A method for partitioning neutral detergent-soluble carbohydrates†

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Abstract: A method was developed to fractionate the neutral detergent-soluble carbohydrates (NDSC) in feedstuffs. Differential solubilities of carbohydrates in 80:20 (v/v) ethanol/water were used to partition NDSC into organic acids (OA) and mono- and oligosaccharides soluble in ethanol/water from starch and neutral detergent-soluble fibre (NDSF) which are insoluble. Mono- and oligosaccharides (total ethanol/water-soluble carbohydrate) were measured on the ethanol/water extract, and starch was measured on the ethanol/water-insoluble residue. The OA and NDSF, the two most compositionally diverse NDSC fractions, were estimated by difference. The method allows partitioning of the NDSC on a nutritionally relevant basis into (1) organic acids, (2) total ethanol/water-soluble carbohydrate, (3) starch and (4) neutral detergent-soluble fibre. The methods involved in this fractionation are relatively simple or are commonly used.

Keywords: soluble carbohydrates; sugars; soluble fibre; starch; ruminant nutrition; feed analysis

INTRODUCTION

Cell components soluble in neutral detergent are a diverse group, both compositionally and nutritionally. These components include organic acids (carbohydrate derivatives; OA), simple sugars, oligosaccharides, starch, fructans, pectic substances, $(1\rightarrow3)(1\rightarrow4)$-$\beta$-glucans and other carbohydrates of appropriate solubility, and may be referred to as neutral detergent-soluble carbohydrates (NDSC). The analyses used to determine individual NDSC fractions have typically been complex[1] and prone to interference from other carbohydrates.[2] This has limited their usefulness for routine food or feed analysis. Currently, NDSC content in feeds is estimated by difference according to a formula similar to that used for more than 100 years to calculate nitrogen-free extract: 100 − (crude protein + neutral detergent fibre + ether extract + ash). This estimate encompasses the cumulative errors of the contributing analyses while disregarding the nutritional differences among NDSC.

Variation in NDSC digestion characteristics precludes incorporation of NDSC into a single nutritional entity. Mammalian enzymes digest some NDSC (OA, simple sugars, some oligosaccharides, starch). The remaining constituents fall into the category of nutritional fibre, because mammalian enzymes cannot hydrolyse them (some oligosaccharides, fructans, pectic substances, $(1\rightarrow3)(1\rightarrow4)$-$\beta$-glucans). Among the NDSC, only OA do not support appreciable microbial growth.[3] Several of the NDSC will continue to ferment at a relatively low ruminal pH, and may ferment to lactic acid. These include simple sugars, oligosaccharides, starch[4] and fructans.[5] The fermentation of pectin is markedly depressed at low pH.[4] These very different digestion characteristics justify fractionation of NDSC on a nutritional basis. A partitioning that reflects ruminal and mammalian digestion properties is (1) organic acids, (2) mono- and oligosaccharides, (3) starch and (4) neutral detergent-soluble fibre (fructans, pectic substances and $(1\rightarrow3)(1\rightarrow4)$-$\beta$-glucans; NDSF). Fructans can be included with either the starch or soluble fibre, depending upon whether it is fermented in the rumen or passes to the small intestine.

In a recent method, NDSF was estimated by making use of the differential solubilities of carbohydrates in aqueous ethanol and in neutral detergent with heat-stable α-amylase.[6] Aqueous ethanol solubilises low-molecular-weight carbohydrates (organic acids, mono- and oligosaccharides), leaving starch, NDSF and neutral detergent fibre (insoluble fibre) remaining in the ethanol-insoluble residue (EIR).[7] The differential solubility of the NDSC in aqueous ethanol and

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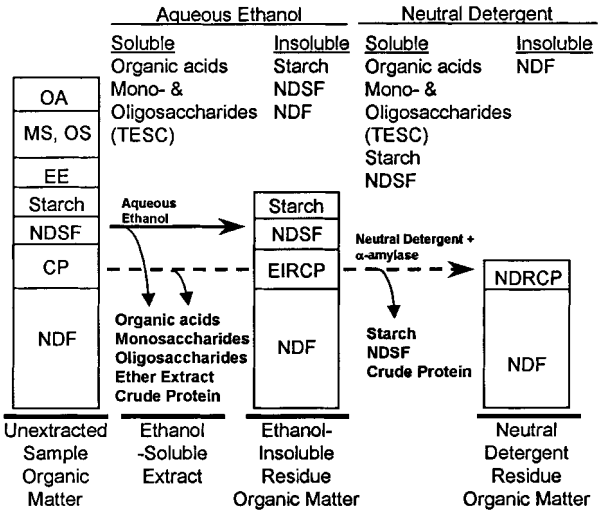


