Fecal particle dry matter and fiber distribution of heifers fed ad libitum and restricted with low and high forage quality

K. Kljak,¹ B. S. Heinrichs,² and A. J. Heinrichs^{2*}

¹Department of Animal Nutrition, Faculty of Agriculture, University of Zagreb, 10000 Croatia

²Department of Animal Science, The Pennsylvania State University, University Park 16802

ABSTRACT

Wet sieving of manure can be used as a means of evaluating the nutrient utilization of diets by dairy cows. A commercially available system for this analysis (Nasco Digestion Analyzer, Nasco, Fort Atkinson, WI; NDA) employs principles of wet sieving for on-farm assessment. The objective of this study was to evaluate effects of diet manipulations and sampling techniques on fecal particle dry matter (DM), neutral detergent fiber (NDF), and starch distribution on NDA sieves. Eight Holstein heifers (means $\pm$ SD; age 18.4 ± 0.6 mo and BW 457.2 ± 27.3 kg), were randomly assigned to a split-plot 4×3 incomplete Latin square experimental design with 18-d periods (15 d of adaptation and 3 d of sampling). Treatment rations differed in forage quality (high ADF $<35\%$, low ADF $>35\%$) and fiber content (high NDF $>45\%$, low NDF $<40\%$) and were offered to heifers for ad libitum and restricted intakes. Diets were fed to allow 900 to 1,000 g/d of body weight gain and fed once daily. Fecal grab samples were collected 0, 6, 12, and 18 h after feeding all 3 d of the sampling period, and the remaining feces was collected in the manner of total fecal collection to represent a daily composite sample. After wet sieving of each sample using a NDA kit, the retained material on sieves was dried and analyzed for DM, NDF, and starch content. Sampling day and feeding regimen did not affect distributions of nutrients. Distributions of total fecal particle DM were greater for high forage quality and high fiber content diets (14.66 to 20.37% of sample DM). The NDF content in retained material decreased with decreasing sieve size (from 89.81 to 86.86%) and was not affected by forage quality and fiber content, whereas NDF retained on NDA (% DM) followed the same pattern as the retained particle DM weight. Starch content in retained material was below 0.5% in the majority of diets. Average retained fecal particle DM and NDF

content varied depending on the time after feeding, resulting in the large differences between grab fecal samples and a daily total composite sample. However, average values of retained particle DM and NDF weight for all sampling time points were similar to ones from total daily composite sample.

Key words: feces sieving, fiber content, forage quality, dairy heifer

INTRODUCTION

Proper rumen function is essential for dairy animal performance, and information obtained with manure evaluation may provide information regarding site and extent of both digestion and fermentation of consumed rations (Hall, 2005). Because manure evaluation includes assessment of both appearance and particle size, a greater proportion of undigested grain and long forage particles may indicate poor rumen fermentation. This is possible because the particle size of feces reflects the particle size of digesta leaving the rumen, as very little particle size reduction occurs in the lower tract (Rinne et al., 2002). Therefore, evaluation of manure can be a useful on-farm diagnostic tool for monitoring nutritional status in dairy cattle.

Jalali et al. (2015) showed that both animal and dietary characteristics strongly affect fecal particle size in small ruminants; particle size increased with increasing BW, forage maturity at harvest, ADL to NDF ratio in forage, concentrate supplementation, and forage NDF intake. However, diet particle size alone had little influence on fecal particle size in lactating cows (Maulfair et al., 2011). Furthermore, Hall (2005) observed that the appearance of large fiber particles or abundant ground grain in feces may be related to a variety of factors that affect the rate of degradability, resulting in the overall reduction in digestibility of the ration.

Measurement of fecal particle procedures commonly involved in scientific research include wet sieving (Rinne et al., 2002; Maulfair et al., 2011) or dry sieving of freeze-dried samples (Rustas et al., 2010; Jalali et al., 2015). Both procedures are time-consuming and

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*Corresponding author: ajh@psu.edu

demand laboratory conditions, and thus could not be routinely performed on farms. Hall (2002) described on-farm fecal sieving using a screen or kitchen strainer with 1.66-mm openings and a steady stream of water as an easy procedure to evaluate manure. Furthermore, a commercially available system, the Nasco Digestion Analyzer (NDA; Nasco, Fort Atkinson, WI), also employs principles of wet sieving and uses 3 stainless-steel screens to evaluate fecal particle size. However, although the NDA has been used for more than 10 yr, very little scientific literature on fecal particle size assessments using this tool exists. To our knowledge, only Carta (2010) and Cotanch and Darrah (2012) reported data about NDA. Whereas Cotanch and Darrah (2012) gave remarks regarding manufacturer recommendation for particle distribution, Carta (2010) compared NDA with laboratory sieving methods and observed that field measures of feces overpredicted the values found in laboratory methods, but did not conduct a detailed study to make further conclusions.

