Comparison of three techniques for estimating the forage intake of lactating dairy cows on pasture[1]

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ABSTRACT: Quantifying DMI is necessary for estimation of nutrient consumption by ruminants, but it is inherently difficult on grazed pastures and even more so when supplements are fed. Our objectives were to compare three methods of estimating forage DMI (inference from animal performance, evaluation from fecal output using a pulse-dose marker, and estimation from herbage disappearance methods) and to identify the most useful approach or combination of approaches for estimating pasture intake by lactating dairy cows. During three continuous 28-d periods in the winter season, Holstein cows (*Bos taurus*; n = 32) grazed a cool-season grass or a cool-season grass-clover mixture at two stocking rates (SR; 5 vs. 2.5 cows/ha) and were fed two rates of concentrate supplementation (CS; 1 kg of concentrate [as-fed] per 2.5 or 3.5 kg of milk produced). Animal response data used in computations for the animal performance method were obtained from the latter 14 d of each period. For the pulse-dose marker method, chromium-mordanted fiber was used. Pasture sampling to determine herbage disappearance was done weekly throughout the study. Forage DMI estimated by the animal performance method was different among periods ($P < 0.001$; 6.5, 6.4, and 9.6 kg/d for Periods 1, 2, and 3, respectively), between SR ($P < 0.001$; 8.7 [low SR] vs. 6.3 kg/d [high SR]) and between CS ($P < 0.01$; 8.4 [low CS] vs. 6.6 kg/d [high CS]). The period and SR effect seemed to be related to forage mass. The pulse-dose marker method generally provided greater estimates of forage DMI (as much as 11.0 kg/d more than the animal performance method) and was not correlated with the other methods. Estimates of forage DMI by the herbage disappearance method were correlated with the animal performance method. The difference between estimates from these two methods, ranging from –4.7 to 5.4 kg/d, were much lower than their difference from pulse-dose marker estimates. The results of this study suggest that, when appropriate for the research objectives, the animal performance or herbage disappearance methods may be useful and less costly alternatives to using the pulse-dose method.

Key Words: Dairy cows, Feed intake, Herbage, Marker, Performance

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Introduction

Quantifying DMI is necessary for the estimation of nutrient consumption by ruminant animals, but it is inherently difficult to quantify forage intake in grazing systems (Burns et al., 1994; Moore, 1996; Reeves et al., 1996). Techniques for estimating intake on pasture can be based on the use of internal or external markers, ingestive behavior, disappearance of herbage mass, prediction from forage characteristics, and animal performance (Moore, 1996). All of the commonly used techniques have unique advantages and disadvantages, and results from all methods are only estimates of intake with an associated error that varies in magnitude (Burns et al., 1994; Moore, 1996). Although none of the methods are completely adequate, it may be possible to obtain precise measurements of differences among pastures at specific times (Moore, 1996).

Moore (1996) suggested that techniques based on the use of markers or on ingestive behavior are considered suitable for estimates of intake by individual animals. He went on to suggest that techniques based on disappearance of herbage mass, prediction from forage characteristics, or calculations of energy requirements for observed animal performance are suitable estimates for groups of animals or a pasture (Moore, 1996).

This study evaluated three techniques for estimating forage DMI by lactating dairy cows (*Bos taurus*) grazing cool-season pastures subject to varying management treatments. The techniques of choice recommended by Moore (1996) were studied: the pulse-dose marker

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method, the animal performance method, and the herbage disappearance method. The null hypothesis was that these three methods would provide similar estimates of forage intake for grazing lactating dairy cows. The objectives were to compare estimates of forage DMI among the three methods and to identify the most useful approach or combination of approaches for estimating pasture intake of grazing lactating cows receiving grain supplement.