Figure 1. Separation of carbohydrates based on their solubilities in aqueous ethanol and in neutral detergent with heat-stable α-amylase (CP = crude protein, EE = ether extract, EIRCP = ethanol/water-insoluble residue crude protein, MS = monosaccharides, NDF = neutral detergent fibre, NDRCP = neutral detergent residue crude protein, NDSF = neutral detergent-soluble fibre, OA = organic acids, OS = oligosaccharides, TESC = total ethanol-soluble carbohydrates).

neutral detergent (Fig 1), combined with selective carbohydrate assays of the ethanol-soluble extract (ESE) and EIR, can be used to expand the NDSF method into a system that separates the NDSC into the four nutritional fractions described above. In this report, investigations of aqueous ethanol extractions for removal and measurement of low-molecular-weight carbohydrates, the incorporation of these and the NDSF methods into a relatively simple system for partitioning NDSC, and comparison of the results with a system for total non-structural carbohydrate analysis are described.

METHODS

Samples

Nine test samples were used to examine the total ethanol/water-soluble carbohydrate (TESC) and fructose contents of aqueous ethanol extracts. Dried citrus pulp, dried sugar beet pulp, alfalfa leaves from immature plants, alfalfa stems from mature plants, almond hulls and timothy hay were ground to pass the 1 mm screen of a Wiley mill (Arthur H Thomas, Philadelphia, PA, USA). Lyophilised broccoli and frozen green peas and oat bran (provided by BA Lewis, Cornell University, Ithaca, NY, USA) were ground to pass a 0.85 mm screen.

Additional samples used to test the NDSC analysis system were corn grain, wheat middlings, two corn silages, two hay crop silages, ground wheat and 48% soybean meal. All were ground to pass the 1 mm screen of a Wiley mill. Composition data for all samples are found in Table 1.

Preparation of ethanol-soluble extract and ethanol-insoluble residue

The ethanol-to-water ratio needed to optimise low-molecular-weight carbohydrate extraction was investigated using different aqueous ethanol solutions in sequential and non-sequential extractions of samples.

Non-sequential extraction

The EIR and ESE were prepared by continuously shaking 0.2 g of air-equilibrated sample with 40 ml of aqueous ethanol solution at room temperature (solution temperature 17–24 °C) for 4 h in a 25 mm × 150 mm Pyrex tube with a Teflon-lined screw-cap. The aqueous ethanol solutions used were 90:10 (90% EtOH) or 80:20 (80% EtOH) (v/v) ethanol/water prepared from 95% ethanol and distilled water.

Table 1. Composition of samples (g kg^{-1} of sample DM)

Sample	*Ash*	*CP*[a]	*EE*[a]	*NDR*[a]	*NDRCP*[a]	*EIR*[a]	*EIROM*[a]	*EIRCP*[a]	*OA*[b]	*TESC*[b]	*Starch*[b]	*NDSF*[b]
Alfalfa leaf	92	293	24	186	16	711	657	259	92	101	34	194
Alfalfa stems	78	124	13	580	23	797	763	96	47	71	3	10
Almond hulls	50	71	38	260	12	498	472	41	82	328	14	169
Broccoli	88	304	47	143	5	606	572	238	50	177	7	189
Citrus pulp	71	64	25	205	23	639	585	47	44	258	0	356
Green peas	32	259	34	182	4	635	618	210	17	250	206	24
Oat bran	26	176	68	100	24	936	910	168	−22	10	550	116
Sugar beet pulp	89	80	4	446	51	847	766	71	4	128	0	300
Timothy hay	50	82	10	673	18	819	787	64	44	91	4	64
Alfalfa silage A	95	191	30	455	21	720	661	99	104	18	7	120
Alfalfa silage B	113	181	45	381	20	675	600	92	142	11	14	134
Corn silage A	49	75	29	509	9	800	776	44	106	9	189	43
Corn silage B	38	70	36	418	6	813	795	21	79	3	304	58
Corn grain	15	90	46	126	7	891	892	47	7	−3	645	81
48% soybean meal	65	527	12	109	14	819	771	526	45	109	10	140
Ground wheat	17	109	14	121	11	936	928	84	−2	18	646	88
Wheat middlings	55	190	22	423	34	843	790	157	46	54	210	34

[a] CP = crude protein, EE = ether extract, NDR = neutral detergent residue organic matter, NDFCP = CP in NDF, EIR = 80:10 ethanol/water-insoluble residue, EIROM = organic matter in EIR, EIRCP = CP in EIR.