Two commonly used approaches to dairy heifer feeding include high-fiber diets fed for ad libitum consumption and limit-feeding using more nutrient dense diets that contain more digestible feedstuffs. With respect to effects of diet characteristics and intake on fecal particle size in ruminants (Jalali et al., 2015), a feeding regimen could affect dairy heifers' fecal particle size distribution as measured by NDA. Our hypothesis in this study was that fecal DM and fiber distribution on NDA sieves varies throughout day and depending on diet manipulations. Hence, this study analyzed effects of feeding regimen, forage quality, and diet fiber content on NDA particle size distribution of feces from dairy heifers. Additionally, different sampling times were evaluated to determine variation during the day and whether single samples could provide results similar to the daily composited fecal output.

MATERIALS AND METHODS

Animals and Feeding

All procedures involving the use of animals were approved by the Pennsylvania State University Institutional Animal Care and Use Committee (Institutional Animal Care and Use Committee #46266). Eight pregnant Holstein heifers, averaging 18.4 ± 0.6 mo and 457.2 ± 27.29 kg of BW at the beginning of the experiment, were randomly assigned to a split-plot 4×3 incomplete Latin square experimental design. The whole plot was ad libitum or restricted feeding, whereas subplots were 2 different forage quality levels and 2 different NDF contents in treatment rations. Forage quality was evaluated based on ADF content; ADF

<35% was considered high forage quality and ADF >35% was low forage quality. Corn silage was a major component of the forage for high, whereas grass hay was used for the low-quality diets. Variability in NDF content was achieved by adding wheat middlings and cottonseed hulls. Each of 3 experimental periods was 18 d in length, consisting of 15 d of adaptation and 3 d of sampling. During all periods, heifers were housed in individual tiestalls with rubber mattress bedding in a mechanically ventilated barn. Heifers had free access to water at all times and were released for exercise in a paved pen 3 to 4 h/d on nonsampling days.

All diets were provided as TMR (Table 1) calculated to provide equal CP intake (13%) and energy requirements based on the NRC (2001) for pregnant heifers. For restricted feeding, energy intake was calculated to provide 0.20 Mcal of ME/kg of BW^{0.75}. Rations were fed once daily at noon. Amount of TMR offered was adjusted weekly based on BW to allow an ADG of 1,000 g/d. Heifers were weighed on d 0, 1, 7, and 8; the value for each day was the average of 2 measurements at 0800 and 1130 h on the same day. For ad libitum feeding, refusals were collected daily and weighed during the sampling period.

Single batches of ration concentrate ingredients were stored to last for the length of the experiment. The concentrate mix was prepared for each treatment at the beginning of each experimental period in a drum mixer (Calan Super Data Ranger, American Calan, Northwood, NH). Using the same equipment, diets were mixed using the concentrate mix and corn silage or grass hay for each treatment every 5 d. An additional 30% of water by weight of grass hay was added to the grass hay diets to reduce dust and improve mixing in TMR. Daily intakes for each heifer were kept in closed and pressed nylon bags to avoid oxygenation and stored in a cooler at 6°C until fed.

Sample Collection and Analysis

Feedstuffs were collected before every period, and TMR was collected during mixing day just before the sampling period, dried in a forced-air oven at 55°C for 48 h, and ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA) for further analysis. By weight, composited and dried feed samples were analyzed by Cumberland Valley Analytical Services, Inc. (Hagerstown, MD) to determine DM, CP, soluble protein, and crude fat (AOAC, 1990). Contents of NDF and ADF (Van Soest et al., 1991) were analyzed using an Ankom 200 fiber analyzer (Ankom Technology Corp., Fairport, NY). Analysis of NDF included the use of heat-stable α -amylase (Ankom Technology Corp.) and sodium sulfite; NDF and ADF values include re-

Table 1. Ingredient and chemical composition of ad libitum and restricted treatment rations differing in forage quality with high (HF) and low (LF) fiber content with dietary intake of DM and major nutrients