Materials and Methods

The study was done in conjunction with a grazing management trial conducted during the 1996-97 winter season at the University of Florida Dairy Research Unit at Hague, 18 km north of Gainesville, FL (29° 40′ north lat.). Evaluated were two different cool-season forage systems (**FS**; N-fertilized rye [*Secale cereale* L.] and ryegrass [*Lolium multiflorum* L.] mixed pastures vs. rye-ryegrass-crimson clover [*Trifolium incarnatum* L.]-red clover [*Trifolium pratense* L.] mixed pastures). Cows grazing pastures of each system were stocked at either 5 or 2.5 cows/ha and were fed two rates of concentrate supplementation (**CS**; 1 kg concentrate [as-fed] per 2.5 or 3.5 kg of milk produced). The 2 × 2 × 2 factorial arrangement of treatments was replicated twice in a completely randomized design, so there were a total of 16 pastures (experimental units). Pasture size was 0.8 ha for the low-stocking rate (**SR**) treatment and 0.4 ha for the high-SR treatment. Starting date for the trial was 13 Jan. 1997. Evaluations were conducted during three continuous 28-d periods, ending 7 Apr. 1997. During the second period, cold weather and drought stress reduced yield of the grass-legume pastures such that animals had to be removed from the high-SR treatment for 14 d.

Two lactating Holstein cows blocked by parity (namely, one primiparous and one multiparous cow) were assigned to each pasture during each of the three 28-d periods. Thus, there was a sample size of n = 32 animals in the study. At the beginning of the trial, primiparous cows were between 149 to 247 d in lactation and multiparous cows were between 130 to 185 d in lactation. Initial BW averaged 542 kg, and milk yield ranged from 32 to 40 kg/cow during the 10 d before being put on the trial. Also, all animals had been managed in a traditional dairy management system based on intensive housing and stall feeding of total mixed ration, and had never been required to graze to meet their nutrient requirements before they were assigned to this study. A given pasture received the same treatment in each period, but cows were reassigned to different experimental units at the beginning of every period. Responses were analyzed for each experimental unit (i.e., each pasture), and not by individual animals, to account for differences in DMI responses by individual animals that could be due to social interactions (dominant or submissive behavior due to body size and/or parity) among animals. In addition, reassignment of animals to different experimental units each period was done to reduce confounding that may be due to animal-to-animal variation. Pastures were subdivided for rotational stocking so that cows were offered a fresh paddock every morning. Nitrogen-fertilized grass pastures were subdivided into 22 paddocks and grass-legume pastures into 29 paddocks to allow for 21- and 28-d rest periods, respectively, between grazing. Animals were milked at 0500 and 1700 each day. Cows walked an average of 4.8 km/d for milking.

Table 1. Ingredient and chemical composition (dry matter basis) of concentrate supplement fed to lactating cows and pasture nutritive value in the winter 1997 grazing study

Ingredient (as-fed basis)	Concentration (g/kg)
Hominy	295
Soybean hulls	225
Whole cottonseed	192
Citrus pulp	150
Dried distillers grain with solubles	60
Fish meal	25
Mineral mix[a]	32
Trace-mineralized salt[b]	2.5
Sodium bicarbonate	16
Magnesium oxide	2.5
Chemical composition	
Dry matter, g/kg	895
NEL, Mcal/kg[c]	1.87
NDF, g/kg	363
ADF, g/kg	238
CP, g/kg	162
Ca, g/kg	9.0
P, g/kg	4.7
Mg, g/kg	4.5
K, g/kg	11.6
Pasture nutritive value (g/kg)	
IVOMD range	763–782
CP range	209–270
NDF range	477–514

[a]Composed of at least 38 g/kg N, 105 g/kg Ca, 30 g/kg P, 45 g/kg K, 20 g/kg Mg, 74 g/kg Na, 11 g/kg S, 54 g/kg Cl, 1,525 ppm Mn, 1,750 ppm Fe, 425 ppm Cu, 1,500 ppm Zn, 12.8 ppm I, 49 ppm Co, 24.2 IU/g vitamin A, 35.2 IU/g vitamin D, and 0.88 IU/g vitamin E.
[b]Composed of at least 920 g/kg NaCl, 2.5 g/kg Mn, 2 g/kg Fe, 0.33 g/kg Cu, 0.07 g/kg I, 0.05 g/kg Zn, and 0.025 g/kg Co.
[c]Calculated using NRC (2001) values for whole cottonseed.

Concentrate (ingredient and chemical composition are presented in Table 1) was fed to animals at the appropriate CS rate after each milking. The feed mixture was provided to the cows in troughs located in each pasture. The amount of concentrate per experimental group was adjusted twice weekly based on average milk production during the preceding 3 to 4 d. A minimum of 4.5 kg (as-fed) concentrate/(cow·d) was fed. All pastures had water tubs fitted with float control devices to ensure continuous availability of drinking water.