[b] Fractions determined with the NDSC method using 80% EtOH; OA = organic acids, TESC = total ethanol/water-soluble carbohydrates, NDSF = neutral detergent-soluble fibre.

Capped tubes were placed horizontally in a rack within a mechanical shaker with the length of the tube parallel to the motion of the shaker for maximum agitation of solution and sample. The crude protein content of the EIR (EIRCP) was determined by Kjeldahl analysis of residues obtained by filtration under either vacuum or gravity through Whatman 541 filter paper (Fisher Scientific, Pittsburgh, PA, USA). Use of filter paper allowed digestion of the entire sample + filter paper in the Kjeldahl analysis. Total EIR and EIR organic matter (OM) (EIROM) were determined on residues obtained after filtration through coarse porosity fritted glass Gooch crucibles. Extracts to be analysed for TESC were collected directly into 100 ml volumetric flasks. All residues were rinsed twice with the ethanol/water solution used for their extraction, followed by two acetone rinses, and were then held under vacuum until dry. Only ethanol/water rinses, not acetone rinses, were collected in ESE. Extracts were brought to volume with the ethanol/water solution used in their extraction. All EIR values for crude protein (CP), OM and starch were expressed as a percentage of the original sample dry matter.

Sequential extraction

Sequential extracts were prepared from two extractions performed with a two-step extraction using 90% EtOH and 80% EtOH, and from a single extraction performed with a three-step extraction using 90% EtOH, 80% EtOH and 70:30 (v/v) ethanol/water (70% EtOH). Sequential extractions proceeded from greatest to lowest concentration of ethanol. As with the non-sequential extractions, 0.2 g air-equilibrated samples and 40 ml aliquots of ethanol/water solution were placed in 25 mm × 150 mm Pyrex tubes with Teflon-lined screw-caps. The samples were shaken continuously at room temperature (solution temperature 17–24 °C) for 4 h. Samples were filtered under vacuum through Whatman 541 or Whatman GF/A filter papers, with the ESE being collected in volumetric flasks. Residues were rinsed twice with the ethanol/water solution used for the extraction. The filtration and rinsing process was repeated after each extraction. Filter papers containing EIR were inserted into screw-cap tubes for the subsequent extraction. Extracts were brought to 100 ml in volumetric flasks with the ethanol/water solution used in their extraction.

ESE Analysis

After filtration under gravity through Whatman 541 filter paper, extracts were analysed for ketohexoses, presumed to be predominantly fructose, by the resorcinol method of Kulka.[8] The fructose measurement was used to assess the efficacy with which ethanol/water solutions extracted oligosaccharides. Total carbohydrates in the extract (TESC) were determined by the phenol–sulphuric acid method of Dubois *et al*[9] using a sucrose standard. All 90% EtOH ESE and non-sequential 80% EtOH ESE were diluted 1:10 with distilled water for fructose and TESC analyses. The sequential 80% and 70% EtOH ESE were analysed without further dilution.

Starch analysis

Enzymatic starch analyses were performed in duplicate on 0.2 g samples extracted with 80% EtOH. The EIR for starch determination were filtered under vacuum through 70 mm diameter Whatman GF/A glass fibre filter paper (Fisher Scientific, Atlanta, GA, USA) in a Büchner funnel. Potassium hydroxide was used for gelatinisation using a modification of Englyst *et al.*[10] The filter paper with EIR was placed in a 100 ml beaker to which was added 10 ml of distilled water. After stirring with a magnetic stir bar, the beaker was capped with aluminium foil and heated in a 90–92 °C water bath for 10 min. Then 0.1 ml of Termamyl (120L, heat-stable, α-amylase, Novo Nordisk Biochem, Franklinton, NC, USA) was stirred in with the sample. The beaker was capped and returned to the water bath for 15 min. After cooling the samples in an ice bath for 20 min, 10 ml of 4 M KOH was added to each sample while stirring on a magnetic stir plate, followed by the immediate addition of sufficient 4 N HCl and dilute HCl to adjust the pH to 6–6.5, according to pH meter readings.

