J. Dairy Sci. 100:6218–6228
https://doi.org/10.3168/jds.2017-12719

Effect of partitioning the nonfiber carbohydrate fraction and neutral detergent fiber method on digestibility of carbohydrates by dairy cows

A. W. Tebbe, M. J. Faulkner, and W. P. Weiss[1]
Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster 44691

ABSTRACT

Many nutrition models rely on summative equations to estimate feed and diet energy concentrations. These models partition feed into nutrient fractions and multiply the fractions by their estimated true digestibility, and the digestible mass provided by each fraction is then summed and converted to an energy value. Nonfiber carbohydrate (NFC) is used in many models. Although it behaves as a nutritionally uniform fraction, it is a heterogeneous mixture of components. To reduce the heterogeneity, we partitioned NFC into starch and residual organic matter (ROM), which is calculated as 100 − CP − LCFA − ash − starch − NDF, where crude protein (CP), long-chain fatty acids (LCFA), ash, starch, and neutral detergent fiber (NDF) are a percentage of DM. However, the true digestibility of ROM is unknown, and because NDF is contaminated with both ash and CP, those components are subtracted twice. The effect of ash and CP contamination of NDF on in vivo digestibility of NDF and ROM was evaluated using data from 2 total-collection digestibility experiments using lactating dairy cows. Digestibility of NDF was greater when it was corrected for ash and CP than without correction. Conversely, ROM apparent digestibility decreased when NDF was corrected for contamination. Although correcting for contamination statistically increased NDF digestibility, the effect was small; the average increase was 3.4%. The decrease in ROM digestibility was 7.4%. True digestibility of ROM is needed to incorporate ROM into summative equations. Data from multiple digestibility experiments (38 diets) using dairy cows were collated, and ROM concentrations were regressed on concentration of digestible ROM (ROM was calculated without adjusting for ash and CP contamination). The estimated true digestibility coefficient of ROM was 0.96 (SE = 0.021), and metabolic fecal ROM was 3.43 g/100 g of dry matter intake (SE = 0.30). Using a smaller data set (7 diets), estimated true digestibility of ROM when calculated using NDF corrected for ash and CP contamination was 0.87 (SE = 0.025), and metabolic fecal ROM was 3.76 g/100 g (SE = 0.60). Regardless of NDF method, ROM exhibited nutritional uniformity. The ROM fraction also had lower errors associated with the estimated true digestibility and its metabolic fecal fraction than did NFC. Therefore, ROM may result in more accurate estimates of available energy if integrated into models.

Key words: starch, nonfiber carbohydrate, neutral detergent fiber, digestibility

INTRODUCTION

Diets for dairy cows comprise predominantly carbohydrates, but this fraction is too chemically and nutritionally heterogeneous to be of value for estimating the available energy concentration of feeds. To partially overcome this problem, many energy models (including NRC, 2001) separate carbohydrates into 2 broad fractions: NFC with a fixed digestibility coefficient (e.g., 0.98 at maintenance in NRC, 2001) and NDF with a variable digestibility based on lignin (ranging from about 0.3 to 0.6). Although this is an improvement over a single carbohydrate fraction, considerable heterogeneity still exists within these fractions.

Starch is a major component of the NFC fraction and is now routinely assayed in feeds for use in ration formulation programs. Starch can range from 65 to 75% of the NFC fraction for typical forage-based diets and from 45 to 55% for diets high in by-products. Across diets, total-tract starch digestibility is not constant and ranges from about 0.85 to 0.99 in lactating dairy cows (Ferraretto et al., 2013). Many sources of the variation are known and have been quantified (Firkins, 2006; Ferraretto et al., 2013; Allen and Piantoni, 2014). Therefore, removing starch from NFC and applying feed-specific (e.g., based on particle size of corn grain) digestibility coefficients to the starch fraction may reduce the nutritional heterogeneity of the NFC fraction.

Other minor components of the NFC fraction include sugars, soluble fibers (e.g., β-glucans, pectin), and organic acids. However, the methods for quantifying many of these minor components are complex (Kagan et al., 2014), lack appropriate standards, and are expensive

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Received February 13, 2017.
Accepted April 6, 2017.
[1] Corresponding author: weiss.6@osu.edu

(Hall, 2003, 2014). Therefore, rather than fractioning nonstarch NFC, an aggregated fraction termed residual OM (**ROM**) is calculated as

$$\text{ROM} = 100 - \text{CP} - \text{LCFA} - \text{ash} - \text{starch} - \text{NDF}, \quad [1]$$

where LCFA = long-chain fatty acids and all values are expressed as a percentage of DM.

By calculating ROM by difference, a large volume of digestibility data previously generated can be used, but an estimate of its true digestibility is needed before ROM can be incorporated into summative equations. However, similar to NFC, ROM has flaws: (1) it is not measured directly and accumulates the errors of analyzing other fractions; (2) it contains a multitude of compounds, including lipid backbones, soluble fiber, sugars, nucleic acids, vitamins, pigments, and waxes; and (3) NDF contains variable amounts of ash and CP, leading to the double subtraction of neutral detergent insoluble CP and ash when ROM is calculated. In most feeds, ash composes 1 to 3% of the NDF (Mertens, 2002) but can exceed 7% of the NDF in some diets (Crocker et al., 1998). Likewise, CP makes up 2 to 10% of NDF but can be more than 20% of the NDF in some by-product feeds (NRC, 2001). Concentrations of ash and CP-free NDF and the resulting ROM fraction can be easily measured, but the effects of removing CP and ash contamination on NDF and ROM digestibility have not been extensively evaluated.

