A 100-Year Review: Carbohydrates—Characterization, digestion, and utilization¹

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ABSTRACT

Our knowledge of the role of carbohydrates in dairy cattle nutrition has advanced substantially in the 100 years of the publication of the *Journal of Dairy Science*. In this review, we trace the history of scientific investigation and discovery from crude fiber, nitrogen-free extract, and “unidentified factors” to our present analytical schemes and understanding of ruminal and whole-animal utilization and effects of dietary carbohydrates. Historically, advances in research and new feeding standards occurred in parallel with and fostered by new methods of analysis. The 100 years of research reviewed here has bequeathed to us an impressive legacy of information, which we will continue to grow.

Key words: carbohydrates, digestion, utilization, 100-year review

INTRODUCTION

In preparing this review on the role of carbohydrates (CHO) in the nutrition of dairy cattle, we were fascinated and humbled by the full scope of work done before and after 1917 that helped to set the stage for our current understanding and use of insoluble fiber and nonfiber CHO (the CHO not included in insoluble fiber). The seeds of most, if not all, of the concepts about CHO that we currently use for dairy cow nutrition were discovered and planted early, and research in the *Journal of Dairy Science* (JDS) served to grow and prune them (Appendix Table A1; Figure 1).

Our knowledge about the characterization, digestion, and utilization of dietary CHO has changed dramatically during the 100 years that JDS has been published. Among the nutrients, CHO are unique because they have both digestive and physiological roles. They have the broadest range in digestibility of any nutrient, rang-

ing from 100% for sugars to 0% for indigestible fiber and they, with lignin, typically make up the greatest portion of diets (70 to 80%). The rumen evolved to selectively retain fiber for optimal utilization; both the chemical and physical attributes of CHO affect rumen function, the pattern of ruminal fermentation, and ultimately the metabolism and production of the dairy cow. Thus, nutritional characteristics of CHO have a tremendous impact on digestible nutrients and net energy. The accuracy of the characterization of CHO for their varied effects similarly has greatly influenced the utility of research, nutrient recommendations, and diet formulation for dairy cows.

Papers published in JDS are products of their time. The journal began in the era of feeding recommendations by Armsby (1917), Haecker (1913), Henry and Morrison (1915), and Savage (1913). Although researchers knew about the diversity of CHO, the most commonly reported fractions were crude fiber (CF) and nitrogen-free extract (NFE). There was some discussion of net energy, but “total digestible nutrients” was the primary “energy” descriptor used for diets. It was not until Moore et al. (1953) unequivocally demonstrated that the relationship of TDN to estimated net energy overvalued low-TDN feeds such as forages that the need to change methods of evaluating feeds, including CHO analyses, became indisputable. During the 100 yr that JDS has published, we moved from a dairy NRC (1945) in which CHO were not mentioned to the most recent dairy NRC (2001) in which a whole chapter is devoted to CHO. Such change is reflective of the changing research information and concepts that were available. Reid (1956), in an excellent review for the 50th anniversary of the American Dairy Science Association, noted at the time that the chemical nature of nutrient groups was better understood than their biochemical, physiological, and pathological effects.

In the early years of JDS, methods were often not very well described; for example, “The proximate constituents... were determined by the recognized methods” (McCandlish, 1920). As more analyses became available and procedures were better defined, method descriptions improved. In the early years, papers gave

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Figure 1. A timeline showing the periods in which different analyses were used (see also Appendix Table A1). CF = crude fiber, CHO = carbohydrate, F:C = forage-to-concentrate ratio, IVNDFD = in vitro neutral detergent fiber digestibility, MN = microbial nitrogen, NFE = nitrogen-free extract.

no description of statistics performed or significance of results: in 1917 when JDS began publication, Fisher's (1925) and Snedecor's (1937) seminal works on statistics were not yet published. Researchers were limited on the chemical and statistical analyses that they could perform. Historically, advances in research and new feeding standards occurred in parallel with and were fostered by new methods of analysis. This simply illustrates that research progress relies on analytical measurements that allow description of biological processes of interest.

Where work with CHO started and how it evolved in JDS is a function of the base of information available and the introduction and evaluation of new insights and methods. Our goal was to review the publications in JDS that had significant impact and were milestones in the characterization, digestion, and utilization of CHO. In some instances, we will discuss research work published in other journals to provide perspective and context.

CHEMICAL CHARACTERIZATION OF CARBOHYDRATES

Insoluble Fiber

Although methods have changed, the concept of nutritional, insoluble fiber has been relatively constant for

nearly 200 years as a feed fraction that was indigestible or resistant to digestion. That nutritional fiber is an empirical measurement is not a failure of understanding or technique, but the result of attempting to measure the nutritional concept of "fiber" using chemical solubility or bioavailability methods. Accordingly, measurement of fiber is defined *only* by the method of analysis, which in turn places extra burden on analysts to follow the method exactly to produce results reflective of the empirical analyte of interest. For ruminants, nutritional, insoluble fiber can be defined as "the slowly digesting or indigestible organic matter of a feed or diet that occupies space in the rumen" (adapted from Mertens, 1997). This is essentially a definition of insoluble fiber that relates to the original desire to measure the indigestible fraction of feeds, with the recognition that slowly digesting matter is resistant to digestion, and that fiber represents the "filling effect" of diets.

The fiber method in place in 1917 was CF, which was developed by Henneberg and Stohmann (1860, 1864) to describe indigestible material in the feed and was part of the Weende or proximate system of analysis. However, Tollens (1897) found that CF was partially digested by ruminants and did not capture all lignin, and there was a variable distribution of lignin and CHO between CF and NFE. Since its inception, the proximate analysis system was considered imperfect and provisional, with Henneberg and Stohmann (1864) and Tollens (1897)

recommending that CF and NFE analyses could be used until better methods were found. Summing up the situation of feed analysis at the time JDS began publication, with no better practical methods available, Henry and Morrison (1915) offered, "By the present method all plant substances are grouped under the terms crude protein, fiber, nitrogen-free extract, and fat, without regard to the differences in composition and feeding value of the different individual proteins, carbohydrates and fats which make up these classes. In many particulars this is unsatisfactory. In time chemists will work out a more accurate, tho necessarily more complicated, classification, but at present for the great majority of feeds there is nothing better than what is given here."