After gelatinisation, all samples were processed according to the method of Holm *et al.*[11] Glucose hydrolysed from starch was measured using glucose oxidase–peroxidase reagent.[12]

TNC procedure

Total non-structural carbohydrates (TNC) were analysed by a modified method of Smith.[13] For starch analysis a 0.2 g sample was weighed into a 25 mm × 150 mm screw-cap test tube. Distilled water (15 ml) was added to each sample. The test tubes were capped and placed in a 110 °C oven for 90 min to gelatinise the starch, with tubes being vortexed at 30 min intervals. After cooling to room temperature, 10 ml each of a pH 4.9 buffer and a 10 g l^{-1} α-amylase solution (*Aspergillus oryzae* A-2611, Sigma Chemical Co, St Louis, MO, USA) were added to the test tubes. Samples were incubated at 38 °C for 44 h, with vortexing of the tubes at 12 h intervals. After incubation, samples were filtered through Whatman No 1 paper into a 250 ml volumetric flask. Tubes and filter paper were washed several times with distilled water and adjusted to volume. Ferricyanide was used to determine reducing sugars photometrically on an aliquot of the filtrate. The ferricyanide method was also used to determine water-soluble monosaccharides (WSM) for the TNC method. Starch was calculated as TNC − WSM.

General methods

Sample dry matter (DM) was determined overnight at 105 °C in a forced draft oven. Organic matter (OM) was determined as the difference in sample weight before and after ashing at 512 °C for 8 h. Crude protein analyses were performed according to the Kjeldahl

nitrogen procedure[14] with the boric acid modification during distillation.[15] Crude protein of all samples was calculated as N × 6.25. Ether extractions (EE) were performed according to the method of the AOAC.[14] Neutral detergent residue (NDR) was prepared on an ash-free basis according to the method of Van Soest *et al*[16] using 0.2 ml of Termamyl during refluxing to remove starch. Samples were filtered through Whatman 541 filter paper for subsequent Kjeldahl nitrogen analysis (NDRCP), or through coarse porosity Gooch crucibles for NDR determination. All analyses were performed in duplicate.

Calculation of NDSF and organic acid values

The NDSF is insoluble in ethanol/water and is contained in the EIROM. The NDSF is calculated as[6]

$$(\text{EIROM} - \text{EIRCP}) - (\text{NDR} - \text{NDRCP}) - \text{EIR starch}$$

Organic acids are soluble in aqueous ethanol and are found in the ESE. The majority of the EE and some CP are co-extracted with OA, with very little EE remaining in the EIR after ethanol/water extraction and acetone rinses.[6] With sample OM minus EIROM setting gravimetric boundaries to the ESE, OA can be calculated as

$$(\text{OM} - \text{CP}) - (\text{EIROM} - \text{EIRCP}) - \text{EE} - \text{TESC}$$

Statistical analysis

Differences between 80% EtOH and 90% EtOH as extractants were evaluated on fructose and TESC contents of ethanol/water extracts and on EIROM yields. Statistical analyses were performed by least squares means ANOVA using the MIXED procedure of SAS[17] (Release 6.12, 1996), using the Satterthwaite option to determine denominator degrees of freedom. Data were analysed as a 9 × 2 factorial arrangement of treatments with feed sample and ethanol/water solution as fixed factors. Feed sample, ethanol/water solution, extraction replication (an ethanol/water extraction run one day) and interaction terms were included in the statistical model. Extraction replication was designated as a random factor.

RESULTS AND DISCUSSION

Ethanol/water extraction analyses

In this study the amount of fructose recovered in the sequential extracts was used as an index of the efficacy of an ethanol/water solution to extract mono- and oligosaccharides. In plant materials, fructose is primarily found as the free monosaccharide, in oligosaccharides (eg sucrose, stachyose, raffinose) and in fructans. The 80% EtOH extracted up to 17 g kg^{-1} more fructose than 90% EtOH in sequential extractions (Fig 2). Only traces of fructose were found in the sequential 70% EtOH extracts. The 80% EtOH