Therefore, the first objective of this project was to determine whether correcting for ash and CP contamination in NDF affected apparent digestibility of NDF and ROM by lactating dairy cows and whether this correction would affect statistical inferences of treatment (i.e., diet) effects on digestibility. The second objective of this experiment was to estimate the true digestibility and metabolic fecal ROM and to determine whether those variables were affected by ash and CP contamination of NDF. This information is needed so that ROM can be incorporated into energy equations.

MATERIALS AND METHODS

All procedures used on animals in these experiments were approved by The Ohio State University Institutional Animal Care and Use Committee.

Data

Retained feed, fecal, and refusal samples from 2 total collection digestibility experiments conducted at the Ohio Agricultural Research and Development Center were used (Faulkner and Weiss, 2017; W. P. Weiss, Ohio Agricultural Research and Development Center, unpublished data). Each sample was a composite of 4 daily samples taken during the collection trials; additional sampling details are presented in Weiss et al. (2009). The samples had been frozen, lyophilized, and ground through a 1-mm screen (Wiley mill; Arthur A. Thomas Co., Philadelphia, PA). Ground samples were analyzed for NDF (Ankom200 Fiber Analyzer; Ankom Technology Corp., Fairport, NY) in quadruplicate with sodium sulfite and amylase (Sigma A3306; Sigma-Aldrich, St. Louis, MO). Two of the 4 NDF residues were ashed in a muffle oven (**NDI-ash**) at 600°C overnight to calculate ash-free NDF (**$\mathbf{NDF_{om}}$**), and 2 of the NDF residues were analyzed for neutral detergent insoluble CP (**NDICP**) using the Kjeldahl method (N × 6.25) to calculate CP-free NDF (**$\mathbf{NDF_{cp}}$**). The NDI-ash and NDICP values were then combined to calculate ash- and CP-free NDF (**$\mathbf{NDF_{om+cp}}$**). Concentrations of DM, OM, CP, starch, and long-chain fatty acids were analyzed as previously described (Weiss et al., 2009). Residual OM was calculated as

$$\text{ROM}_x = 100 - \text{CP} - \text{LCFA} - \text{ash} - \text{starch} - \text{NDF}_x, \quad [2]$$

where all values are expressed as a percentage of DM, ROM_x was calculated using the 4 different NDF methods, and NDF_x was from the 4 different methods (i.e., NDF, NDF_{om}, NDF_{cp}, and NDF_{om+cp}). Total-tract digestibilities were then calculated for the 4 different NDF fractions and the 4 different resulting ROM fractions.

Experiment 1 (Faulkner and Weiss, 2017) was designed to examine interactions between source of fiber (conventional forage-based diet or by-product-based diet) and source of supplemental trace minerals (sulfate or hydroxy; Micronutrients Inc., Indianapolis, IN) on nutrient digestibility. The experiment was a split-plot, Latin square design (fiber was whole plot, minerals was split plot) with 9 cow periods per dietary treatment. Experiment 2 was designed to determine digestibility of nutrients from diets comprising silage made from 3 different corn hybrids (W. P. Weiss, unpublished data): a conventional dual-purpose corn (Hy-A), a brown midrib corn (Hy-B), and an experimental brown midrib corn (Hy-C). The experiment was a replicated, incomplete 3 × 2 Latin square with 4 cows per treatment. Ingredients and chemical composition for the diets and ingredients are shown in Tables 1–5.

The ROM calculated from experiments 1 and 2 was combined with data generated from 8 other experiments conducted at the Ohio Agricultural Research

Table 1. Ingredient and nutrient composition of the diets

Item	Experiment 1[1]		Experiment 2[2]		
	Conventional	By-product	Hy-A	Hy-B	Hy-C
Ingredient					
Corn silage	44.0	20.0	45.0	45.0	45.0
Alfalfa silage	20.0	15.0	11.0	11.0	11.0
Ground corn	18.5	—	7.41	10.6	11.1
Soybean meal, 48% CP	14.9	8.5	15.5	16.4	16.5
Distillers grains	—	—	5.02	4.97	4.90
Rolled oats	—	14.3	—	—	—
Dried corn gluten feed	—	11.0	—	—	—
Dried beet pulp	—	15.0	—	—	—
Soy hulls	—	14.1	12.7	8.67	8.10
Animal or vegetable fat	0.51	0.65	0.44	0.48	0.44
Vitamins and minerals	2.09	1.45	2.93	2.88	2.96
Nutrient,[3] %					
DM	55.0	71.6	62.5	61.6	60.7
Starch	35.4	17.6	23.7	22.3	23.0
CP	14.3	14.8	16.5	17.0	17.3
NDICP	0.89	2.03	1.52	1.30	1.31
NDI-ash	0.62	1.34	0.80	0.75	0.69
LCFA	3.66	3.54	3.52	3.53	3.52
Ash	6.25	7.23	6.75	6.89	6.48

[1] Dietary treatments: conventional forage fiber diet or by-product fiber diet with supplemental Cu, Zn, and Mn from sulfate or hydroxy mineral sources.

[2] Conventional dual-purpose corn (Hy-A), a brown midrib corn (Hy-B), and an experimental brown midrib corn (Hy-C).

[3] NDICP = neutral detergent insoluble CP; NDI-ash = neutral detergent insoluble ash; LCFA = long-chain fatty acids.

and Development Center, resulting in 214 cow periods representing 36 dietary treatments (referred to as the full data set). Most studies used Latin square type designs, and digestibility was measured using the protocol outlined in Weiss et al. (2009). Samples were no longer available; therefore, ROM was calculated using only the standard NDF method. Objectives varied across experiments and included evaluation of forage sources, by-product diets, sources of supplemental fat, and mineral supplementation. Generally, the diets met or exceeded NRC (2001) recommendations for CP, fiber, and minerals.