Item	Ad libitum intake				Restricted intake			
	Low forage quality		High forage quality		Low forage quality		High forage quality	
	HF	LF	HF	LF	HF	LF	HF	LF
Ingredient, % of DM								
Grass hay	60.0	60.0	—	—	60.0	60.0	—	—
Corn silage	—	—	60.0	60.0	—	—	60.0	60.0
Wheat middlings	4.0	—	2.0	18.3	1.1	—	1.0	14.05
Ground corn	25.4	36.9	—	5.2	26.5	36.9	—	7.0
Cotton hulls	7.55	—	33.0	12.5	9	—	32.25	14.6
Canola meal	—	—	—	—	0.25	—	2.0	—
Optigen ¹	0.35	0.4	2.3	1.4	0.5	0.4	2.05	1.55
NaCl	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral mix ²	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.8
Chemical composition								
DM, %	71.14	71.29	48.38	47.41	70.18	69.55	48.19	48.53
CP, % of DM	12.79	12.85	12.87	12.83	12.88	12.85	12.83	12.88
Soluble CP, % of CP	33.96	29.05	65.02	56.06	34.92	29.05	62.44	59.35
NDF, % of DM	45.79	40.40	47.20	39.41	46.00	40.42	46.86	39.54
ADF, % of DM	28.27	23.53	33.53	23.82	28.74	23.59	33.33	24.39
Starch, % of DM	17.37	25.11	21.61	29.83	17.45	25.13	21.47	29.98
Sugar, % of DM	4.08	4.08	1.70	2.14	4.03	4.08	1.71	2.06
Crude fat, % of DM	2.89	2.88	3.66	3.91	2.86	2.88	3.68	3.86
ME, ³ Mcal/kg	2.26	2.47	2.02	2.42	2.24	2.47	2.03	2.38
ME, ⁴ Mcal/kg of BW ^{0.75}	0.23	0.24	0.25	0.29	0.20	0.20	0.20	0.20
Particle size, %								
>19.0 mm	61.75	55.95	1.62	2.21	52.72	46.51	1.38	1.40
19.0–8.0 mm	6.67	10.36	63.98	50.69	9.79	11.77	62.05	47.21
8.0–4.0 mm	10.12	6.58	17.79	16.58	9.61	10.00	19.03	18.96
<4.0 mm	21.46	27.10	16.60	30.51	27.87	21.74	17.27	32.43
Intake								
DM, kg	10.69	10.02	13.04	12.96	9.30	8.49	10.50	8.99
CP, kg	1.36	1.29	1.68	1.66	1.20	1.08	1.36	1.20
NDF, kg	4.88	4.04	6.19	5.13	4.29	3.45	4.93	3.57
Starch, kg	1.74	2.52	2.84	3.89	1.58	2.13	2.72	2.30

¹Slow-release urea (Alltech, Nicholasville, KY) contained 94.8% DM and 269.9% CP.

²Mineral mix (US Feeds, Eldora, IA) contained 94.28% DM, 11.65% CP, 1.7% soluble CP, 5.46% RUP, 8.29% ADF, 19.2% NDF, 5.5% fat, 12.4% Ca, 0.36% P, 2.63% Mg, 0.44% K, 0.39% S, 1,628.87 mg/kg of Mn, 542.71 mg/kg of Cu, 1,639 mg/kg of Zn, 232.94 mg/kg of Fe, 9.90 mg/kg of Se, 9.2 mg/kg of Co, 22.2 mg/kg of I, 70.748 IU/g of vitamin A, 17.637 IU/g of vitamin D, and 1.230 IU/g of vitamin E.

³Estimated as ME = TDN × 0.04409 × 0.82.

⁴ME expressed as Mcal/kg of metabolic BW.

sidual ash. Starch and soluble sugar were determined by the method of Hall (2009) using Hazyme enzyme (Centerchem, Norwalk, CT). Metabolizable energy intake was estimated for each heifer within each period using the observed OM intake × 0.04409 × 0.82 (NRC, 2001).

Fecal grab samples (approximately 500 g) were collected from the rectum of the animal on all 3 d of the sampling period 0, 6, 12, and 18 h after feeding (1200, 1800, 0000, and 0600 h). Remaining feces were collected in the manner of total fecal collection to obtain daily composite samples and total fecal output (hourly from waterproof boards and stored in airtight containers). To avoid fecal contamination with urine, noninvasive cup collectors (Lascano et al., 2010) were used during sampling days. After feeding, daily feces were mixed,

and a subsample of approximately 500 g was taken. Wet sieving of fecal samples started immediately after taking the first samples; if needed, fecal samples were refrigerated at 4°C for a short period.