The early work in JDS focused on CF and NFE as contributors to TDN, but there were also efforts to find alternatives to proximate measures. The first mention of CHO in feedstuffs in JDS is found in volume 1 by Woll (1918), who listed "Digestible carbohydrates and fat" in a table, with CHO reflecting the sum of CF and NFE multiplied by tabular digestion coefficients for each feed. Bennett (1936) reported the first detailed feed analysis in JDS that included CF and NFE, but also lignin, cellulose, hemicelluloses, pentosans, starch, sucrose, and reducing sugars for pasture grass. Despite efforts to use better analyses than CF and NFE, in a review about roughage quality (Huffman, 1939), only total sugars, reducing sugars, CF, and NFE were summarized in tables, suggesting that little data were available for the other analyses. Attempts were made to develop alternative analyses for cellulose (Crampton and Maynard, 1938), lignin (Norman and Jenkins, 1934a,b; Crampton and Maynard, 1938; Ellis et al., 1946), and pentosans (hemicelluloses; AOAC, 1907). A limitation of the Crampton and Maynard (1938) system was that hemicelluloses were lumped together with sugars, starches, pectins, and organic acids in the "other" CHO fraction. Comparison of methods of lignin analysis that used 72% sulfuric acid showed potential CHO and nitrogen (N) contamination of lignin and the harmful effects on lignin measurement of drying samples at >60°C (Thomas and Armstrong, 1949).

Ely et al. (1953a) initiated an extensive analysis of CF, NFE, and lignin (Ely et al., 1953b) with the goal of all components summing to 100% of DM. Their analyses included sugars, starch, organic acids, pentosans, celluloses, lignin, undetermined, CF, and NFE. They also calculated several fractions, including holocellulose_{calc} [(CF + NFE - lignin) - starch - sugars], which should contain cellulose and hemicellulose. The sum of (pentosan + celluloses) was 10 percentage units of DM less than holocellulose_{calc}, which was about the magnitude of the undetermined fraction. This differ-

ence should contain pectic substances and noncellulose hexosans. Ely et al. (1953a) concluded that similarity of apparent digestibility for celluloses, pentosans, and holocellulose_{calc} could justify grouping them into an analytical holocellulose fraction. In a series of papers, Ely and Moore (1955a,b, 1956) attempted to develop an analysis for holocellulose directly. However, when it was found that the same method could not be used on feeds and feces, the method was abandoned. Other analytical schemes for measuring CHO in plant materials were offered (e.g., Gaillard, 1958; Smith, 1969), but were not extensively used for dairy cattle nutrition.

In 1957, Peter Van Soest joined the USDA-Agricultural Research Service at Beltsville, Maryland, which was the same location where Ely was stationed, and he continued the quest to measure the indigestible matter in feeds. At that time, Lane Moore assembled a research group at Beltsville that included Bill Flatt, Peter Van Soest, Marv Bryant, Bill Thomas, and Dale Waldo, all research award winners. Aware of the problems in measuring holocellulose and lignin, Van Soest began work with acid detergent extraction to reduce the protein and CHO contamination of lignin.

The initial methods for ADF and acid detergent lignin using sulfuric acid (ADL; Van Soest, 1961) and for NDF (Van Soest and Marcus, 1964) were published as abstracts in JDS. The papers describing the logic and process for the development of detergent fibers (Van Soest, 1963a,b, 1965a; Van Soest and Wine, 1967, 1968) are recommended reading for anyone interested in fiber and method development. In 1973, ADF and ADL became "official methods" of the Association of Official Analytical Chemists (AOAC) based on the results of a collaborative study (Van Soest, 1973). The development of the detergent fiber methods represented a revolutionary change in how feeds would be characterized. They largely avoided the issues of CF and NFE of indigestible lignin being found in more than one fraction. Further improvements with the incorporation of heat-stable α -amylase reduced interference from starch (Van Soest et al., 1991). A further refined and standardized method for NDF became an AOAC official method (Mertens, 2002). The relatively rapid replacement of CF by detergent analyses indicates that researchers and nutritionists were ready for a change.

In a symposium paper, Van Soest (1964) made it clear that his initial analytical goal was to measure lignin as the truly indigestible portion of forages (what CF was supposed to be). He reported that the recovery of ADL in total collection digestion trials was close to 100% and, when used as a marker, it obtained better correlations with total collection digestibilities than did chromic oxide. He discussed the difficulty both in obtaining lignin that was not contaminated with pro-

tein and in identifying the manner in which it affects digestibility. He demonstrated that ADL was correlated with DM digestibility (**DMD**), but the relationship was different for grasses and legumes. However, when expressed as fiber digestibility, the ratio of lignin to fiber was similar for grasses and legumes (Figure 2 in Van Soest, 1964). It is interesting to note that ADF was not discussed in this paper, which may be due to his recognition that ADF was a preparatory step in the measurement of lignin, and was never intended to be a measure of total insoluble fiber because hemicellulose was dissolved in acid detergent.

The measurement of indigestible lignin was important, but Van Soest's (1965b, 1967; Van Soest and Moore, 1965) greater contribution was the hypothesis that uniform nutritional availability, rather than chemical purity, was the most important criterion for developing methods to measure total insoluble fiber. He postulated that a clear definition of nutritional fiber should take the place of a primary standard in developing analytical methods for fiber. He observed that extractable cellular contents of forages (proteins, lipids, sugars, starches, pectin, and other neutral detergent-soluble CHO) had uniform and nearly complete (98%) nutritional digestibility, and that NDF (lignin, cellulose, and hemicelluloses) were indigestible or only partially available. Note that pectin, which is a component of plant cell walls, is not included in NDF. Thus, NDF does not include all of the cell wall constituents. Likewise, the detergent system does not separate its soluble and insoluble fractions by the classic definition of fiber applied to simple-stomached animals: "the fraction of the feed or diet that cannot be hydrolyzed by mammalian enzymes." Although NDF fits this definition, neutral detergent-soluble CHO contains pectins, fructans, galacto-oligosaccharides, and other CHO that are not digestible by mammalian enzymes (soluble fiber), as well as starch and sugars, which are.