Calculations and Statistical Analysis

Data were analyzed using PROC MIXED (SAS Institute, 2015) to evaluate the effect of NDF method and its interaction with dietary treatments on NDF and ROM apparent digestibility. For experiment 1, the model included the fixed effects of fiber (1 df), mineral source (1 df), and NDF method (3 df); the 2- and 3-way interactions of the fixed effects; and the random effects of group (2 df), period within group (2 df), cow within group × fiber (12 df), cow × group × fiber × period, and residual error. No 3-way interactions of fixed effects were significant ($P > 0.10$). For experiment 2, the model included the fixed effects of hybrid (2 df), period (1 df), and method (3 df) and the random effects of cow (5 df) and interaction of cow × hybrid × period (10 df). For both models, degrees of freedom were adjusted using the Kenward-Roger approximation. Interactions of fixed effects were evaluated using the SLICE option, and if significant ($P < 0.05$), means were separated by Fisher's least significant difference test. Because NDF methods are highly correlated and inherently measured on the same observation, the fixed effect of method was also evaluated as a repeated measure with an autoregressive covariance structure but produced almost identical results and was not further investigated.

Estimating the True Digestibility of ROM

True digestibility and metabolic fecal excretion of ROM calculated using the standard NDF method of the full data set were estimated using the Lucas test (Van Soest, 1994). Cow period data (n = 214) were used in the regression of concentration of digestible ROM on concentration of ROM using PROC MIXED with experiment and period within an experiment as random class effects (St-Pierre, 2001). The intercept and slope from the regression are estimates of metabolic fecal ROM and the true digestibility of ROM, respectively

Table 2. Concentrations of dietary carbohydrate fractions and digestibility coefficients using 4 different NDF methods (experiment 1)

Item	Treatment[1]						SEM[3]	P-value[4]	
	Conventional		SD	By-product		SD		Fiber	Source
Dietary NDF concentration,[5] % of DM									
NDF	24.6		1.01	36.2		1.24			
NDF_{om}	24.0		1.13	34.9		1.15			
NDF_{cp}	23.7		1.05	34.2		1.21			
NDF_{om+cp}	23.1		1.17	32.9		1.13			
Dietary ROM concentration,[6] % of DM									
ROM	15.7		1.58	20.6		1.20			
ROM_{om}	16.4		1.59	21.9		1.06			
ROM_{cp}	16.7		1.56	22.6		1.14			
ROM_{om+cp}	17.3		1.59	23.9		1.00			
	Sulfate	Hydroxy		Sulfate	Hydroxy				
DMI, kg/d	24.5	24.3		23.9	24.0		1.40	0.76	0.96
NDF digestibility coefficient								M: <0.01	F×M: <0.01
NDF	0.414^a	0.440^a		0.495^a	0.507^a		0.013	0.01	0.05
NDF_{om}	0.424^b	0.451^b		0.510^b	0.521^b		0.014	0.01	0.06
NDF_{cp}	0.423^b	0.450^b		0.496^a	0.507^a		0.013	0.01	0.06
NDF_{om+cp}	0.434^c	0.463^c		0.512^b	0.521^b		0.014	0.01	0.07
ROM digestibility coefficient								M: <0.01	F×M: <0.01
ROM	0.632^a	0.610^a		0.725^a	0.714^a		0.016	0.01	0.28
ROM_{om}	0.607^b	0.583^b		0.680^c	0.677^c		0.015	0.01	0.37
ROM_{cp}	0.606^b	0.583^b		0.699^b	0.696^b		0.014	0.01	0.34
ROM_{om+cp}	0.584^c	0.558^c		0.660^d	0.664^d		0.014	0.01	0.40
Digestible NDF, % of DM								M: <0.01	F×M: <0.01
NDF	9.68^a	10.6^a		17.9^a	18.1^a		0.454	0.01	0.13
NDF_{om}	9.78^a	10.5^a		17.6^a	18.0^a		0.441	0.01	0.13
NDF_{cp}	9.46^a	10.5^a		17.0^b	17.0^b		0.458	0.01	0.16
NDF_{om+cp}	9.62^a	10.3^a		16.7^b	16.9^b		0.413	0.01	0.15
Digestible ROM, % of DM								M: <0.01	F×M: <0.01
ROM	8.72^a	7.99^a		12.5^a	12.6^a		0.456	0.01	0.49
ROM_{om}	8.72^a	8.02^a		12.6^a	12.8^a		0.442	0.01	0.50
ROM_{cp}	8.87^a	8.15^a		13.4^b	13.7^b		0.429	0.01	0.56
ROM_{om+cp}	8.88^a	8.17^a		13.6^b	13.8^b		0.412	0.01	0.55

[a–d] Values within the same column, dietary treatment, and NDF or residual OM (ROM) digestion measurement with the same superscripts are similar ($P > 0.05$).

[1] Conventional sulfate or hydroxy = conventional forage fiber diet with supplemental Cu, Zn, and Mn from sulfate or hydroxy mineral sources. By-product sulfate or hydroxy = by-product fiber diet with supplemental Cu, Zn, and Mn from sulfate or hydroxy mineral sources.

[2] Standard deviations of concentrations determined by Monte Carlo simulation.

[3] Data were unbalanced because of 1 missing observation; therefore, the greatest SEM values were reported.

[4] Where M = P-value for method; F×M = P-value for fiber × method interaction.