Forage nutritive value of pastures was considered to be of high quality. Across periods, FS, and SR, forage in vitro organic matter digestibility (**IVOMD**) ranged from 763 to 782 g/kg, CP ranged from 209 to 270 g/kg, and NDF ranged from 477 to 514 g/kg.

Intake Measurements

Pulse-dose Marker Method. Total organic matter intake (**OMI**) was computed based on fecal output (**FO**) and diet digestibility using the equation

$$\text{OMI} = \text{OM output of feces}/[1 - (\text{OM digestibility}/100)]$$

Fecal output was estimated using a pulse-dose marker technique (Pond et al., 1986). Chromium-mordanted fiber was used as the external marker. Hand-plucked samples that represent the grazed portion of the pastures were used as the source of fiber for mordanting. The forage was dried at 60°C in a forced-air oven until constant weight was achieved (usually 72 to 120 h) and then ground in a Wiley mill (Thomas Scientific, Philadelphia, PA) to pass a 2-mm stainless steel screen. The neutral detergent-soluble component of the ground forage was extracted by boiling in detergent, and the fiber was washed and mordanted with chromium following recommended procedures (Uden et al., 1980). The mordanted fiber was dried in a convection oven at 105°C for 72 to 96 h until constant weight was achieved. Then 2.5 g of oven-dried mordanted fiber was packed into gelatin capsules (Jorgenson Laboratories, Loveland, CO).

Experimental animals were orally dosed with 12 gelatin capsules (30 g mordanted fiber/cow) at approximately 1800 on d 24 of each period. Capsules were administered with a multiple dose-balling gun (Nasco, Ft. Atkinson, WI). Fecal samples were obtained from rectal grab samples when animals were in the milking parlor or collected in the pasture by following the animals and grabbing a sample upon defecation. These samples were taken at dosing time (0 h) and at approximately 12, 15, 18, 21, 24, 27, 36, 42, 48, 60, 72, and 84 h after dosing.

Fecal samples were dried at 60°C in a forced-air oven until constant weight was achieved (usually 10 to 14 d) and then ground in a Wiley mill to pass a 2-mm stainless steel screen. Marker concentration in the feces was assayed by atomic absorption spectrophotometry following the procedure outlined by Williams et al. (1962). For each animal, the marker concentration data were fitted to recommended models (Pond et al., 1988), and, based on the adequate fits and peaks achieved, the one-compartment model with time delay and gamma-2 age dependency (Pond et al., 1986; Pond et al., 1988) was used to describe the marker appearance curve from which FO was then computed (Pond et al., 1986). No background Cr was detected in any of the samples taken at the 0-h sampling, so no adjustment for this was necessary. Marker concentration data from two animals each in Periods 1 and 2 did not give adequate fit to the marker appearance curve and were not included in subsequent analysis, but they were treated as missing data.

Forage OMI was calculated using an iterative SAS (SAS Inst. Inc., Cary, NC) program developed by J. E. Moore. In this program, total OMI is computed from estimates of FO (called *observed FO* [**OFO**]) obtained from the marker appearance curve, and total diet digestibility (called *expected digestibility* [**EXPD**]) to balance for the estimated proportion of forage and concentrate supplement OM consumed and their respective digestibilities. Estimated forage OMI is the difference between total OMI and known supplement OMI. The equation to calculate EXPD is

$$\text{EXPD} = [(\text{forage OMI} \times \text{forage OMD}) + (\text{supplement OMI} \times \text{supplement OMD})]/\text{total OMI}$$

This EXPD was further adjusted to help account for associative effects (Moore, 1992; Dixon and Stockdale, 1999) that may result from mixed forage-concentrate diets. This new calculation of total diet digestibility, called *adjusted digestibility* (**AJD**), was obtained using an equation developed from a wide range of published data of mixed diet digestibilities showing deviation from the expected (based on calculations from the weighted intake and digestibilities of the forage and concentrate supplement components) when mixed diets are fed (Moore et al., 1999). The equation is

$$\text{ADJ} = 59.71 - (0.8948 \times \text{EXPD}) + (0.01399 \times \text{EXPD}^2)$$

Using this adjusted digestibility value, a prediction of FO (**PFO**) was computed:

$$\text{PFO} = \text{Total OMI} \times (1 - \text{ADJ})$$

Given that supplement OMI is fixed, the iterative SAS program then adjusted estimates of forage intake until the difference between OFO and PFO was less than 0.01 kg OM/d. Forage OMI estimates were converted to DMI estimates by dividing the OMI by OM concentration of the forage.