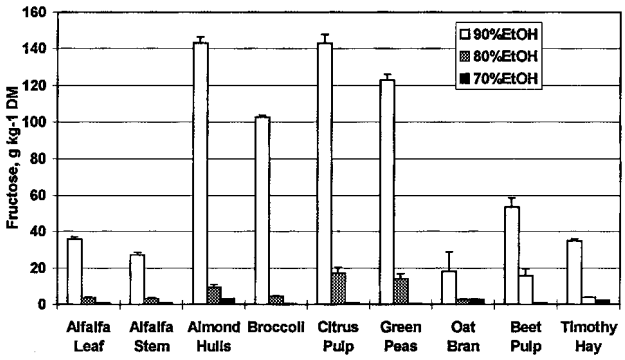


Figure 2. Fructose content of sequential 90:10, 80:20 and 70:30 ethanol/water extracts. Values are least squares means with standard error bars. %EtOH = ethanol concentration of the ethanol/water solution.

extracted numerically, but not significantly, more fructose than did 90% EtOH in non-sequential extractions (least squares means ± standard errors 77 ± 3.8 and 71 ± 3.4 g kg^{-1} of DM respectively) ($P = 0.2578$).

The portion of TESC accounted for by fructose was examined. Fructose averaged 480, 400 and 98 g kg^{-1} of TESC in the 90%, 80% and 70% EtOH sequential extracts respectively. Among samples, fructose proportions ranged from 360 to 560 g kg^{-1} of TESC in 90% EtOH ESE and from 380 to 610 g kg^{-1} in of TESC 80% EtOH ESE. In the non-sequential extracts the proportion of fructose in TESC did not differ significantly between 80% and 90% EtOH (498 and 505 g kg^{-1} respectively) ($P = 0.9027$), but did differ among samples ($P = 0.0064$) (Table 2). That the proportion of fructose in TESC did not differ between 90% and 80% EtOH suggested that 80% EtOH removed more of the low-molecular-weight carbohydrates not extracted by 90% EtOH. Differences in fructose/TESC ratios among samples reflected differences in their ethanol/water-extractable carbohydrate compositions and interactions with other components of the feed. Ethanol/water solutions are not a perfectly precise tool for partitioning of carbohydrates based on molecular weight, because the carbohydrate components and their associations are not uniform among feeds. The choice of extractant could change depending upon the population of samples tested. Based on our results, 80% EtOH was deemed an acceptable extractant for the NDSC method. The selection of 80% EtOH places the NDSC methodology on common ground with the current analytical methods for dietary fibre which use precipitation in 78–80% ethanol to separate mono- and oligosaccharides from polysaccharides.[18]

In the non-sequential extractions, 80% EtOH extracted more TESC than 90% EtOH (least squares means 168 and 143 g kg^{-1} of DM respectively) ($P = 0.0001$) (Table 2). This agrees with reports that greater amounts of carbohydrate were extracted as the proportion of water in an ethanol solution was increased.[19] The standard errors on the 80% EtOH TESC measures were quite small, indicating good repeatability of the assay. The second filtration of ESE

	Ethanol/water (v/v) of extracting solution							
	90:10				80:20			
Sample	*n*[a]	*Mean*[a]	*SE*[a]	*Fru/TESC*[b]	*n*[a]	*Mean*[a]	*SE*[a]	*Fru/TESC*[b]
Alfalfa leaf	6	91	4.9	400	3	101	1.8	380
Alfalfa stem	6	59	5.3	510	3	71	3.8	420
Almond hulls	5	309	5.6	480	2	328	2.6	480
Broccoli	6	177	6.3	580	3	177	7.8	590
Citrus pulp	6	235	17.6	530	5	258	21.3	510
Green peas	5	235	10.0	530	2	250	2.6	540
Oat bran	4	18	8.4	570	2	10	2.2	610
Sugar beet pulp	5	93	8.7	510	3	128	2.7	530
Timothy hay	5	83	4.8	430	2	91	2.1	420

Table 2. Total ethanol/water-soluble carbohydrate content ($g\,kg^{-1}$ of sample DM) and fructose proportion of non-sequential ethanol/water extracts

[a] *n* = number of separately extracted ethanol solutions analysed, mean = arithmetic mean, SE = standard error.

[b] Fru/TESC = fructose content of TESC ($g\,kg^{-1}$). Values are least squares means.

through filter paper improved repeatability by removing very fine particles that had passed through in the first filtration. The greater variation in citrus pulp TESC data likely reflects problems with subsampling of the ground feed. The values of the first two extractions were $\sim 90\,g\,kg^{-1}$ of DM lower than the three subsequent extractions.