[5] NDF = NDF analysis with amylase and sulfite; NDF_{om} = NDF − NDI-ash; NDF_{cp} = NDF − NDICP; NDF_{om+cp} = NDF − NDI-ash − NDICP. (NDI-ash = NDF residues ashed in a muffle oven at 600°C overnight; NDICP = neutral detergent insoluble CP.)

[6] ROM_x (% of DM) = 100 − % CP − % long-chain fatty acids − % ash − % starch − % NDF_x, where x = NDF method.

(Van Soest, 1994). The same data and NDF method were used in regressions with digestible NFC and NFC (calculated as 100 − NDF − CP − LCFA − ash) and digestible starch and starch. The slopes and intercepts of these regressions (ROM, NFC, and starch) were compared using paired t-tests. The same regression analyses using ROM calculated with each of the 4 different NDF methods (experiments 1 and 2 only) were also conducted to determine the effect of NDF method on true digestibility of ROM. The Y-intercepts of the 4 regressions were the same ($P > 0.19$), so a common intercept model was used to estimate true digestibility of ROM for each method. Slopes were evaluated using contrast statements (effect of ash correction, effect of CP correction, and NDF_{om} vs. NDF_{cp}). Simple statistics for the full data set were calculated using PROC UNIVARIATE (Table 6).

RESULTS AND DISCUSSION

Composition

The ROM concentration of corn silage ranged from about 12 to 16% of DM; concentrations in alfalfa silage were higher, ranging from about 20 to 30% (Table 4). Concentrations of ROM in predominantly corn grain

Table 3. Concentrations of diet carbohydrate fractions and digestibility coefficients using 4 different NDF methods (experiment 2)

	Diet[1]							
Item	Hy-A	SD[3]	Hy-B	SD	Hy-C	SD	SEM	Hybrid *P*-value[2]
Dietary NDF concentrations,[4] % of DM								
NDF	34.4	1.38	33.1	1.19	33.4	1.42		
NDF_{om}	33.6	1.44	32.4	1.20	32.8	1.39		
NDF_{cp}	32.9	1.10	31.8	1.05	32.1	1.20		
NDF_{om+cp}	32.1	1.07	31.1	0.97	31.4	1.08		
Dietary ROM concentration,[5] % of DM								
ROM	15.1	1.44	17.1	0.78	16.3	0.54		
ROM_{om}	15.9	1.39	17.9	0.63	17.0	0.54		
ROM_{cp}	16.6	1.90	18.4	1.00	17.6	0.86		
ROM_{om+cp}	17.4	1.84	19.2	0.85	18.3	0.86		
DMI, kg/d	25.5		25.9		25.6		1.53	0.96
NDF digestibility coefficient								M: <0.01
NDF^{a}	0.532		0.511		0.527		0.033	0.75
NDF_{om}^{b}	0.539		0.516		0.534		0.034	0.68
NDF_{cp}^{b}	0.538		0.521		0.532		0.040	0.82
NDF_{om+cp}^{c}	0.545		0.526		0.540		0.041	0.75
ROM digestibility coefficient								M: <0.01
ROM^{a}	0.750		0.774		0.755		0.035	0.89
ROM_{om}^{b}	0.723		0.756		0.732		0.030	0.79
ROM_{cp}^{c}	0.703		0.751		0.706		0.035	0.68
ROM_{om+cp}^{cd}	0.695		0.723		0.708		0.025	0.77
Digestible NDF, % of DM								M: <0.01
NDF^{a}	17.9		16.8		17.4		1.08	0.59
NDF_{om}^{a}	17.8		16.5		17.3		1.13	0.48
NDF_{cp}^{b}	17.5		16.5		16.9		1.37	0.60
NDF_{om+cp}^{b}	17.3		16.2		16.8		1.36	0.54
Digestible ROM, % of DM								M: <0.01
ROM^{a}	11.4		13.6		12.4		0.571	0.19
ROM_{om}^{a}	11.4		13.8		12.5		0.380	0.06
ROM_{cp}^{ab}	11.5		14.0		12.7		0.486	0.06
ROM_{om+cp}^{c}	12.0		14.1		13.1		0.342	0.07

[a–d] NDF methods within an NDF or residual OM (ROM) measure with similar superscript letters do not differ ($P > 0.05$).

[1] Conventional dual-purpose corn (Hy-A), a brown midrib corn (Hy-B), and an experimental brown midrib corn (Hy-C).

[2] Where M = *P*-value for method.

[3] Standard deviations of concentrations determined by Monte Carlo simulation.

[4] NDF = NDF analysis with amylase and sulfite; NDF_{om} = NDF − NDI-ash; NDF_{cp} = NDF − NDICP; NDF_{om+cp} = NDF − NDI-ash − NDICP. (NDI-ash = NDF residues ashed in a muffle oven at 600°C overnight; NDICP = neutral detergent insoluble CP.)

[5] ROM_x (% of DM) = 100 − % CP − % long-chain fatty acids − % ash − % starch − % NDF_x, where x = NDF method.

and soybean meal mixes averaged about 10% and more than 20% in concentrates based on high-fiber by-products (Table 5). For alfalfa silage, concentrations of NFC and ROM were similar, but ROM was substantially smaller than NFC for corn silage and the concentrates. The smaller concentration of ROM compared with NFC will reduce the influence this fraction has on estimated energy values. Because of the heterogeneous nature of both NFC and ROM, a smaller fraction may result in less error when estimating energy.