Herbage Disappearance Method. Forage DMI predictions based on herbage disappearance were calculated as the difference between pregrazing and postgrazing herbage mass. Because grazing periods were 1 d, forage growth during the grazing period was not accounted for (Mannetje, 1978). Sampling for pregrazing and postgrazing herbage mass was done weekly in each period using a double-sampling technique (Burns et al., 1989). Indirect estimates of herbage mass were done using a 0.25-m^2 disk meter at 20 randomly selected sites per paddock. These indirect estimates were calibrated with direct estimates taken at the first and third sampling times during each period. Samples to quantify the direct estimates were taken at three sites in each paddock (one paddock per experimental unit) selected to represent low, intermediate, and high disk meter readings. Circular quadrats of the same area covered by the disk meter (0.25 m^2) were harvested using battery-powered hand clippers. Samples were clipped to a 3-cm stubble height, below the expected grazing height as suggested by Burns et al. (1989). Harvested samples were dried

at 60°C in a forced-air oven until constant weight was achieved (usually 72 to 120 h). Regression was used to develop equations relating direct (harvested sample) and indirect (disk meter height) measures of herbage mass on pastures. Equations developed for a particular double-sampling date (first and third week of each period) were used to estimate herbage mass for that date and for the indirect measures taken the following week. Herbage removed from pasture during grazing (herbage disappearance) was the difference between pregrazing and postgrazing herbage mass.

Animal Performance Method. Energy requirements were estimated by computing NE of lactation (**NEL**; Mcal/d) requirements for maintenance (**NELM**), for lactation (**NELL**), for BW changes (**NELBW**), for walking (**NELW**), and for grazing activity (**NELG**). Total NEL requirement was obtained by summing all of these. The NELM was calculated using appropriate NRC equations based on BW and cows' parity, that is, NELM = $1.2 \times (0.080 \times BW^{0.75})$ for first-lactation cows, NELM = $1.1 \times (0.080 \times BW^{0.75})$ for second-lactation cows, and NELM = $0.080 \times BW^{0.75}$ for third or greater lactation cows (NRC, 2001). Estimates of NEL requirements for lactation were calculated based on daily milk production and milk fat concentration according to NRC (2001), with NELL = kg milk per day × (0.3512 + [0.0962 × % milk fat]), and estimated from the latter 14 d of each 28-d study period. Average daily BW gain was charged a 5.12 Mcal/kg BW NEL requirement, whereas daily BW loss provided 4.92 Mcal/kg BW to available energy, additional to that provided by feed intake (NRC, 2001). Energy requirements for walking were calculated using the estimate for horizontal walking of 0.62 cal/(kg BW·m) (AFRC, 1993). The distance walked each day by animals in this study was 4.8 km (representing the average distance from pasture to milking parlor, 1.2 km, × 4, the number of times they walked to or from the parlor), and there was negligible slope in the lanes the cows walked. Calculation of the energy requirement for grazing activity was done using an equation suggested by Rochinotti (1998): NELG = 1.2 kcal × grazing time (h) × $BW^{0.75}$ (divided by 1,000 to convert to megacalories). The energy requirement value used in this equation is the average of estimates made by Di Marco et al. (1996). Grazing time was determined from a study of grazing behavior done simultaneously on the experimental animals in the current study. Animals were observed for 24-h periods beginning when animals returned from the milking parlor in the morning and continued until they left for the parlor the following morning. Observations were done approximately every 2 wk (six times during the study) on days selected to represent the range of weather conditions expected during the cool season. The weather conditions considered in the selecting observation days included ambient temperature, solar radiation, cloud cover, and rainfall. Data were recorded on animal activity, such as eating concentrate, grazing, and loafing (standing or lying). Based on data from that study, the values used in the computation were 8.25 h when animals grazed the low-SR treatment of the N-fertilized forage system and 7 h when animals were on all other treatments (our unpublished data).

