heat increment in hays or silages integrating the magnitude and duration of spontaneous heating during storage. The metric is based on the increment that daily internal bale or silage temperatures exceed a predetermined threshold. For hays, this threshold is usually set at 30 or 35°C (86 or 95°F) depending on location and expected local temperature norms.

N, *nitrogen*. Concentration of N within a forage or concentrate feedstuff.

NDF, *neutral detergent fiber*. The fibrous forage or feedstuff residue remaining after extraction in neutral detergent, typically including hemicellulose, cellulose, lignin, residual ash, and some N. The assay is performed directly on the ground sample, but often sequentially precedes determination of other entities, such as ADF or neutral detergent insoluble CP (NDICP). Various lower-case identifiers are frequently added to the NDF acronym, such as ‘om’, ‘s’, or ‘a’, thereby indicating the NDF assay was performed on an organic matter basis (i.e., corrected for ash content), or using sodium sulfite and/or heat-stable α-amylase during the extraction. The use of sodium sulfite is especially noteworthy because of its (negative) effect on recovery of N bound within NDF residual fiber.

NDF_{corr}, *NDF concentrations corrected for residual N or CP*. Throughout this publication, NDF_{corr} represents the concentration of NDF corrected for any residual N. The removal of N from residual NDF is important adjustment in certain energy calculations. NDF_{corr} also can be abbreviated as NDF_n in some publications.

NDFD, *neutral detergent fiber digestibility*. In-vitro or in-situ determinations of NDF digestibility, most commonly conducted with 48-hour incubations. Concentrations of NDFD are most commonly reported on a percentage of NDF basis.

NDICP, *neutral detergent insoluble CP*. Measurement of the nitrogen N associated with residual (NDF) fibers following extraction in neutral detergent. This metric can be measured as N or CP (N × 6.25) and subsequently expressed as either a percentage of dry matter (DM) or total CP in the sample. Inclusion of sodium sulfite within NDF extraction procedures solubilizes NDICP, reducing its recovery in any forage/concentrate analysis.

NFC, *non-fiber carbohydrate*. Comprised of sugars, starch, and other highly digestible components and calculated by difference as: 100% - [(NDF - NDICP) + CP + ether extract + ash]. The NFC is a key component of energy calculations carrying a digestibility coefficient of 0.98 within the summative model described in NASEM (2001).

NFE, *N-free extract*. An obsolete metric that is an artifact of the proximate analysis system and associated early estimates of energy as total digestible nutrients (TDN). It was calculated by difference as: 100% - [ether extract + crude fiber + ash + (N × 6.25)] and assumed to primarily represent highly digestible carbohydrates (such as sugars).

NIRS, *near-infrared reflectance spectroscopy*. A secondary method of forage and feedstuff analysis based on absorbance/reflectance of light throughout the near-infrared range of wavelengths.

OM, *organic matter*. Concentrations of OM are determined by subtracting ash from the total pool of DM within a sample (OM = total DM – ash), where the ash concentration is quantified by combustion in a muffle-type furnace at high temperature (500 to 600°C).

ROM, *residual organic matter*. Within the NASEM (2021) nutritional model, ROM represents a fraction (including sugars) determined by difference that largely replaces NFC in the NASEM (2001) model; however, it does not include starch, which is analyzed separately. The ROM fraction carries a revised digestibility coefficient of 0.96.

TDN, *total digestible nutrients*. A summative measure of energy based on true digestibilities of various feedstuff components. Formulas have been refined over time, particularly after the change from the proximate analysis system to one based on Van Soest concepts of fiber analysis.

tdCP, tdFA, tdFiber, tdNFC. Truly digestible forage/feedstuff components included in the summative equation for TDN described within the NASEM (2001) nutritional model.

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