The carbohydrate selected as the standard influences the values obtained with the phenol–sulphuric acid assay.[9] A sucrose standard was selected for this study because it is the predominant low-molecular-weight carbohydrate found in plant materials. If materials known to contain other low-molecular-weight carbohydrates are analysed, another carbohydrate that better represents the composition of that fraction will be preferable. The phenol–sulphuric acid assay was used as a fast, simple method for TESC that is relatively non-toxic and inexpensive. Other methods that allow quantification of TESC can be used for this purpose.

Consistent with previous work,[6] significantly less sample DM was recovered in EIROM after extraction with 80% EtOH (least squares mean $675\,g\,kg^{-1}$) than with 90% EtOH (least squares mean $736\,g\,kg^{-1}$) ($P = 0.0001$). The standard errors of the EIROM measurements were small (Fig 3). As mentioned previously, the greater variation associated with the citrus pulp was likely due to subsampling difficulties.

Starch analysis

Extraction with aqueous ethanol was reported to make starch resistant to analysis.[20] With 90% EtOH-extracted EIR samples, base was found to be a more efficient gelatinising agent than boiling alone, and provided higher starch recoveries.[21] Consequently, a base was used for gelatinisation of all ethanol/water-extracted samples in this study. Subsequent studies in our laboratory indicated that starch recoveries did not differ between alkali- and (water + heat) gelatinised 80% EtOH-extracted samples (data not shown). Therefore a gelatinisation procedure using water and high temperatures is likely adequate for analyses of 80% EtOH-extracted samples in the NDSC method.

Use of an extracted sample recovered on filter paper eliminated the need for subsampling extracted material for starch analysis. The glass fibre filter paper was superior to other filter paper for its ability to be manipulated without tearing during filtration and subsequent handling.

Estimation of NDSF and organic acids

Values determined for OA, TESC, starch and NDSF for all samples based on their 80% EtOH extractions are in Table 1.

Organic acids and NDSF are the most compositionally diverse carbohydrate fractions defined by the NDSC procedure. The OA pool may contain lactate, citric acid cycle components and secondary plant compounds such as oxalate and shikimate. The volatile OA are not included in the OA estimate as they are lost upon drying the samples. Fructans, pectic substances, β-glucans and other non-starch polysaccharides insoluble in 80% EtOH are included in NDSF. The compositional diversity of these fractions precludes the use of simple procedures to directly

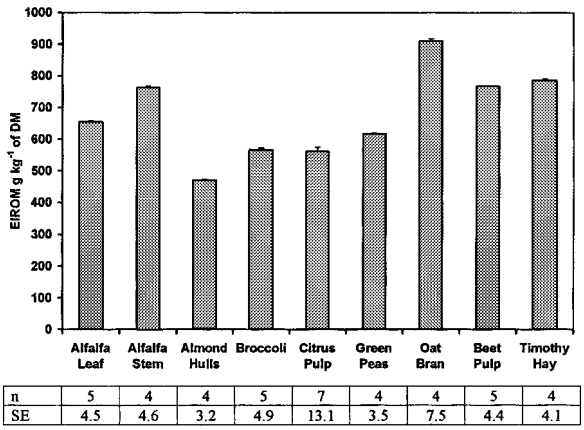
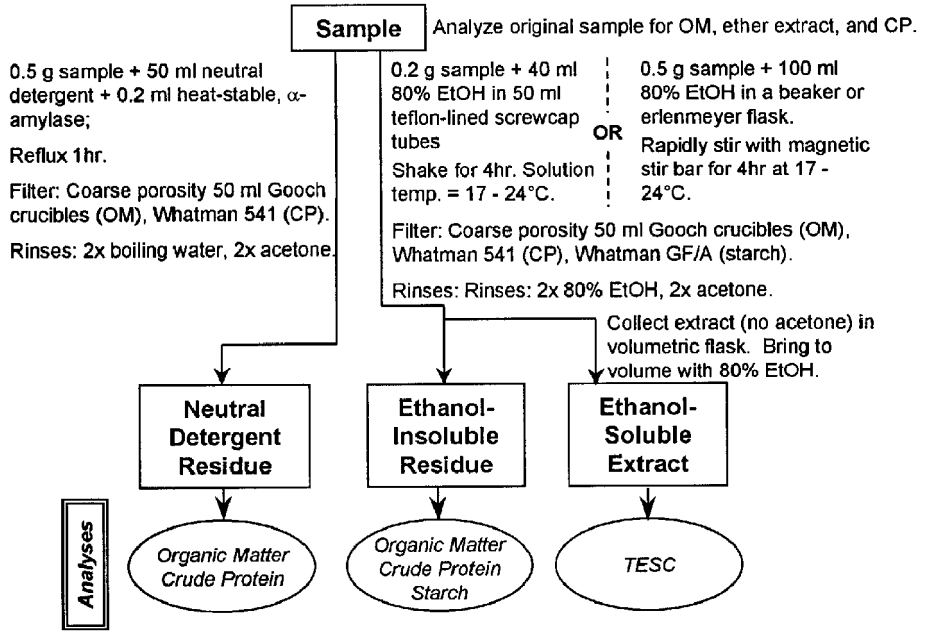