Each sample was wet sieved in duplicate using items from the NDA kit (Nasco), which included 3 stainless-steel screens (top, 4.76 mm; middle, 3.17 mm; bottom, 1.59 mm), 22.8-L plastic bucket, adjustable pistol spray nozzle, and flat-bottom measuring scoop (1.89 L). Based on pretrial wet sieving, 200 g of subsample was determined to be sufficient to obtain a satisfactory distribution on each sieve. The remains of the original fecal sample were used to determine DM content by drying in a forced-air oven at 55°C for 48 h.

After attaching pistol spray nozzle, the “shower” position was set and water flow was adjusted to 10 L/

min. The same water flow was used for all samples. The subsample was mixed with water filled to the label in the flat-bottom measuring scoop; if necessary, the subsample was soaked for 10 min before mixing into solution. The feces solution was transferred to the top of the NDA sieve set positioned in the bucket. After draining of the sieves was completed, water was discarded from the bucket. Wet sieving was performed in 30-s shower intervals using circular movements from a distance that ensured complete covering of the sieve set; after each wet sieving interval, drained water was discarded from the bucket. Showering of the sieve set was performed in as many 30-s intervals as necessary until water easily passed through the top sieve. After visual inspection to ensure the absence of small particles, the top sieve was detached from the sieve set. The middle sieve was then visually inspected for the presence of small particles; if present, the remaining sieve set was showered for 30 s until small particles were washed. After removal of the middle sieve, the bottom sieve was again showered in 30-s intervals to ensure passage of clear water through the sieve. Retained particulates from each sieve were then rinsed into a funnel with rumen *in situ* bags (5 × 10 cm, 53 µm pore size; Ankom Technology Corp.) attached to the stem to collect samples. Bags were then dried in a forced-air oven at 55°C for 24 h to determine DM retained on each sieve.

Dry duplicates of each sample were combined to ensure sufficient material for further analyses. Sieve samples were ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA) or, in the case of a small amount of sample, in a coffee grinder. All sieve samples were analyzed for NDF content using an Ankom 200 fiber analyzer. Analysis included use of heat-stable α-amylase (Ankom Technology Corp.) and sodium sulfite, and calculated NDF and ADF values include residual ash. Sieve samples from the second day of each period were analyzed for starch content (Hall, 2009) but without correction for free glucose because of its absence in feces. Because analyzed samples had starch content below 3%, further analysis of all samples was not performed.

Statistical Analysis

All statistical analyses were conducted in SAS (version 9.3, SAS Institute Inc., Cary, NC) using the MIXED procedure. Dependent variables were first analyzed as a split-plot 4 × 3 incomplete Latin square design with fixed effects of period, feeding regimen, forage quality, fiber content, sampling day and time, sieve, and interactions among feeding regimen, forage quality, fiber content, sampling time, and sieve. Heifer was treated as a random effect, and repeated statement in-

cluded heifer nested in sampling period and day. Starch content was also analyzed to determine its variability; because it was analyzed only 1 d/period, sampling day was omitted from the model and repeated statement for this variable. Residual variances were assumed to be normally distributed, and all data are presented as least squares means.

Further analysis of dependent variables included determination of similarity between sampling time and composited daily samples. For that purpose, results for dependent variables were calculated as a difference between each sampling time and composited sample for each treatment ration. The best predictive model was selected based on Akaike's information criterion with intent to select the model that explained the greatest amount of variation. Akaike's information criterion is an estimate of the amount of information unexplained by a given model (Anderson et al., 2000). Because feeding regimen and fiber content were not found to have a significant effect on any dependent variables, forage quality level was selected to show variability between sampling time and composited sample.

Furthermore, to address how fecal grab sampling in overall gives sieving results compared with total collection, all dependent variables were averaged across sampling time points and compared with composited sample using the MIXED model previously described but with collection method instead of sampling time in fixed effects. Least squares means and standard errors were determined using the LSMEANS statement, and differences of least squares means were determined using the PDIF statement. Differences were declared significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Wet-Sieving Procedure

Nasco's procedure for fecal wet sieving with NDA includes washing a 2-quart sample at a 30° angle in quarterly parts until the whole sample is washed through the screens (Nasco). Additionally, in the case of plugging of the middle and bottom screens, Nasco recommends pulsating the NDA separator in a bucket three-quarters full of water past the level of the plugged screen until all water is removed. This procedure was adapted as described previously to more quickly and uniformly process a large number of samples (smaller sample size, mixed with water and washed with standardized water flow rate and washing intervals).