When correcting for ash and CP, the change in NDF or ROM concentrations was not constant. Corrections had a greater effect for alfalfa than for corn silage and a greater effect for by-product-based concentrate mixes than for corn- and soybean-based mixes (Tables 4 and 5). The NDI-ash of forages typically ranges from about 15 to 25% of the total ash content but can differ based on the amount of soil contamination or harvesting methods (Mertens, 2016). The NDICP content of forages is also dissimilar (5–40% of the total CP; Krishnamoorthy et al., 1982) and is affected by heat damage (Weiss et al., 1986), kernel processing (Weiss and Wyatt, 2000), plant varieties, maturities, and growing conditions (Valk et al., 1996; Nordheim-Viken and Volden, 2009). In this study, the NDICP averaged 1.9 and 7.6% of total NDF for corn silage and alfalfa silage, respectively, and NDI-ash averaged 1.6 and 4.6% of the total NDF, respectively. For corn silages, correcting for CP or ash contamination was similar quantitatively, but for concentrates, correcting for CP contamination typically had a greater effect on NDF (and subsequent ROM) than did correcting for ash. The contamination of NDF by CP was much greater than ash in the alfalfa silage in experiment 2, but ash and CP contamination were

Table 4. Analyzed nutrient composition of forages for experiments 1 and 2

	Experiment 1				Experiment 2[1]							
					Corn silage hybrid							
Item	Corn silage	SD[2]	Alfalfa silage	SD	Hy-A	SD	Hy-B	SD	Hy-C	SD	Alfalfa silage	SD
NDF concentration,[3] % of DM												
NDF	31.9	1.27	36.0	1.27	38.0	2.22	40.0	1.13	41.3	1.99	46.5	1.82
NDF_{om}	31.5	1.26	34.0	1.66	37.3	2.27	39.3	1.20	40.5	1.85	44.8	2.23
NDF_{cp}	31.2	1.26	34.1	1.33	37.1	1.79	39.4	1.18	40.7	1.83	41.8	0.80
NDF_{om+cp}	30.9	1.25	32.1	1.76	36.4	1.84	38.7	1.26	39.9	1.69	40.2	0.39
ROM concentration,[4] % of DM												
ROM	15.7	1.68	27.2	1.72	11.0	1.05	14.9	0.59	15.2	0.32	19.9	2.72
ROM_{om}	16.1	1.69	29.3	1.71	11.7	1.00	15.7	0.52	15.9	0.46	21.5	2.31
ROM_{cp}	16.4	1.64	29.2	1.67	11.9	1.49	15.6	0.54	15.8	0.48	24.5	5.34
ROM_{om+cp}	16.7	1.64	31.2	1.70	12.5	1.43	16.3	0.47	16.5	0.62	26.1	4.93
Starch, % of DM	39.3	1.83	1.56	0.23	37.2	3.51	31.0	0.78	30.3	1.83	1.30	0.59
NFC,[5] % of DM	55.0	1.70	28.8	1.65	48.2	2.46	45.9	0.19	45.4	1.51	21.2	2.13

[1] Conventional dual-purpose corn (Hy-A), a brown midrib corn (Hy-B), and an experimental brown midrib corn (Hy-C) (n = 2).

[2] Standard deviation of samples taken during the experiment.

[3] NDF = NDF analysis with amylase and sulfite; NDF_{om} = NDF − NDI-ash; NDF_{cp} = NDF − NDICP; NDF_{om+cp} = NDF − NDI-ash − NDICP. (NDI-ash = NDF residues ashed in a muffle oven at 600°C overnight; NDICP = neutral detergent insoluble CP.)

[4] Residual OM. ROM_x (% of DM) = 100 − % CP − % long-chain fatty acids − % ash − % starch − % NDF_x, where x = NDF method.

[5] NFC (% of DM) = 100 − % CP − % long-chain fatty acids − % ash − % NDF.

Table 5. Nutrient composition of concentrate mixes for experiments 1 and 2

	Experiment 1[1]				Experiment 2[2]					
Item	Conventional concentrate	SD	By-product concentrate	SD	Hy-A	SD	Hy-B	SD	Hy-C	SD
NDF concentration,[3] % of DM										
NDF	9.4	0.55	37.6	1.22	27.7	0.42	22.7	1.10	22.2	0.74
NDF_{om}	9.2	0.69	36.1	1.00	27.0	0.39	22.1	0.93	21.8	0.70
NDF_{cp}	8.8	0.62	35.2	1.17	26.3	0.47	21.5	0.99	21.0	0.66
$\text{NDF}_{\text{om+cp}}$	8.6	0.75	33.7	0.94	25.6	0.45	21.0	0.82	20.6	0.62
ROM concentration,[4] % of DM										
ROM	9.4	1.38	20.5	0.92	18.1	1.52	18.7	0.49	16.6	0.21
ROM_{om}	9.6	1.40	22.0	0.71	18.8	1.54	19.2	0.33	17.0	0.17
ROM_{cp}	10.1	1.43	23.0	0.86	19.5	1.47	19.8	0.39	17.8	0.13
$\text{ROM}_{\text{om+cp}}$	10.3	1.46	24.5	0.65	20.2	1.49	20.3	0.22	18.2	0.09
Starch, % of DM	49.4	1.51	14.7	0.21	15.5	1.80	18.8	0.35	20.9	0.79
NFC,[5] % of DM	58.9	0.71	35.2	0.82	33.6	0.27	37.5	0.84	37.4	0.59

[1] Conventional concentrate fed in a typical-forage-based diet; By-product concentrate fed in a low forage, high by-product-based diet (n = 6).

[2] Conventional dual-purpose corn (Hy-A), a brown midrib corn (Hy-B), and an experimental brown midrib corn (Hy-C) (n = 2).