The first paper in JDS to report ADF and ADL was that of Simkins and Baumgardt (1963), who reported that ADL was better than other laboratory assays (in vitro cellulose or DM digestion, "digested laboratory nutrients" or ADF) in predicting in vivo DMD. When hay-crop and other forages were separated, the correlations of ADL to in vivo DMD were -0.84 and -0.78 , respectively. Waldo et al. (1965) published the first trial in JDS that reported detergent fibers and their in vivo digestibility.

Alternate methods to evaluate insoluble fiber continue to be explored, such as monomeric analyses of NDF, cell wall, and neutral detergent solubles, which provided information about the composition and digestibility of fiber and nonfiber components. (Wedig et al., 1986; Miron et al., 2002; Jung et al., 2011). Cell

wall or total monosaccharide analysis is a laborious and expensive analysis that varies in technique and monomer recovery among analysts. It is unlikely to compete with detergents for routine analysis, but does provide research information for investigating the effect of fiber composition on digestibility, especially when done in conjunction with the routine detergent assays. Unfortunately, detailed analysis of monomers in fiber does not provide information about the indigestibility of these components, which is a key to nutritional relevance.

Nonfiber Carbohydrates

In the last 100 yr, CHO not included in insoluble fiber, commonly referred to as "nonfiber CHO," has been even more problematic than insoluble fiber to describe in analytically feasible and nutritionally relevant terms. As NFE in the proximate analysis system and nonfiber CHO (NFC) in the detergent fiber system, the most digestible CHO were estimated by difference as $(100 - \text{moisture} - \text{CP} - \text{CF} - \text{insoluble fiber} - \text{ash})$, with insoluble fiber represented by CF or NDF. This "by difference" fraction is a repository for all CHO not in the insoluble fiber, feed components not included in the analyzed fractions, and analytical error. For NFE, the issue of analytical errors accumulating in NFE calculated by difference was known (Tollens, 1897), and the same problem applies to calculated NFC. The method for NFE originated with Henneberg and Stohmann (1864), though they accounted for crude fat by pre-extraction. A reason that NFE was called an "extract" rather than "carbohydrate" was the recognition that it included non-CHO compounds such as lignin, tannic acids, glycosides, gums, alcohols, organic acids, and alkaloids, in addition to hexoses, cane sugar (sucrose), di- and trisaccharides, starch, glycogen, dextran, inulin, mannans, galactans, mucins, gums, pectic substances, glycosides, pentoses, and pentosans (Tollens, 1897). Digestibility of NFE was not uniform among its constituents, with a portion of it reported to be indigestible (Henneberg and Stohmann, 1860). Stone (1897) was blunt about the problems with NFE, "... it is now generally recognized that that portion of food material broadly included under the term "nitrogen-free extract," consists of a considerable number of definite chemical compounds, mostly of the nature of carbohydrates, but of such evident variation as regards their food value or digestibility, as well as their chemical and physical properties, that it is highly inconsistent, if not absurd, to continue to regard them as of homogenous character or worth in food valuation." The major reason that NFE continued to be used from 1860 through the 1960s was the lack of viable alternatives to account for the nonfiber CHO. Alternative methods for measur-

ing specific CHO in NFE (e.g., Stone, 1897; Horwitt et al., 1936) required sequential or parallel extractions that could take more than 24 to 44 h, not including the time spent preparing the samples and measuring the products. At a 1940 AOAC symposium on NFE, it was suggested that extensive changes not be made to the analyses of CF and NFE for routine feed analysis because of the complicated and time-consuming nature of the alternative assays, although researchers should use the latest improved methods (Browne, 1940). Efforts to improve measurement of CHO and remove lignin from the noncellulose CHO included methods proposed by Crampton and Whiting (1943) and Wilcox and Moxon (1949), but these never fully displaced the proximate analysis system.

The first mention of NFE in JDS came in a paper by Shaw and Norton (1920), but they also used AOAC methods to report furfurals (pentosans), starch, total sugars, and invert sugars in fresh and ensiled corn to attempt to better characterize NFE CHO. In a later effort to determine the effect of weather on forage quality, Archibald (1961) used the Lane-Eynon reducing sugar assay to show that the amounts of sugars in green forages were negatively correlated with air temperature and rainfall occurring before harvest. This was a forerunner of work that evaluated the relationship between climatic conditions and digestibility of fiber (Van Soest et al., 1978).

That new assays for the nonfiber CHO were not developed and widely adopted from 1917 through the 1970s is because analysts lacked many tools that are now available. In 1917, most assays relied on partitioning CHO based on their solubility in ethanol, water, and alkali, on hydrolysis with acid or crude plant extracts, followed by detection with polarimetry or titration. Takadiastase (Takamine, 1894), an enzyme preparation that could hydrolyze or synthesize starch with limited hydrolysis of other CHO (Southgate, 1976), was one of the few commercial purified carbohydrases available. Chromatography was performed with paper, not columns. As is still the case today, broad-spectrum colorimetric assays for CHO such as anthrone (Morris, 1948), phenol-sulfuric acid (Dubois et al., 1956), or reducing sugar assays (Southgate, 1976) could not accurately differentiate and measure specific CHO when they were in the mixtures present in feed extracts.

As technology changed, methods changed. The first magnetic stirrer was patented in the 1940s (Rosinger, 1944), reducing the time required to perform nonrefluxed extractions. Starting in the 1960s, purified enzymes became increasingly available and could be used to hydrolyze specific CHO and catalyze reactions for their detection. This allowed for specific measurement of starch (Thivend et al., 1971) and of glucose (Trinder,

1969). The 1950s saw the invention of gas chromatography using columns and detectors (James and Martin, 1952), with high performance liquid chromatography being developed during the 1960s and 1970s (e.g., Huber, 1969). The more refined and rapid chromatographic methods allowed for specific, sensitive detection of CHO and fermentation products. From the 1980s on, development of a variety of analyses, analytical systems, and refinements of existing methods allowed determination in animal feeds of sugars (Larsson and Bengtsson, 1983; Bach Knudsen, 1997), fructans (Bach Knudsen, 1997), soluble fiber (Prosky, et al., 1992; Hall et al., 1999), and starch (Karkalas, 1985; Bach Knudsen, 1997; Hall, 2015) with fewer interferences and artifacts. Starting in the late 1950s and early 1960s, the availability of computers for analyzing data and solving equations also provided needed tools to advance CHO research.