The top sieve was usually washed in one 30-s interval; however, some samples (mostly low forage quality and low fiber diets) needed a second washing interval. After the top sieve was washed it was removed, because

we observed passage of large particles to the middle sieve in pretrial wet sieving with prolonged washing. The middle sieve was also usually washed in one 30-s interval, and then removed for the same reason as the top sieve. Up to 2 washing intervals were sufficient to obtain clear water passing through the bottom sieve; 2 intervals were mostly needed in feces from heifers fed low forage quality.

Diets and Nutrient Intakes

Treatment rations were formulated to differ in fiber content (Table 1), and forage quality did affect ADF and starch content ($P < 0.01$). Diet starch and ADF were not different between feeding regimens ($P > 0.05$). Diet particle size distribution varied across sieves, especially in the longest fractions of the diet but similar between ad libitum and restricted-fed diets. Restricted-fed heifers had lower intakes of DM, CP, fiber, and starch when compared with ad libitum-fed heifers ($P < 0.05$) by design. Due to the effect of forage quality and NDF content in rations ($P < 0.05$), intake of DM, CP, and fiber was higher for high forage quality and high NDF diets regardless of the feeding regimen.

Effect of Diet Manipulations on Fecal Characteristics

Fecal particle DM and NDF distributions on NDA sieves (Table 2) were not affected by sampling day, suggesting that the variability caused by animal was minimal. Feeding regimen affected neither retained particle DM nor NDF and starch content in retained samples. Retained particle DM of fecal samples, presented as a percentage of sample DM, increased with decreasing aperture size of sieve ($P < 0.01$). The total retained weight on all 3 sieves ranged from 14.66 to 20.37% of sample DM depending on treatment ration. These values are comparable with ones obtained by Carta (2010) for the NDA separator (averaged 21.4%) and also for a laboratory sieve with 6 screens (averaged 15.2%) of feces from cows in different lactation phases fed rations with corn silage. Although Carta (2010) concluded that on-farm methods may overestimate the weight of retained particles when compared with a laboratory sieve because of the creation of a fiber mat on the screen, which acts as an additional filtering system, the procedure in the present study was done to minimize this effect. Using a wet sieving procedure with 6 screens, Maulfair et al. (2011) obtained a similar range of values: 16.2 to 17.8% of sample DM larger than 1.18 mm from feces of lactating cows fed treatment rations with grass hay varying in particle size.

The percentage of DM retained on each sieve was affected by forage quality and fiber content in the diet,

regardless of ad libitum or restricted intake ($P < 0.01$; Table 2) but not particle size of the diet, similar to previous studies (Maulfair et al., 2011). Diets with higher forage quality and higher forage content produced feces with a greater fraction retained on the sieves (20.86 vs. 16.45% and 20.18 vs. 17.13%, respectively), implying more efficient particle size reduction by heifers fed lower forage quality and lower fiber content. The effect of higher forage quality may be related to the more efficient particle size reduction during rumination of longer particles in hay used in the low forage quality diets (Rustas et al., 2010). The effect of higher fiber content is in agreement with results from Schulze et al. (2014) who obtained higher fecal particulate DM in heifers fed highly digestible grass/clover silage.

Contrary to the retained particle DM, the content of NDF in retained samples decreased with decreasing aperture size of sieves regardless of treatment ($P < 0.05$). Forage quality level and fiber content did not affect NDF content in the retained sample. However, NDF content in retained DM of feces from heifers fed the ad libitum diet with low forage quality and low fiber content was 94.48 compared with an average 87.87%.