[3] NDF = NDF analysis with amylase and sulfite; NDF_{om} = NDF − NDI-ash; NDF_{cp} = NDF − NDICP; $\text{NDF}_{\text{om+cp}}$ = NDF − NDI-ash − NDICP. (NDI-ash = NDF residues ashed in a muffle oven at 600°C overnight; NDICP = neutral detergent insoluble CP.)

[4] Residual OM. ROM_{x} (% of DM) = 100 − % CP − % long-chain fatty acids − % ash − % starch − % NDF_{x}, where x = NDF method.

[5] NFC (% of DM) = 100 − % CP − % long-chain fatty acids − % ash − % NDF.

almost identical in the alfalfa silage used in experiment 1. The alfalfa silage in experiment 2 was drier (54.4 vs. 39.8% DM), which is related to increased heat damage.

The full correction (CP and ash) decreased NDF on average 3.6% for corn silages and 13.9% for alfalfa silages, whereas the average increase for ROM was 8.6 and 18.3%, respectively (Table 4). For concentrate mixes, the full correction reduced NDF content by 9.0%, whereas the average increase in ROM was 10.3% (Table 5). The percentage change varied based on the amount of by-products in the concentrate mixes (especially distillers grains and corn gluten feed) because of their large NDICP content (Krishnamoorthy et al., 1982; DePeters et al., 1997).

Table 6. Descriptive statistics of the full data set (n = 214 cow observations from 36 treatment means)

Item	Mean	SD	10th–90th percentile
Concentration, % of DM			
NDF[1]	33.3	7.40	24.4–39.2
CP	16.1	2.03	13.6–18.1
Ash	6.40	0.65	7.31–5.67
Long-chain fatty acids	3.96	1.22	2.87–5.87
Starch	27.0	5.69	18.2–35.0
NFC[2]	40.3	5.98	33.8–48.8
ROM[3]	13.2	4.11	7.58–18.3
DMI, kg	22.2	4.38	15.8–26.9
Digestibility coefficient			
DM	0.666	0.032	0.626–0.705
Starch	0.912	0.043	0.857–0.971
NFC	0.841	0.047	0.784–0.898
ROM	0.651	0.260	0.473–0.825

[1] NDF = NDF analysis with amylase and sulfite.

[2] NFC (% of DM) = (100 − % CP − % long-chain fatty acids − % ash − % NDF).

[3] Residual OM (% of DM) = (100 − % CP − % long-chain fatty acids − % ash − % starch − % NDF).

In experiment 1, total dietary NDF concentration decreased 6.5 and 10.0% for the conventional and by-product-based diets, respectively, when ash and CP contamination was removed. Conversely, ROM increased 11.8 and 13.8%, respectively. In experiment 2, the total diet NDF concentration decreased 6.7% on average and ROM content increased 11.7%, similarly to the conventional diet. Overall, with full correction of ash and CP contamination, NDF concentration decreased about 2 percentage units with a concomitant increase in ROM concentration.

Digestibility

Type of NDF method affected ($P < 0.01$) the digestibility of NDF and ROM (Tables 2 and 3). In all diets, digestibility of NDF was less ($P < 0.05$) than digestibility of NDF_{om} and $\text{NDF}_{\text{om+cp}}$ (Tables 2 and 3). Crocker et al. (1998) also reported that digestibility of NDF_{om} was greater than digestibility of NDF, and for most of their diets the differences were quantitatively similar to the differences we observed. Differences between digestibility of NDF and NDF_{cp} were not consistent across diets. Digestibility of NDF and NDF_{cp} were similar for by-product-based diets in experiment 1, but digestibility of NDF_{cp} was greater ($P < 0.05$) than NDF digestibility for the other diets in experiments 1 and 2 (i.e., method × fiber source treatment interaction for experiment 1; $P < 0.01$). The effect of NDF method on ROM digestibility was the opposite of what was observed for NDF.

Although the effect of method on NDF and ROM digestibility was statistically significant, quantitatively the effects were not great. Adjusting for both ash and CP resulted in the greatest increase in NDF digestibility. However, even full correction increased NDF digestibility only by 2.4 to 5% (average correction across diets = 3.4%) compared with uncorrected NDF. Full correction of NDF resulted in a 6.5 to 9% increase in ROM apparent digestibility (average increase = 7.4%). Correcting NDF for ash and CP contamination did not have a substantial average effect on NDF digestibility. However, the observed NDF method ×diet interactions on NDF and ROM digestibility limit extrapolation of that conclusion to diverse diets.

The effects of removing ash and CP from NDF on concentrations of NDF was opposite the effects observed on digestibility of those fractions (i.e., removing contamination reduced the concentration of NDF but increased the digestibility of NDF). The same was true for ROM. Therefore, the effects of method on the concentration of digestible NDF or digestible ROM were generally less than the effect on concentrations or digestibility (Tables 2 and 3). Because summative energy equations (e.g., NRC, 2001) use concentrations of digestible matter (i.e., both digestible NDF and digestible ROM), NDF method had little effect on the final estimation of digestible energy. On average for the diets in Tables 2 and 3, the sum of digestible NDF and ROM was equal (30.5% of DM) for uncorrected NDF and full correction of NDF. In the worst case (by-product diets in Table 2), the difference between NDF methods for the sum of digestible NDF and ROM was <0.1% of DM.