Figure 3. Ethanol-insoluble residue organic matter (EIROM) arithmetic means for 80:20 ethanol/water extractions (*n* = number of replicates, SE = standard error).



Express all values as a percentage of the original sample's dry matter.

80% EtOH: 845 ml 95% ethanol + 155 ml high quality distilled water (dH2O).

Organic Matter (OM): Obtain dry weight of residue and Gooch Crucible. Ash residue and crucible overnight at 512°C. Obtain dry weight of crucible and ash. OM = residue - ash

Crude Protein (CP): Analyze residue in Whatman 541 filter paper for total Kjeldahl nitrogen. CP = nitrogen x 6.25. Use a Whatman 541 filter paper blank in the analysis.

Total Ethanol/water-Soluble Carbohydrate (TESC): Analyze the 80% EtOH extract for total carbohydrates by phenol-sulfuric acid assay or other broad spectrum carbohydrate assay. Dilute the extracts 1 part extract:9 parts dH2O. Use the carbohydrate most likely to predominate in the extract as the assay's standard (sucrose, glucose, fructose, lactose, etc.).

Starch: Perform gelatinization through released glucose measurement on same day. Example procedure: Extract sample with 80% EtOH. Filter under vacuum through Whatman GF/A glass fiber filter paper. Transfer filter paper + residue to a 100 ml beaker on a magnetic stir plate. Add a small stir bar. Add 15 ml dH2O; may need more dH2O for samples larger than 0.2g. Add 0.1 ml heat-stable, α-amylase. Stir to moisten sample. Cap beaker with foil. Incubate beaker in 92-93°C water bath for 1 hour. Cool sample on bench at ambient temperature. Filter through glass wool plug in funnel into 100 ml volumetric flask. Thoroughly rinse beaker, funnel, magnet and glass wool with dH2O into flask; bring to volume. After repeated inversions to mix, transfer 1 ml of sample to a 50 ml volumetric flask. Add 8 ml 0.1 M Na acetate buffer (pH~4.5) and 0.5 ml amyloglucosidase. Incubate foil-capped flask in 60°C waterbath for 30 min with gentle swirling. Cool to room temperature. Bring to volume with dH2O. Analyze for glucose. Starch = glucose x 0.9.

Calculations: **Organic Acids = (OM - CP) - (EIROM - EIRCP) - EE - TESC**

NDSF = (EIROM - EIRCP) - (NDROM - NDRCP) - Starch

Figure 4. Recommended procedures for partitioning neutral detergent-soluble carbohydrates.

quantify them. However, their estimation by difference makes them prone to the same errors suffered by nitrogen-free extract. It seems likely that the error in NDSF would be smaller than that associated with OA. The majority of low-molecular-weight nitrogenous compounds that invalidate the use of a 6.25 factor for CP estimation are not present in the EIROM[6] but are extracted into ESE. This lack of certainty that CP accurately defines the mass of protein in ESE may make the OA estimate prone to error.

Other authors have indicated that pre-extraction of samples containing more than $100\,g\,kg^{-1}$ EE is necessary for accurate carbohydrate analysis.[1,22] Such pre-extraction should be applied to samples for EIROM analysis but not to the preparation of ESE for TESC analysis. The pre-extraction may remove some of the low-molecular-weight carbohydrates prior to ethanol/water extraction, thereby excluding them from the ESE.

The recommended scheme of analysis for the NDSC method is presented in Fig 4.