The NDF (DM total sample) retained on NDA sieves followed the same pattern as the retained particle DM weight; the retained NDF increased with decreasing aperture size of the sieves ($P < 0.01$) and was higher for high compared with low-quality forage diets (avg. 15.50 vs. 13.38% DM; $P < 0.01$) and for high compared with low forage content in diets (15.48 vs. 13.41% DM; $P < 0.01$). In accordance with the decrease of NDF content, starch content in the retained sample increased with decreasing aperture size of sieves ($P < 0.05$). However, starch content in samples retained on NDA sieves was below 2%, and in the majority of treatment rations even below 0.5%, suggesting efficient degradability of starch as would be expected in low-starch heifer diets. Efficient starch degradability is in accordance with not observed retention of visible ground corn particles from feces on NDA sieves because they may contain 6 to 18% starch (Hall, 2005). More starch was detected in fecal samples from diets with low forage quality and low fiber content. This most likely reflects effects of lower NDF intake accompanied with higher starch intake in these diets compared with diets with high forage quality and high fiber content.

Effect of Sampling Time on Fecal Characteristics

Analysis of fecal samples collected over the course of a day revealed considerable variations in the percentage of retained particle DM, NDF of the total sample, and NDF content in the retained sample depending on the time after feeding. Starch content was low and

Table 2. Fecal particle DM, NDF, and starch distribution of ad libitum and restricted feeding treatment rations differing in forage quality with high (HF) and low (LF) fiber content

Feeding regimen	Sieve	Low forage quality		High forage quality		SEM	Effect, <i>P</i> -value			
		HF	LF	HF	LF		Feeding regimen	Forage quality	Fiber content	Sampling time ¹
Retained particle DM weight, % of total sample DM										
Ad libitum	Top	2.25	1.92	1.74	2.58	0.43	0.19	<0.01	<0.01	<0.01
	Middle	3.98	3.07	3.81	4.03					
	Bottom	11.20	9.67	13.91	12.09					
Restricted	Top	3.48	2.59	3.33	4.33					
	Middle	4.44	3.16	5.43	4.27					
	Bottom	10.98	9.05	16.15	11.77					
NDF, % in retained sample										
Ad libitum	Top	87.69	96.26	90.82	89.58	2.96	0.14	0.36	0.80	<0.01
	Middle	86.71	95.33	90.47	90.10					
	Bottom	84.41	91.85	88.30	87.35					
Restricted	Top	87.73	86.10	91.55	88.40					
	Middle	87.83	84.27	91.42	89.53					
	Bottom	85.48	81.54	89.46	86.51					
Retained NDF weight, % of total sample DM										
Ad libitum	Top	1.55	1.25	0.98	1.58	0.35	0.33	<0.01	<0.01	<0.01
	Middle	3.09	2.42	2.71	2.90					
	Bottom	9.22	9.07	10.85	9.95					
Restricted	Top	2.58	1.78	2.36	2.79					
	Middle	3.45	2.28	3.95	2.90					
	Bottom	9.13	7.33	12.04	8.98					
Starch, ² % in retained sample										
Ad libitum	Top	0.26	0.00	0.09	0.27	0.34	0.64	0.02	0.03	0.33
	Middle	0.25	1.54	0.07	0.19					
	Bottom	0.72	1.64	0.07	0.21					
Restricted	Top	0.18	0.00	0.07	0.13					
	Middle	0.23	1.46	0.07	0.20					
	Bottom	0.49	1.92	0.08	0.36					

¹Time points throughout day when fecal grab samples were taken.²Results from samples for second day of each period.

similar among time points (Table 2). Daily variations of average retained particle weight, NDF content, and retained NDF of fecal samples are presented in Figures 1, 2, and 3, respectively. The highest average retained particle DM weight per NDA sieve was detected at 0 h for diets with high and 6 h after feeding for diets with low fiber content, and it was greater for diets with higher forage quality at all sampling times, reflecting the overall effect of these 2 variables on fecal particle size. Additionally, compared with lower forage quality diets, average retained particle weight in fecal samples decreased more rapidly during the time after feeding, possibly implying more rapid increase in efficiency of particle size reduction. On the other hand, high-quality diets had low content of particles larger than 19 mm, which implies that longer chewing activity would be necessary for more extensive particle size reduction and more rapid decrease of average retained particle weight in fecal samples for low-quality diets.