The NEL from forage intake was estimated as total NEL minus NEL supplied by concentrate supplement. The NEL concentration of the supplement was computed by the equation NEL = 0.0245 × [(supplement digestible OM concentration, **DOM**) + (1.25 × digestible ether extract, **DEE**)] – 0.12, derived from NRC (2001). The DEE concentration of the concentrate supplement was 0.06 g/kg (converted to 6% to use in the equation) (DHIA forage testing laboratory, Ithaca, NY). Forage NEL concentration was predicted by the equation NEL = (0.0245 × forage DOM) – 0.12 (the DEE component of the equation was omitted because DEE concentration of forage is considered negligible).

Forage Digestibility Analysis

To determine forage digestibility, hand-plucked samples of forage—selected to represent what the animals were consuming—were composited from 15 to 20 random sites in the paddock that was to be grazed the following day. Samples were collected fortnightly. These samples were dried at 60°C for at least 96 h in a forced-air oven and then ground in a Wiley mill to pass a 1-mm stainless steel screen. Herbage IVOMD was determined using a modification of the two-stage procedure (Moore and Mott, 1974).

Statistical Analysis

To assess responses within each method, that is, considering forage DMI estimates determined by each method as response variables in three separate analysis, the data were analyzed by fitting mixed-effects models (Littell et al., 1996) using the PROC MIXED procedure in SAS (SAS Inst. Inc., Cary, NC). All effects were considered fixed except for the error term, which was considered random. Period was considered to be a repeated measure in time because it did not have a chance to be assigned randomly (Littell, 1989). Subject (pasture or animal pair) was the experimental unit, that is, each replicate × FS × SR × CS combination. Summarization of the results from the foregoing analysis suggested that there were similarities as well as differences among intake estimation methods. In order to test intake estimation method × treatment interaction, method was considered as a fixed experimental variable so that the design resembled a randomized complete block design with methods as blocks, and the random error term was considered to be each replicate within method (Littell et al., 1996). This allowed method and error effects to be independent of one another (Littell et al., 1996). Similar to the model for separate analysis of each intake method, period was considered to be a repeated measure in time and subject was each replicate × FS × SR × CS combination. Additionally, correla-

Table 2. Estimated forage DMI and selected animal measurements associated with each treatment including amount of supplement fed (Suppl.), of lactating dairy cows grazing two cool-season forage systems (N-fertilized grass [Grass/N] or grass-legume mixed [Grass/L] pastures) at two stocking rates (SR) and two concentrate supplement rates (CS) during three consecutive 28-d periods by the animal performance (APM), pulse-dose marker (PDM), and herbage disappearance (HDM) methods

Treatment				Selected measurement					Forage DMI, kg/d		
Period	Forage system	SR	CS	BW, kg	ADG, kg/d	Milk, kg/d	FCM, kg/d	Suppl., kg/d	APM	PDM	HDM
1	Grass/L	High	High	565	–0.82	19.68	18.59	7.09	5.2	16.1	7.7
1	Grass/L	High	Low	556	–0.67	20.25	18.82	5.23	7.1	18.1	8.2
1	Grass/L	Low	High	555	–0.20	20.01	18.14	7.02	6.3	14.7	9.8
1	Grass/L	Low	Low	545	–0.02	22.38	21.09	5.70	9.2	15.9	10.5
1	Grass/N	High	High	571	–1.03	21.30	19.59	7.52	4.1	14.3	5.0
1	Grass/N	High	Low	496	–0.70	17.14	16.34	4.11	6.3	12.9	6.5
1	Grass/N	Low	High	545	–0.49	22.13	22.09	7.54	6.3	13.8	10.5
1	Grass/N	Low	Low	500	–0.28	19.45	17.91	4.81	7.7	16.0	7.3
2	Grass/L	High	High	ND[a]	ND	ND	ND	ND	ND	ND	ND
2	Grass/L	High	Low	ND	ND	ND	ND	ND	ND	ND	ND
2	Grass/L	Low	High	526	–0.40	19.45	17.90	7.06	5.3	10.3	5.7
2	Grass/L	Low	Low	503	–0.29	17.28	16.69	4.30	8.1	9.5	7.0
2	Grass/N	High	High	515	–0.99	13.04	12.95	4.44	4.3	9.5	3.7
2	Grass/N	High	Low	547	–1.29	16.79	15.91	4.36	5.2	8.8	4.0
2	Grass/N	Low	High	534	0.65	19.52	18.49	6.84	8.6	10.7	9.8
2	Grass/N	Low	Low	557	0.04	21.18	19.09	5.27	9.1	11.0	7.7
3	Grass/L	High	High	526	0.12	14.04	13.05	4.77	7.3	10.3	6.3
3	Grass/L	High	Low	545	0.80	13.68	12.78	4.08	10.1	10.2	7.7
3	Grass/L	Low	High	542	1.22	18.48	17.81	6.70	10.4	8.8	10.5
3	Grass/L	Low	Low	532	0.95	18.99	18.74	4.63	12.4	10.2	11.5
3	Grass/N	High	High	507	0.10	15.38	13.98	5.23	6.8	11.0	5.0
3	Grass/N	High	Low	523	0.13	15.08	14.08	3.98	8.6	10.4	5.3
3	Grass/N	Low	High	539	1.04	22.31	21.10	7.84	9.9	14.8	11.2
3	Grass/N	Low	Low	561	0.62	20.71	19.70	5.11	11.3	17.2	9.5
								SE[b]	1.03	2.47	0.96
								SE[c]		1.66	