Although several methods are available, we do not yet have the final word on how nonfiber CHO should be analyzed to be most relevant in the nutrition of dairy cows. In the most highly cited paper on CHO in JDS (10,061 citations per Web of Science; accessed May 21, 2017), Van Soest et al. (1991) stated, "The more readily digestible carbohydrates in animal feeds lack a satisfactory system of classification. However, they are the major energy yielding components of feedstuffs. This lack of definition arises from their diversity and from the relative lack of basic research into their specific nutritive characteristics . . . they include sugars, starches, fructans, galactans, pectins, β -glucans, etc." Only in the last 10 to 20 years, have we made appreciable progress in measuring and fractionating the nonfiber CHO for the purposes of dairy cattle nutrition. The relevance of the available nonfiber CHO analyses to animal performance and practical application remains to be fully tested.

PHYSICAL FORM: CHARACTERIZATION AND IMPACT

Insoluble Fiber

From early in their evaluation, feeds were classified into roughage and concentrate based on their coarse or fine texture and fiber concentration. It was recognized that roughages contained less available energy, but they were useful, if not necessary, for cow health and productivity. Powell (1939) reported that milk fat in dairy cows could be varied by as much as 60% by regulating physical characteristics and intake of roughage. At the time, relationships between fiber, physical form, and milk fat percentage were controversial. Pursuing this work, Tyznik and Allen (1951) showed that decreasing roughage allowance reduced milk fat production, and

Porter et al. (1953) found that ground and pelleted alfalfa resulted in lower milk fat percentage than did chopped or unchopped alfalfa. Although not fully appreciated at the time, roughage affects chewing activity and the biphasic nature of ruminal contents. Balch (1971) and Sudweeks et al. (1981) suggested that total chewing time per kilogram of DM would be a quantitative measure of roughage value.

Fiber characteristics affect much more than just milk fat. Particle size of fiber affects its passage rate (Moore, 1964), the amount of chewing needed to reduce particle size, and the rumen environment (Welch, 1982). Smith and Waldo (1969) and Waldo et al. (1971) developed methods (vertical shaking) for measuring the particle size of fiber useful in describing and modeling particle size and its reduction (Smith et al., 1983). Lammers et al. (1996) noted that forage particle size can affect ruminal function (pH and VFA patterns), milk fat percentage, and passage from the rumen, and described a simple on-farm method for forage particle length (horizontal shaking). The concept of physically effective fiber (peNDF) was developed as a way to combine physical and chemical characteristics of fiber that could be used to meet the roughage or minimum fiber requirements for lactating cows (Mertens, 1997). Physically effective fiber was shown to affect animal behavior and ruminal pH (Beauchemin and Yang, 2005). A recent meta-analysis suggests that dietary characteristics of physical form needed to maintain a desired ruminal pH may be predicted with equations that include dietary characteristics such as starch content and digestibilities of NDF and starch, and in which TMR particle size and NDF content are treated as separate factors (White et al., 2017).

Particle size and fiber not only affect the minimum fiber requirement of lactating cows, but they also have the potential to alter intake and digestibility. Porter et al. (1953) first reported sorting differences with baled, chopped, ground, or pelleted alfalfa, as well as lower intake with hard pellets. When rumen fill limits intake, reducing the fiber particle size should reduce fiber volume and increase fiber passage, thereby increasing intake. However, increasing passage can reduce fiber digestibility. Fine processing of corn silage (0.95-cm theoretical length of cut) decreased in vivo NDF digestibility (NDFD; 0.284) compared with the control (0.339) (Bal et al., 2000).

Nonfiber Carbohydrates

Starch is a major nonfiber CHO in diets of lactating cows for which particle size and gelatinization can have a significant role its digestion and utilization. Particle size for grains is most commonly evaluated by dry siev-

ing using a series of sieves in a vertical shaker (e.g., Cook et al., 2016). Atkeson and Beck (1942) were the first to publish in JDS that fine grinding improved the digestion of sorghum grain, although they noted that most grinding studies for grains were reported in experiment station bulletins between 1898 and 1936. Evans and Colburn (1967) found that in situ digestibility NFE increased from 0.60 for cracked corn to 0.72 for ground corn. The dairy NRC (2001) included a "processing adjustment factor" that was intended to describe the effect of processing on NFC digestibility, even though the focus was on starch, not total NFC. More recently, Hoffman et al. (2012) suggested that particle size is a primary determinant of corn grain digestion and that fermentation during storage effectively reduces particle size by granule and protein matrix disruption. Using meta-analysis, Ferraretto et al. (2013) reported that in vivo starch digestibility decreased from 0.933 to 0.777 when mean particle size increased from an average of 750 μm to 3,750 μm . The processing of corn silage to maintain fiber particle length but disrupt grain has been shown to improve starch digestibility, with processing kernels in corn silage improving in vivo starch digestibility (0.993) compared with unprocessed (0.951) (Bal et al., 2000). Characterizing particle size of kernels in corn silage as a means of detecting potential differences in starch digestion continues to be studied (Dias Junior et al., 2016).

For water- or neutral detergent-soluble CHO other than starch, there is little clear evidence that particle size affects their digestion and utilization, unless perhaps for CHO that are entrapped in a larger, insoluble particle that limits access by microbes or enzymes.