Similarly to the average retained particle DM weight per NDA sieve, NDF content in samples retained on

the NDA sieves was greater for diets with higher forage quality at all sampling times. Additionally, although the variations in samplings times in high forage quality diets were small, low-quality forage diets showed pronounced variability with the level of fiber content. The largest variations within a treatment during the course of the day were determined for heifers fed a ration with low forage quality and low fiber. It seems that rations prepared with hay resulted in higher variability of NDF digestibility during the day than rations prepared with corn silage. In accordance with our finding, Maulfair et al. (2011) determined that fecal NDF was affected by time after feeding in diets varying in particle size but with the same composition. Although diets in this study varied in composition as well as particle size distribution, a possible explanation for higher variability in NDF content in samples retained on NDA in low-quality diets could be attributed to the higher content of particles >19 mm. Higher proportions of these particles in hay-based diets most likely caused higher chewing and ruminating activity of heifers (Beauchemin and

Figure 1. Changes in average fecal particle weight distribution of treatment rations differing in forage quality with high (HF) and low (LF) fiber content using the Nasco Digestion Analyzer (Nasco, Fort Atkinson, WI) at different sampling time points (solid line with diamonds: low forage quality and LF; dashed line with squares: low forage quality and HF; dotted line with x: high forage quality and HF; dashed line with triangles: high forage quality and LF). Error bars represent SE. Asterisks indicate a significant difference ($P < 0.05$) among treatment rations at a given time point.

Yang, 2005), which in combination with a longer retention time due to the lower DMI resulted in lower NDF content in comparison to the corn-silage-based diets. Furthermore, it is possible that at feeding time, heifers sorted hay in favor of consumption of fine particles (Leonardi and Armentano, 2003) so NDF content in samples retained on NDA sieves slowly increased from time of feeding for the next 12 h. On the other hand, decrease in NDF content during next 12 h could be the

result of increased ruminating activity of heifers during the night (Schirrmann et al., 2012).

Grab Samples Versus Composite Sample

The most representative sample for determination of fecal particle size distribution would be collected from total daily fecal output due to the variations presented in Figures 1, 2, and 3 of this paper, or as Maulfair et al. (2011) showed for lactating dairy cows. However, total fecal collection is not easily accomplished in many cases. In some research trials, grab fecal samples are taken at 1 selected time (Rustas et al., 2010; Leiber et al., 2015; Mgbearhuruike et al., 2016) or at several points and combined into 1 representative sample (Rinne et al., 2002; Schulze et al., 2014, 2015). For this reason, our intention was to evaluate how grab samples differ from daily composite samples, or perhaps find a time point that would accurately represent this composited sample (i.e., total daily fecal output). Treatment rations were compared based on forage quality level, and resulting graphs for weight of retained particle DM on sieves, NDF content in retained sample, and retained weight of NDF are shown in Figures 4, 5, and 6, respectively.

Regardless of the analyzed variable, the largest variations and difference from the composite sample were detected for the bottom sieve. This observation reflects variability in the weight of retained particles and NDF content in the retained sample during the course of the day (Figures 1 and 2), which was highest for the bottom sieve (Table 2). Forage quality treatments were accomplished using hay and corn silage to prepare rations,

Figure 2. Changes in average distribution of NDF content in retained particulates of treatment rations differing in forage quality with high (HF) and low (LF) fiber content using the Nasco Digestion Analyzer (Nasco, Fort Atkinson, WI) at different sampling time points (solid line with diamonds: low forage quality and LF; dashed line with squares: low forage quality and HF; dotted line with x: high forage quality and HF; dashed line with triangles: high forage quality and LF). Error bars represent SE. Asterisks indicate a significant difference ($P < 0.05$) among treatment rations at a given time point.

Figure 3. Changes in average distribution of NDF content in total DM of sample of treatment rations differing in forage quality with high (HF) and low (LF) fiber content using the Nasco Digestion Analyzer (Nasco, Fort Atkinson, WI) at different sampling time points (solid line with diamonds: low forage quality and LF; dashed line with squares: low forage quality and HF; dotted line with x: high forage quality and HF; dashed line with triangles: high forage quality and LF). Error bars represent SE. Asterisks indicate a significant difference ($P < 0.05$) among treatment rations at a given time point.

Figure 4. Difference in fecal particle DM weight distribution on (A) top, (B) middle, and (C) bottom sieve of the Nasco Digestion Analyzer (Nasco, Fort Atkinson, WI) of treatment rations with high (dashed line with squares) or low (solid line with diamonds) forage quality between sampling time points and composited sample representing total daily feces. Error bars represent SE. Asterisks indicate a significant difference ($P < 0.05$) between sampling time point and composited sample for each diet.

and hence dietary effects of these forages could be the reason for larger variations during the day, rather than the difference in forage quality. In terms of this trial, those dietary effects are related to particle size distribution of the diet and hence DM and NDF intake and eating behavior of heifers throughout day (Beauchemin and Yang, 2005; Schirmann et al., 2012). Additionally, when considering digestibility of corn silage and hay, Jalali et al. (2012) showed that more digestible forage caused thinner and longer fecal particles than less digestible forage in small ruminants, which agrees with

higher proportion of particles retained on bottom sieve of high forage quality in this trial.