In addition to determining whether NDF method affected digestibility of NDF and ROM, we evaluated whether method would affect statistical interpretation of dietary treatment effects. If the P-value for significance was set at $P < 0.05$, different conclusions may have been reached in experiment 1. Mineral source affected ($P < 0.05$) NDF digestibility but did not affect NDF_{om}, NDF_{cp}, or NDF_{om+cp} digestibility (Table 4). However, method did not affect interpretation of the effect of fiber source on NDF digestibility; by-product diets had greater ($P < 0.05$) NDF digestibility than forage diets regardless of method. For experiment 2, the same conclusion relative to NDF digestibility was reached regardless of NDF method (i.e., hybrid did not affect NDF or ROM digestibility; Table 2). However, different treatment conclusions may have been reached relative to ROM digestibility. Based on the limited number of diets we evaluated, treatment inferences were similar for the different measures of NDF, but more diets with greater or more variable amounts of NDI-ash and NDICP should be investigated.

Estimating True Digestible ROM

For a nutrient fraction to be incorporated into a summative energy equation, its true digestibility and contribution to metabolic fecal excretion must be known. The Lucas test can be used to estimate true digestibility when a fraction displays nutritional uniformity over a range of feedstuffs or diets and has a nonnegative metabolic fecal content and a true digestibility <1 (Lucas, 1964; Van Soest, 1994). The full data set (10 digestibility experiments, 38 treatments; Table 6) was used to estimate the true digestibility coefficient of ROM (i.e., the slope) and metabolic fecal ROM (i.e., Y-intercept). When random effects of experiment and period within an experiment were included in the model, the Lucas test confirmed that ROM behaved as a nutritionally uniform fraction (Figure 1; Table 7). The Y-intercept was negative and different from zero ($P < 0.01$), indicating that ROM has a significant metabolic fecal component. Starch and NFC also approximated nutritionally uniform fractions, but true digestibility and the metabolic fecal fraction differed between the 3 fractions (Table 7).

Metabolic fecal excretion of ROM was greater ($P < 0.01$) than that for NFC (Tables 6 and 7). In theory, the metabolic fecal extraction of NFC and ROM should be essentially the same because starch does not have a metabolic fecal component. However, the estimates were statistically different ($P < 0.01$). The lower intercept for NFC compared with ROM could in part be a statistical issue. The NFC intercept has a large standard

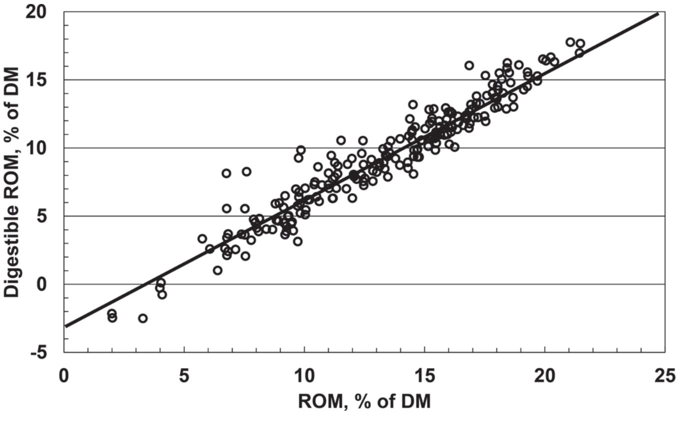

Figure 1. Relationship between residual OM (ROM) and digestible ROM concentrations (% of DM) using the standard NDF method of the cumulative data set (Y = −3.43 + 0.961X). Each point represents a cow period (n = 214) and is adjusted for random effects of experiment and period within an experiment (St-Pierre, 2001).

Table 7. Lucas test[1] for the full data set and data from experiments 1 and 2

Item	Intercept[2]	SEM	Slope[3]	SEM	BIC[4]
Full data set[5]					
Digestible ROM[6]	−3.43[a]	0.295	0.961[a]	0.021	688
Digestible NFC[6]	−2.08[b]	0.800	0.903[b]	0.020	806
Digestible starch[7]	−0.17[c]	0.359	0.931[ab]	0.013	578
Experiments 1 and 2[8]					
Common intercept	−3.76	0.604			204
Digestible ROM			0.985[a]	0.032	
Digestible ROM_{om}			0.909[b]	0.032	
Digestible ROM_{cp}			0.938[ab]	0.031	
Digestible $\text{ROM}_{\text{om+cp}}$			0.874[b]	0.025	

[a–c] Values in the same column and data set followed by different superscripts are significantly different ($P < 0.05$).

[1] Regression of dietary concentration and digestible concentration (% of DM) of a nutrient.

[2] The estimate of the metabolic fecal content (g/100 g of DMI).

[3] An estimate of the true digestibility coefficient.

[4] Bayesian information criterion.

[5] The full data set (n = 214) using the standard NDF method.

[6] Residual OM (ROM) = NFC − starch. NFC (% of DM) = 100 − % CP − % long-chain fatty acids − % ash − % NDF.

[7] Y-intercept was not different from zero ($P < 0.65$).

[8] Experiments 1 and 2 data set measuring NDF using all 4 methods (n = 47). ROM_x (% of DM) = 100 − % CP − % long-chain fatty acids − % ash − % starch − % NDF_x, where x = NDF method.

error (SE divided by the intercept is 4.5 times greater for NFC than for ROM) and has a greater extrapolation distance to zero than does ROM. The overall fit as assessed with the Bayesian information criterion was better for ROM and starch compared with NFC. The combination of better fit and smaller pool size (i.e., concentration in the diet) for ROM compared with NFC should result in more precise estimates of energy when incorporated into summative equations.