CARBOHYDRATE DIGESTION AND PRODUCTS

The primary purpose of feed characterization is to estimate the effects of the feed fractions on nutrient supply and other factors that affect animal performance. Although chemical and physical characterization of CHO provides useful nutritional information, they are one step removed from this goal. Direct in vivo measurement of CHO digestion and nutrients supplied is the gold standard for determining nutritive value, but in vivo digestibility of nutrients is not a constant intrinsic property of the CHO in feeds—it can vary with species, intake level, measurement method, and ration ingredients. Donefer (1966) and Barnes (1968) reported significant variation in the in vivo measurement of digestibility for the same forage. Volatile fatty acids and microbial mass from ruminal fermentation and glucose from small intestinal digestion of starch are the main products of CHO that provide nutrients to the cow.

Insoluble Fiber

In early research trials, digestibilities were not measured; values for proximate analytes were usually multiplied by tabular digestion coefficients to calculate TDN (e.g., Woll, 1918). First reports of measured CHO digestibility in JDS were by Mead and Goss (1935), who reported the *in vivo* digestibilities of NFE (0.85) and CF (0.38) in a no-roughage, barley grain-based diet. Cave et al. (1936) reported the *in vivo* total-tract digestibilities of CF (0.446) and NFE (0.617) in thistle hay. Researchers subsequently attempted to separate ruminal and total-tract digestibilities of CHO. Hale et al. (1940) were the first to report the measurement of both rumen and total-tract digestibilities of CHO and lignin. They used iron oxide and lignin as markers and showed that both the cellulose and “other carbohydrates” in alfalfa were primarily digested in the rumen, but with some digestion occurring postruminally.

Recognizing that the rumen was the primary location of digestion for most CHO, interest in understanding rumen function grew as researchers worked to understand the nutritional value of feeds for meeting animal requirements. This led to development of *in vivo* and *in vitro* ruminal techniques and investigations starting in the late 1930s. Time-course *in vivo* investigations with ruminally cannulated cows demonstrated how ruminal pH changed over the course of the day, declined after feeding, differed by feed offered, and differed by sampling location in the rumen (Monroe and Perkins, 1939). The first JDS report on counts of ruminal microbes and cellulolytic activity was published in 1947 (Gall et al., 1947). Bryant and Burkey (1953) noted differences by diet and by animal in the types of bacteria found in the rumen.

In 1954, Huhtanen et al. (1954) developed an *in vitro* system to evaluate fiber digestion by ruminal microbes. Although there had been more diversity in *in vitro* fermentation systems (Barnes, 1967), most *in vitro* systems used today are based on the 2-step methods of Tilley and Terry (1963) and Van Soest et al. (1966) (also cited as Goering and Van Soest, 1970). Both systems were compared with *in vivo* digestibilities. The Tilley and Terry (1963) *in vitro* digestion system measured apparent DMD (48 h) and was highly correlated with *in vivo* DMD (148 forages). The *in vitro* fermentation system of Van Soest et al. (1966) measured true DMD, which was highly correlated with *in vivo* DMD (20 hays) and NDFD. Subsequent evaluation of the precision of Goering and Van Soest-derived *in vitro* methods at 30 h of fermentation demonstrated that the values within laboratory had an average precision of ± 5 percentage units (Hall and Mertens, 2012); this gave direction as to the precision with which the values should be used.

Modifications to *in vitro* fermentation systems used to determine forage digestibility continue to be explored (Goeser and Combs, 2009).

Conrad et al. (1958) were the first to use *in vitro* gas production when studying bloat. Pell and Schofield (1993) were the first to report in JDS on an automated method for measuring gas production during feed fermentation. It is an indirect method because gas production can be generated directly by the microbes and indirectly through the interaction of bicarbonate buffer and the pattern of fermentation acids produced. Because it measured the pressure of the gas generated from all components in substrates, it measured fiber and nonfiber fermentation simultaneously. Results of *in vitro* gas production are commonly dealt with as single or “fast” and “slow” pools because of the challenges of decomposing the gas production curve into components that relate to the routinely measured feed analytes.

Batch *in situ* “bag” systems for determination of fiber digestibility and feed evaluation were first reported in JDS by Archibald et al. (1961). Although it would seem that *in situ* measurements would be more direct and simpler than *in vitro* measurements, numerous factors affect *in situ* results (Nocek, 1988; Wilkerson et al., 1995; Vanzant et al., 1998). Estimating digestibility using *in situ* methods has potential for substrates to leave *in situ* bags by solubilization or by particle escape, depending on the sample grind and porosity of the bags. Varel and Kreikemeier (1995) reported that *in situ* NDFD had shorter lag, faster fractional rates, and greater extent of digestion compared with *in vitro* methods when NDF residues of alfalfa and brome grass were fermented. Bossen et al. (2008) observed the opposite, suggesting the differences in either method might change the comparison. Dieho et al. (2017) reported very slow *in situ* fractional rates, suggesting that the filter bags used may have inhibited fermentation. Hoffman et al. (1993) did an extensive *in situ* study of the digestion kinetics of DM, NDF, and CP of perennial forages at several maturities. At every maturity, the legumes had a larger undigested proportion of NDF but a faster rate than grasses, which resulted in similar ruminal digestion of the forages.

Various approaches to describing the fermentation of insoluble fiber have been used, such as monosaccharide disappearance (e.g., Wedig et al., 1986), as single or multiple pools of fermentable fiber, and as undigested fiber. The simple summative equation (Van Soest, 1967) treats NDF as a single pool with varying digestibility and this is how NDFD is typically reported in JDS; that is, $\text{NDFD} = \text{digested NDF} / \text{consumed NDF}$. Waldo et al. (1965) was the first to measure *in vivo* NDFD using heifers. Nousiainen et al. (2009) evaluated the effects of forage and concentrates on total diet digestibility. They

concluded that NDFD decreased linearly as the concentrate proportion in the diet increased and that replacing starch with fibrous by-products improved NDFD. Lippke et al. (1986) were the first to use fermentations of 6, 7, or 8 d (144, 168, or 196 h) for NDF using both in vitro and in situ methods to determine fiber values to be used as an internal marker for digestibility studies on C4 grasses. In general, recoveries of undigested NDF were less than 100%, and the authors speculated that this might be due to smaller particle size of feces and potential for loss of undigested NDF through crucibles.