[a]ND = no data because cows were removed from these treatments for 14 d.

[b]SE of four-way interaction means within each intake estimation method (NS, $P > 0.10$). Within methods, the meaningful main effects or interactions are: APM—period effect ($P < 0.001$; SE = 0.36), SR effect ($P < 0.001$; SE = 0.27), CS effect ($P = 0.003$; SE = 0.29); PDM—period effect ($P = 0.014$; SE = 0.87), period × FS interaction trend ($P = 0.108$; SE = 1.23); and HDM—SR effect ($P < 0.001$; SE = 0.32), period × FS interaction ($P = 0.015$; SE = 0.48).

[c]SE to compare among all interaction means across methods (NS, $P > 0.10$). Across all data, the meaningful main effects or interactions are: method × period × FS ($P = 0.044$; SE = 0.87), method × SR ($P = 0.007$; SE = 0.48), FS × SR ($P = 0.022$; SE = 0.33), method × CS ($P = 0.082$; SE = 0.48).

tion analysis (PROC CORR in SAS) was used to determine whether any relationships existed among estimates of forage intake determined by the various methods.

Results and Discussion

Estimates of Forage DMI by Different Methods

Animal Performance Method. Estimates of forage DMI based on NEL requirements for observed animal performance were affected by main effects of period ($P < 0.001$), SR ($P < 0.001$), and CS ($P = 0.003$) (Table 2). The forage DMI predicted for Period 3 (9.6 kg/d) was greater than that for Period 1 (6.5 kg/d) or Period 2 (6.4 kg/d). Estimates for Periods 1 and 2 were not different from each other ($P > 0.10$). Cows grazing high-SR pastures had lower forage DMI (6.3 kg/d) compared to low-SR (8.7 kg/d) treatments. Also, cows fed the higher CS rate had lower forage DMI (6.6 kg/d) compared to animals on the low-CS treatment (8.4 kg/d). The period and SR effect on forage DM intake seemed to be associated with forage allowance (our unpublished data). Forage allowance was greater on low-SR compared to high-SR pastures and also was greater during Period 3 compared to Periods 1 and 2. These results conform with findings typically reported for grazing intensity effects on forage intake responses, that is, higher intake with increased forage availability (Kristensen, 1988; Dougherty et al., 1992; Hoogendoorn et al., 1992; Fisher et al., 1996) and reduced forage intake with increased intake of supplemental feed, likely due to substitution