Comparison of TNC and NDSC methods

The TNC method was designed to measure the total carbohydrate present in cell contents and is not commonly used to provide separate sugar and starch values. Tests of the amylase used in the TNC procedure indicated that it hydrolysed sucrose and inulin (923 and $339\,g\,kg^{-1}$ hydrolysed respectively), thereby including those carbohydrates in the starch fraction. Particularly in the case of almond hulls, citrus pulp and soybean meal, the enzyme's activity explains the difference between the starch values in the NDSC and TNC systems in Table 3. All three feeds contain

Table 3. Calculated non-structural carbohydrate (NSC),[a] NDSC systemb and measured non-structural carbohydrate comparison[c] ($g\,kg^{-1}$ of sample DM)

		NDSC system				*TNC system*[c]		
Sample	*Calc NSC*	*OA*[d]	*TESC*[d]	*Starch*	*NDSF*[d]	*WSM*[d]	*Starch*	*OA+NDSF*[e]
Alfalfa silage A	250	104	18	7	121	40	14	196
Alfalfa silage B	300	142	11	14	133	34	44	222
Almond hulls	593	82	328	14	169	30	453	110
Citrus pulp	658	44	258	0	356	101	258	299
Corn silage A	347	106	9	189	43	12	253	82
Corn silage B	444	79	3	304	58	13	345	86
Corn grain	730	7	−3	645	81	5	713	12
48% soybean meal	301	42	109	10	140	7	160	134
Ground wheat	750	−2	18	646	88	20	638	92
Wheat middlings	344	46	54	210	34	37	278	29

[a] 100 − CP − NDR − EE − ash + NDRCP.
[b] Using 80:20 ethanol/water extractions.
[c] Modified total non-structural carbohydrate (TNC) method of Smith.[13]
[d] OA = organic acids, TESC = total ethanol/water-soluble carbohydrates, NDSF = neutral detergent-soluble fibre, WSM = water-soluble monosaccharides.
[e] Calculated as calc NSC − TNC.

sucrose, stachyose or raffinose, which are included in the TESC fraction in the NDSC procedure and in the starch fraction in the TNC system. By subtracting the total TNC from the calculated value for NSC, an estimated of the combined OA + NDSF can be made. A similar value can be estimated using the proposed NDSC procedure. The method comparisons are in Table 3.

The WSM + starch as estimated using the TNC procedure gave higher values than were determined by adding the TESC and starch values from the NDSC system. Part of this discrepancy can be attributed to known differences in procedures, such as the inclusion of fructans in the TNC but not in the TESC or starch values of the NDSC system. Other reasons for the differences are very likely due to gelatinisation procedures, side activities of the amylase preparation used, and incubation times and temperatures as described in the procedures. As a consequence of the higher values for TNC, the combined OA + NDSF values are lower for most feeds than are the estimates using the NDSC methods. This emphasises the need for standardisation of the procedures for routine starch and general carbohydrate analysis.

There are few values in the literature describing the NDSC in feeds. The non-volatile OA have been estimated at 20–90 $g\,kg^{-1}$ in fresh plant materials[23] and up to 120 $g\,kg^{-1}$ in fermented silages.[24] Reported mono- and oligosaccharide contents of alfalfa, almond hulls, soybean meal, broccoli, green peas and timothy are 51 $g\,kg^{-1}$,[25] 264–317 $g\,kg^{-1}$,[26] 128 $g\,kg^{-1}$,[27] 227 $g\,kg^{-1}$,[28] 196 $g\,kg^{-1}$ [28] and 68 $g\,kg^{-1}$ [25] respectively. The sugar contents of silages and fully mature grains would be expected to be low. Starch values tend to be low (<20 $g\,kg^{-1}$) in the vegetative portions of plants, and higher in seeds of corn and small grain crops. Reported starch contents of alfalfa, soybean meal, broccoli and green peas are 4 $g\,kg^{-1}$ [29] to 60 $g\,kg^{-1}$,[25] trace,[27] trace[28] and 163 $g\,kg^{-1}$ [28] respectively. The values determined with the NDSC fractionation system appear similar.

CONCLUSIONS

The proposed method partitions neutral detergent-soluble carbohydrates into nutritionally relevant pools. The combination of separation on the basis of solubility followed by direct measurements of isolated carbohydrates and calculated estimations of more compositionally diverse fractions offers an approach that lends itself to practical application. The methods required are simple or are commonly used, and have good precision. The separation of neutral detergent-soluble carbohydrates into organic acids, TESC, starch and neutral detergent-soluble fibre will allow more accurate prediction of the overall nutritional value of the NDSC fraction to ruminant and non-ruminant species. The diversity among feeds in the specific carbohydrates present in organic acids, TESC and neutral detergent-soluble fibre advises their further characterisation. More complete knowledge of the specific carbohydrates typically found in different classes of feedstuffs will permit refinement of the nutritional application of this system.

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