In a system still used today, the 2-pool approach to describing fiber digestion recognized the existence of digestible and indigestible fiber fractions. Wilkins (1969) used a 6-d in vitro fermentation to define potentially digestible cellulose. Based on this work, Waldo (1969) and Waldo et al. (1972) made a conceptual breakthrough when they suggested that any cellulose still present after long fermentation times should be excluded from a model of cellulose digestion, which created 2 pools: potentially digestible and indigestible cellulose. If the indigestible fraction of cellulose was subtracted from the total cellulose, the remaining potentially digestible cellulose might be digested according to first-order kinetics. Gill et al. (1969) tested this concept and determined that, using 48 h as the endpoint of cellulose digestion, the potentially digestible cellulose followed first-order kinetics (linear semi-logarithmic plot). Waldo (1969) noted that chemical analysis cannot distinguish between the digestible and indigestible cellulose. Potentially digestible or indigestible matter can only be estimated by fitting data to mathematical models, not by direct measurement, regardless of the fermentation time.

In attempting to relate in vivo digestibilities to feed composition, Lancaster (1943) reported that lignin affected both cellulose and pentosan digestibility and was more highly correlated with OM digestibility than were CF or cellulose. This suggested that cellulose and hemicellulose would be fibrous CHO with similar digestibilities and relationships to lignin. He also remarked that digestibility is not simply a function of lignin and CHO composition, but a function of the whole mechanical (physical) structure of the plant. This prescient conclusion agrees with our current understanding that fiber indigestibility may be related to the matrix of the fiber complex as much as to its chemical composition.

Nonfiber Carbohydrates

It appears that there is little or no indigestible fraction for nonfiber CHO in the feeds typically fed to dairy cows, although some portion may remain undigested. The diversity in nonfiber CHO can give variation

in digestion end products in terms of microbial mass and fermentation acids. Because they generally ferment more rapidly and extensively than insoluble fiber, they provide a valuable source of energy and carbon for microbial fermentation, but excessive feeding of readily fermentable CHO can cause ruminal acidosis and digestive disturbances (Tremere et al., 1968). Cattle do have the capacity to digest starch, lactose, and the microbial storage CHO, glycogen and trehalose, as evidenced by enzymes present in pancreatic secretions and the lining of the small intestine (Kreikemeier et al., 1990).

The early forays into nonfiber CHO utilization by rumen microbes published in JDS provided some of the basic principles upon which we still work today. The theory that microbes could convert NPN to protein with fermentation of CHO was explored by Krauss (1927). Wegner et al. (1940) developed a buffered in vitro system that demonstrated that NFE sources of CHO could be used by ruminal microbes to convert NPN into microbial N, and the disappearance of NPN depended on the amount of CHO. As scientists evaluated catabolic microbial products produced in vitro from mono- and disaccharide substrates, they reported VFA production and noted a positive iodine test in rumen microbial cultures largely devoid of protozoa (Doetsch et al., 1953). The researchers had discovered that ruminal microbes could convert the NFE to glycogen, a microbial storage polysaccharide of both bacteria and protozoa that is similar to starch, though further work was required to identify the material. Formation of glycogen can slow fermentation and acid production relative to the rate of the rapidly available CHO from which it was formed. But it costs 1 ATP to add a glucose to the glycogen chain (Ball and Morell, 2003), an energy expense that could reduce the energy available to produce microbial cells. Glycogen may offer a supply of "starch" to the animal, even on diets containing no starch, as it can pass from the rumen to the small intestine (Branco et al., 1999).

Other work focused on ruminal function explored how bacterial lactate and glycogen production were affected by amount and type of CHO, as well as by pH and the length of fermentation (Robinson et al., 1955). The relationship between ruminal lactate production, time after feeding, and CHO type was parsed out by Waldo and Schultz (1956). In working to define the nutritional characteristics of measurable CHO fractions, Lancaster (1943) reported that the total-tract digestibilities of water-soluble CHO were 1.00, indicating that these CHO are an ideal nutritive entity that is completely digested. The first report in JDS of the use of an in vitro system using ruminal microbes to assess starch digestibility was provided by Tonroy and Perry (1974), whereas Herrera-Saldana et al. (1990) used in

situ incubations to show that the ranking of different grains for starch degradation followed the same pattern as CP degradation. The latter work illustrated the effect of the protein matrix around starch granules on starch digestibility.

Exploration of the digestion products of CHO and the effect of different types of CHO continued in earnest, particularly as they affected the yield of microbial products or ruminal conditions. The fermentation acids were of specific interest as they support glucogenic or lipogenic needs of the animal and affect ruminal pH. Microbial fermentation of sugars such as sucrose (Strobel and Russell, 1986) and lactose (Schingoethe, 1976) were reported to give greater molar percentages of butyrate than does starch, and pectins gave a greater proportion of acetate than either sucrose or starch (Strobel and Russell, 1986). Fermentation of sugars such as sucrose have the potential to yield more lactate than other CHO (Strobel and Russell, 1986); however, lactate is normally present only transiently, being converted by rumen microbes largely to acetate or propionate (Baldwin et al., 1962). Starch has been suggested to provide a greater molar proportion of propionate; however, propionate production from starch can differ between diets with higher or lower amounts of forage (Murphy et al., 1982).

The yield of microbes from CHO was shown to increase in vitro with increasing supplementation of amino acids or peptides (Cotta and Russell, 1982; Argyle and Baldwin, 1989). The magnitude of response declined as more N was added, but there was no absolute plateau in response. Increasing dietary ruminally degradable protein has also been shown to increase lactate production by ruminal microbes by increasing the flux of CHO through glycolysis (Counotte and Prins, 1981), and to reduce microbial glycogen production (McAllan and Smith, 1974). The proportion of ruminally degradable protein from feedstuffs (Hoover and Stokes, 1991) and nonfiber CHO type (Hall and Herejk, 2001) were also shown to affect yields of microbial N from CHO.