The NRC (2001) true digestibility coefficient of NFC at maintenance intake was set at 0.98, which is substantially higher than true digestibility of NFC in this experiment (Equation 4; Table 7). Van Soest (1994) and Girard and Dupuis (1988) reported that true digestibility of NFC can range from 0.83 to 0.98 depending on intake. The average DMI in this data set was approximately 3.5 times maintenance (Table 6). After applying the NRC (2001) discount, the estimated true digestibility of NFC in our data set approximated the NRC (2001) value. Variation is partially caused by intake, but variation in starch digestibility (Ferraretto et al., 2013) also contributes to the variation in NFC digestibility. Many of the components of ROM, such as organic acids and sugars, should be essentially completely digested, which could explain the greater true digestibility of ROM compared with NFC (Table 7). However, other components, such as waxes, would be poorly digested, suggesting that our estimate of true digestibility of ROM could be too high.

In the data set measuring ROM using 4 different NDF methods, all intercepts were different from zero ($P < 0.05$), but method did not affect ($P > 0.19$) estimated metabolic fecal ROM (Table 7). A common intercept was then calculated (−3.78) and was similar to the value obtained for the full data set (Equation 3; Table 7). However, method did affect ($P < 0.01$) the estimates of true digestibility (Table 7). Estimated true digestibility of ROM calculated with NDF corrected for ash or CP contamination was lower than that for ROM calculated using uncorrected NDF (Figure 2).

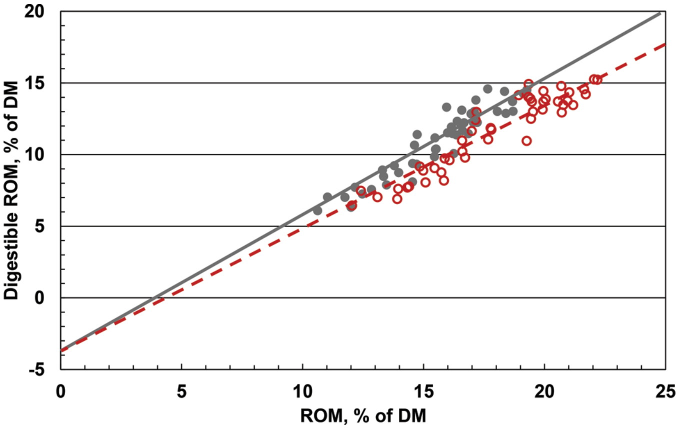

Figure 2. Relationship between residual OM (ROM) and digestible ROM concentrations (% of DM) using the standard NDF (filled circle, solid line, online gray; Y = −3.76 + 0.985X) and the ash- and CP-free NDF (open circle, dashed line, online red; Y = −3.76 + 0.874X) methods. Each point represents a cow period (n = 47) and is adjusted for random effects of experiment and period within an experiment (St-Pierre, 2001). Color version available online.

This suggests that ash and CP contaminated the fiber as it passed through the digestive tract, resulting in a decrease in apparent fecal ROM when NDF is not corrected for ash and CP contamination.

Correcting NDF for ash and CP contamination should result in the most accurate estimates of diet energy because it avoids double subtraction of NDI-ash and NDICP and eliminates the error in measuring NDF digestibility caused by contamination of the fraction as it flows through the digestive system. This correction is also biologically relevant because ash does not contribute to digestible energy. However, the number of diets available to generate Equation 9 was limited, resulting in a greater standard error and less confidence in the coefficients than the equation using NDF in the full data set (Equation 3). Quantitatively, the differences in estimating digested ROM will usually not be great between Equations 3 and 9. For example, the greatest difference between ROM and $\mathrm{ROM}_{\mathrm{om+cp}}$ concentrations was observed for the by-product diet in experiment 1 (Table 2), but the estimated digestible $\mathrm{ROM}_{\mathrm{om+cp}}$ was only about 4% greater than estimated digestible ROM.

Integrating ROM into Energy Models

Incorporating ROM into the summative equation used by NRC (2001) requires replacing the NFC term (0.98 × NFC, where NFC is expressed as percentage of DM and is calculated using $\mathrm{NDF}_{\mathrm{cp}}$) with a term for starch and a term for ROM:

$$(\text{starch digestibility} \times \text{starch}) + 0.96 \times \text{ROM} - \text{metabolic fecal ROM} \quad [10]$$

or

$$(\text{starch digestibility} \times \text{starch}) + 0.87 \times \mathrm{ROM}_{\mathrm{om+cp}} - \text{metabolic fecal } \mathrm{ROM}_{\mathrm{om+cp}}. \quad [11]$$

Note that the total metabolic fecal fraction would comprise more than ROM (e.g., metabolic fecal nitrogen) but that the ROM contribution to the metabolic fraction would vary depending on NDF method.

CONCLUSIONS

When correcting for ash and CP in NDF and ROM fractions, their digestibilities increased and decreased, respectively, and the effects were greater for diets based on by-products (i.e., significant diet interaction). Removing starch from NFC to produce the ROM fraction increased the precision for estimating true digestibility and metabolic fecal excretion. Correcting NDF for ash and protein contamination reduced the true digestibility of the resulting ROM fraction. The data provided in this paper allow for replacing NFC with starch and ROM in summative energy equations, but the equation used depends on whether NDF was corrected for ash and protein contamination. Additional data on the apparent digestibility of $\mathrm{ROM}_{\mathrm{om+cp}}$ are needed to increase the precision of the estimated true digestibility and metabolic fecal fraction.

ACKNOWLEDGMENTS

The authors thank Donna Wyatt (The Ohio State University) for assistance in conducting many of the assays and calculations and for organizing the data. They also thank Kevin Miller and the rest of the Ohio Agriculture Research and Development Center Krauss Dairy Farm staff for animal care and assistance during the digestion trials.

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