The NDF content in the retained sample and weight of NDF retained on NDA sieves was less variable between the grab and composite samples, suggesting that the limiting factor to determine which time point is the most suitable to represent daily fecal output is the particle DM retained on the sieves. Based on the values of difference between time points and composited sample, each time from feeding to 12 h after feeding could be considered. However, significant similarity for

Figure 5. Difference in NDF content in retained particulates on (A) top, (B) middle, and (C) bottom sieve of the Nasco Digestion Analyzer (Nasco, Fort Atkinson, WI) of treatment rations with high (dashed line with squares) or low (solid line with diamonds) forage quality between sampling time points and composited sample representing total daily feces. Error bars represent SE. Asterisks indicate a significant difference ($P < 0.05$) between sampling time point and composited sample for each diet.

all 3 sieves was detected only for grab samples taken 12 h after feeding. Thus, this time is the only one at which a grab sample will produce results for DM and NDF distribution on NDA sieves from feces from dairy heifers that closely represent the average daily fecal output. However, this would be dependent on the timing of feeding and eating that occurred in this study.

In the overall comparison of fecal grab sampling and total collection method, values obtained for each time point were averaged to simulate results of a represen-

tative sample in spot sampling. By doing this, daily variations were included in sieving results obtained by this method and differentiated from total daily composite sample only in NDF content in the retained sample (Table 3). The observed effect of feces collection method on this variable ($P < 0.05$) was most likely the result of approximately 10% higher NDF content in retained particle DM in total daily composite from heifers fed ad libitum, and in accordance with higher NDF content in retained DM of feces from heifers fed the ad libitum diet with low forage quality and low fiber (Table 2). Retained particle DM and NDF weight per NDA sieve were similar between collection methods ($P > 0.05$), although the values were somewhat higher on middle sieves and lower on bottom sieves in total daily composite regardless of feeding regimen.

CONCLUSIONS

We evaluated the Nasco Digestion Analyzer for fecal samples and found that forage quality and fiber content of the diet did affect the results, whereas feeding regimen and sampling day did not. Retention of particle DM from fecal samples (expressed as % sample DM) increased with decreasing sieve aperture size and was affected by forage quality and fiber content of the diet. In contrast, the content of NDF in retained samples decreased with decreasing aperture size of sieves for all diets. When comparing the analysis of single grab fecal samples to a daily total composite sample, large differences were found in DM and NDF results. Only the grab sample collected at 12 h after feeding was similar between the 2 methods. However, if more grab fecal samples could be taken throughout day to prepare a representative sample, results would be more similar to the ones from the total daily composite sample. The 4 time points every 6 h during the day used in this study resulted in a distribution of retained particle DM and NDF weight similar to the total collection method.

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Figure 6. Difference in NDF content in total DM of sample on (A) top, (B) middle, and (C) bottom sieve of the Nasco Digestion Analyzer (Nasco, Fort Atkinson, WI) of treatment rations with high (dashed line with squares) or low (solid line with diamonds) forage quality between sampling time points and composited sample representing total daily feces. Error bars represent SE. Asterisks indicate a significant difference ($P < 0.05$) between sampling time point and composited sample for each diet.

Table 3. Particle DM and NDF distribution of ad libitum and restricted feeding treatment rations in feces sampled by spot sampling, averaged for time points throughout day, and total collection techniques

Sieve	Ad libitum		Restricted		SEM
	Spot sampling	Total collection	Spot sampling	Total collection	
Retained weight, % of total sample DM					
Top	2.15	2.50	3.45	3.69	0.52
Middle	3.81	5.77	4.39	6.53	
Bottom	11.89	8.58	12.14	9.05	
NDF, % in retained sample					
Top	88.06	97.75	88.29	88.14	4.82
Middle	87.70	96.61	88.05	87.62	
Bottom	85.24	95.62	85.49	87.19	
Retained NDF weight, % of total sample DM					
Top	1.32	1.79	2.36	2.68	0.49
Middle	2.77	4.72	3.17	5.12	
Bottom	9.55	8.00	9.43	7.00	

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