Although acidic ruminal pH has been shown to reduce ruminal fiber digestion (Hoover, 1986), non-pH factors can also alter fiber digestion. An apparently proteinaceous material produced during fermentation of glucose by ruminal microbes inhibited fiber fermentation in vitro (Piwonka and Firkins, 1996). Competition between fiber and nonfiber CHO utilizers for N resources may also affect insoluble fiber fermentation. Starch, sucrose, fructose, or glucose supplemented (at 0.3% of BW) depressed ruminal NDF digestion relative to the control when a low dietary concentration of ruminally degradable protein (0.031% of BW) was provided, though the average ruminal pH was >6.2 (Heldt et al., 1999). However, NDF digestion was equal to or greater

than the control with CHO supplements when degradable protein levels in the diet were increased (0.122% of BW), even though ruminal pH decreased slightly.

IMPACT IN DAIRY CATTLE DIETS

Carbohydrates provide nutrients to support the performance requirements of dairy cows, but the impacts are not the straightforward effects on TDN that were perceived in 1917.

Insoluble Fiber

Insoluble fiber has a substantial effect on feed intake. Conrad et al. (1964) concluded that physical and physiological factors regulating intake change in importance with increasing digestibility of the diet. At low digestibility, BW and undigested DM per unit of BW limited intake, and at high digestibility, metabolic size and production controlled intake. For cows averaging 414 kg of BW and producing 16 kg/d of 4% FCM, the breakpoint between the 2 mechanisms occurred at about 66% digested DM. When less than the breakpoint diet digestibility, intake was related to undigested DM (ballast) and BW. It is interesting that Huffman and Duncan (1952) suggested that undigested CHO in CF and NFE were good estimates of ballast. At greater than breakpoint digestibility, intake was related to energy needed for production. The difficulty in using this approach is that total-tract DMD of the diet must be known, and laboratory methods for estimating the in vivo value are unavailable or inaccurate. However, fiber is inversely related to DMD and is routinely measured. Given fiber's resistant digestibility and its bulk, NDF may be an alternative for describing the fill or physical limitations on intake (Mertens, 1987). Lippke (1986) suggested that indigestible fiber might be a factor in limiting intake of forages.

Dado and Allen (1995) observed that inert bulk added to the rumen did not affect intake when a low fiber (25% NDF; 45% forage) ration was fed but lowered intake when a high fiber (35% NDF; 81% forage) was fed. This suggests that NDF in forages may serve as a proxy for the "fill factor" in diets. Dado and Allen (1995) suggested that the effects of ruminal fiber digestion, fiber particle size, and animal characteristics needed additional study when intake was fill-limited. Dado and Allen (1994) indicated that mechanisms affecting individual meals and daily DMI might differ with parity, rumen capacity, and BW.

Allen (2000) provided a thorough review of the physical and chemical characteristics of diets that affect the DMI of lactating cows. Although the mechanisms are not understood, he described characteristics of dietary

CHO (e.g., fiber content, ease of hydrolysis of starch and fiber, particle size, particle fragility) that should be considered when formulating diets allocating feeds to dairy cows. He noted that DMI decreased when NDF in the diet increased above 25% of DM and suggested that experiments often did not show the quadratic response expected by the model of Mertens (1987), which predicts that DMI of low fiber diets should decrease when milk yields are low. However, many interactions among ingredients can modify animal responses. Allen (2000) noted, for example, that when nonforage fiber sources are substituted for forage, they might increase DMI when fill is limited but might decrease DMI if substituted for grains. He also noted that site and extent of starch digestion can affect intake. Oba and Allen (1999) observed that the *in vitro* or *in situ* digestibility of NDF in forages were correlated with intake and milk production and reported that increases in NDFD measured *in vitro* or *in situ* were associated with increases in DMI and 4% FCM.

However, research has also demonstrated that laboratory measures of CHO characteristics may differ from their *in vivo* metrics and effects. In a comparison of brown midrib (BMR) and conventional corn silage, total-tract NDFD in dairy cows did not differ when the 2 silages were substituted to provide equivalent proportions of total dietary NDF (Oba and Allen, 2000). The total-tract NDFD were 0.302 and 0.303 (29% diet NDF), and 0.381 and 0.421 (38% diet NDF), for the BMR and conventional corn silages, respectively. This is in contrast to the 0.559 (BMR) and 0.465 (conventional) *in vitro* 30-h NDFD values obtained for these forages. Dry matter intake was greater for cows consuming diets containing the BMR variety, suggesting that increased passage with BMR corn silage could have reduced NDFD *in vivo*.

Nonfiber Carbohydrates

The different organic acid profiles from the ruminal fermentation of CHO have potential to change cow responses to diets. Increasing amounts of ruminally infused propionate substituted for acetate were related to a decrease in DMI (Oba and Allen, 2003). The generally positive but variable association of feeding sugars, such as sucrose, and increased milk fat production (Nombekela and Murphy, 1995; Broderick et al., 2008) may be associated with the greater molar proportion of butyrate produced from those sugars. Butyrate may have a greater effect on milk fat than the maximum of 8% it constitutes of the carbon in milk fatty acids: 50% of the 4 carbon atoms at the methyl-terminal ends of the likely *de novo* synthesized C₄ to C₁₂ fatty acids in milk fat arose from β -hydroxybutyrate (Palmquist et

al., 1969). Additionally, butyrate constitutes approximately 30% of the fatty acids in the *sn*-3 position in milk triglycerides (Jensen, 2002).

Another potential effect of nonfiber CHO on milk fat production may come from the biohydrogenation activity of CHO-utilizing microbes. Some species of glucose-utilizing microbes have been shown to perform biohydrogenation on fatty acids in the rumen (e.g., *Butyrivibrio fibrosolvens*; McKain et al., 2010). A partially unsaturated fatty acid, the *trans*-10 isomer of the 18:1 fatty acid, has been implicated in milk fat depression, so a current hypothesis is that more complete ruminal biohydrogenation of fatty acids could reduce the potential for milk fat depression. When sucrose was supplemented as 4.7% of ration DM, the concentration in the milk of total *trans* 18:1 fatty acids declined and butterfat yield tended to increase (Penner and Oba, 2009). It would seem that there would need to be enough polyunsaturated fatty acids in the diet for this effect to influence milk fat.

FUTURE DIRECTIONS

The ultimate goal of all research on carbohydrates in dairy cattle nutrition is to improve the efficient use of resources to feed people sustainably, maintain the welfare of dairy cattle, and maintain a healthy environment. Planning future improvements involves considering what did or did not work well in the past. Three basic elements that were crucial to making advances in carbohydrate research in the last 100 years were (1) evaluating whether a method measured what people thought it did or wanted it to, (2) knowing how accurate and precise the measurements were, and (3) providing complete descriptions of experiments.

It is critical to ensure that we are accurately measuring the characteristic we intend (i.e., that the method works). This is particularly challenging with *in vitro* or *in situ* digestibility measures intended to correlate with *in vivo* assessments. It can be at least partly addressed by comparison of a method to a "gold standard" (e.g., total collections vs. spot sampling) over a range of samples, or by using internal standards or control samples as appropriate. And an assay may not work for all samples; even the AOAC indicates which samples are not suitable for measurement by an official method. Additionally, there is a critical need to assess whether methods are actually measuring the same analyte when they are using different procedures. If they are not, the variation among methods can devalue the data for describing any relationship to animal responses. To the second point, if we do not know the precision of an assay, we cannot know how the variation may or may not be clouding our ability to detect relationships or affect-

ing the power of a study, or how many decimal points to be concerned with when applying the measures. Very few analytical methods, except for AOAC methods, report this information. Finally, describing our experiments in sufficient detail is essential to interpretation of our work by others. Meta-analysis has become an increasingly useful tool for discerning relationships and patterns from multiple experiments, but it is best done when studies provide enough of the descriptive data on diets, animals, methods used, and animal responses to give a more complete picture of the experiment. This may mean attempting to make more measurements (particle size? composition? animal behavior?) than we commonly have, which may require more collaborations. We need this information to decide whether the chemical, digestion, and biological measures of CHO that we are using now are sufficient, or whether new fractions and approaches are needed. It is reasonable to assume that the greatest change in concepts on the utilization of CHO may be related to the dynamics of digestion and passage in extremely high-producing cows. Are our models and methods adequate to describe in vivo, non-steady-state kinetics that dictate nutrient supply to the animal?

We can expect that the knowledge we gain will increase our understanding and add complexity to diet formulations for dairy cows. One of our challenges may be how to decide what level of complexity and aggregation matters for use in the field to achieve desired animal performance and in the laboratory to move the science ahead. Nutritional concepts and applications must evolve as the field is integrated with genomics, immunology, microbiology, ecology, and other disciplines. As in the past, we must continue reaching out to cooperate with and learn from other disciplines to improve our knowledge in our own field.

The last 100 years of the *Journal of Dairy Science* provides us with a substantial research legacy regarding the role of carbohydrates in dairy cattle nutrition. As we move forward and build upon that legacy, we need to remember that it is our responsibility to faithfully and intensely evaluate and judge the worth of our concepts and methods so that we can improve upon that legacy in the next 100 years.

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APPENDIX

Table A1. A timeline of major events in carbohydrate research for dairy cattle nutrition (see also Figure 1)

Date	Milestone	Reference
1918	Carbohydrate (CHO) and fat total digestible nutrient (TDN) contribution is first reported.	Woll, 1918
1920	Starch and sugars in corn silage are first reported.	Shaw and Norton, 1920
1927	The ability of microbes to convert urea to microbial protein is investigated.	Krauss, 1927
1936	Digested crude fiber (CF) and nitrogen-free extract (NFE) are shown to relate to cow performance.	Cave et al., 1936
1939	Physical feed characteristics affect milk fat production.	Powell, 1939
1939	Time course measurement of rumen pH is reported.	Monroe and Perkins, 1939
1940	Nonprotein nitrogen use by microbes is dependent on CHO amount.	Wegner et al., 1940
1950	Effect of increasing CF on milk production is described.	Nordfeldt et al., 1950
1951	Roughage level (forage-to-concentrate ratio) affects milk fat.	Tyznik and Allen, 1951
1953	Proof is given that the relationship of TDN to net energy differs for roughages and concentrates; glycogen is detected in rumen bacteria.	Doetsch et al., 1953; Moore et al., 1953
1954	In vitro fermentation system is used to evaluate cellulose and fiber digestion.	Huhtanen et al., 1954
1964	Impact of physical form and digestibility on DMI is described.	Conrad et al., 1964
1971	In vitro neutral detergent fiber digestibility (IVNDFD) is used to investigate kinetics of fiber fermentation; a method is proposed for measuring particle size of forage.	Smith et al., 1971; Waldo et al., 1971
1972	Two-pool model of cellulose digestion and relationship of digestion and passage rates are described.	Waldo et al., 1972
1974	In vitro starch digestibility is tested in an in vitro fermentation system.	Tonroy and Perry, 1974
1980	Starch and pH affect IVNDFD kinetics.	Mertens and Lofton, 1980
1989	Increased RDP:CHO ratio increases microbial protein yield.	Argyle and Baldwin, 1989
1991	Relationship of RDP and CHO for optimum microbial yield is described.	Hoover and Stokes, 1991
1996	A field method is developed for particle sizing diets.	Lammers et al., 1996
1997	Physically effective (pe)NDF is defined and related to rumen function.	Mertens, 1997
1999	Increased IVNDFD of forage is related to increased DMI in lactating cows.	Oba and Allen, 1999

Continued

Table A1 (Continued). A timeline of major events in carbohydrate research for dairy cattle nutrition (see also Figure 1)

Date	Milestone	Reference
2001	CHO affects short-term intake regulation.	Allen, 2000
2001	Microbial N yield differs among nonfiber CHO sources.	Hall and Herejk, 2001
2005	peNDF affects chewing and ruminal pH.	Beauchemin and Yang, 2005
2009	Dietary sugars affect ruminal biohydrogenation of fats.	Penner and Oba, 2009
2012	The relationship between effective particle size and starch digestion is described.	Hoffman et al., 2012
2017	The ruminal effects of NDF and particle size are separated for their impact on ruminal pH.	White et al., 2017