of grain for forage (Berzaghi and Polan, 1992; Holden et al., 1995; Reeves et al., 1996). Cows in the Kristensen (1988) study grazed perennial ryegrass (*Lolium perenne* L.), and in the week prior to being put on pastures were averaging 110 d in lactation, mean daily milk yield was 28.4 kg, and mean BW was 495 kg. All the animals were in first or second lactation and received concentrates at a rate of 2.2 kg OM/d during twice daily milking. Herbage OM intake was 8.1 kg/d at the lower forage allowance compared to 9.2 kg/d at the higher forage allowance treatment in that study, and the corresponding milk yields were 14.4 (high forage allowance) and 13.6 kg/(cow·d) (low forage allowance). When estimated using the herbage disappearance method, cows (mean BW = 475 kg) in the Hoogendoorn et al. (1992) study had daily DMI of 17.0 kg at a high forage allowance and 13.5 kg at a low forage allowance, with corresponding daily milk yield of 19.6 and 21.5 kg, respectively. In the Berzaghi and Polan (1992) study, cows (average BW 557 kg and 130 d in milk) had lower pasture OM intake when fed 5.7 kg/d cracked corn compared to no supplemental feed (8.0 vs. 10.3 kg/d). Supplemented animals had greater milk production (23.7 vs. 19.5 kg/d). Holden et al. (1995) studied 32 Holsteins cows, averaging 133 d in lactation and 32 kg/d milk at the start of the trial, grazing primarily orchardgrass (*Dactylis glomerata* L.) pastures. All the animals received 1 kg of grain DM per 4 kg of milk, and half the animals received an additional 2.3 kg/d of corn silage DM. Total DMI was not different between treatments (21.8 kg/d for the control animals and 21.5 for the animals fed corn silage), but pasture DMI was greater (14.0 kg/d) for the control animals compared to the animals fed corn silage (10.56 kg/d). Cows fed corn silage did not have improved BW gain or body condition score. In addition, milk yield was not different between the cows fed corn silage (28.8 kg/(cow·d) and the control animals (29.1 kg/[cow·d]). In our study, milk yield ranged from 13.0 to 22.4 kg/(cow·d) (Table 2).

Pulse-Dose Marker Method. Forage DMI estimated by the pulse-dose marker method was affected by a period main effect ($P = 0.014$). Estimates for Period 1 (15.2 kg/d) were greater than those for Period 2 (10.3 kg/d) or Period 3 (11.6 kg/d), but estimates for Periods 2 and 3 were not different from each other (Table 2). This was contrary to the pattern of responses obtained using the animal performance method. Examination of the pasture characteristics (herbage mass, forage nutritive value) and forage allowance data did not provide evidence of any relationship between these variables and the greater forage DMI during Period 1. Upon examination of the grazing management effects on forage DMI estimated by this method, there is no clear trend suggested. The highest estimates in Period 1 were detected at the high SR on the grass-legume pastures (Table 2). These pastures also had lower forage allowance (our unpublished data), so it is illogical that forage intake will be as high as estimated, suggesting that the pulse-dose method may be inaccurate in this situation. It may be crucial to consider that one of the characteristics (and a disadvantage, as suggested by these results) of the pulse-dose marker technique is that intake is being estimated only for the period when the marker is administered, in this case, for an 84-h period. These estimates are then used to explain intake during a much longer grazing period, which may be inaccurate. It is possible that in the current experiment, intake estimated by the pulse-dose technique when measurements were done was indeed as high as the results show, but was not an accurate reflection of the average intake of animals during the 28-d grazing period.

Herbage Disappearance Method. Forage DMI estimates by the herbage disappearance technique were affected by SR ($P < 0.001$). Cows grazing low-SR pastures had greater forage DMI (9.3 kg/d) compared to high SR (5.4 kg/d) (Table 2). This was similar to the results obtained for estimates of forage DMI by the animal performance method, but the magnitude of difference was larger in this case. There was also a period × FS interaction ($P = 0.015$). In both FS, forage DMI estimates were similar during Periods 1 and 3. In the grass-legume system, mean forage DMI estimates during Periods 1 and 3 were 9.1 and 9.0 kg/d, respectively, and in the N-fertilized grass system they were 7.3 and 7.8 kg/d, respectively. Forage DMI estimated for Period 2 (4.4 kg/d) was lower than for Periods 1 and 3 in the grass-legume system. In N-fertilized grass pastures on the other hand, forage DMI estimated for Period 2 (6.3 kg/d) was not different from that for Period 1, but it was different from that for Period 3. Greater forage DMI was estimated on the grass-legume system compared to N-fertilized grass pastures during Period 1 and Period 3 but the opposite occurred during Period 2.

Estimate of Method × Management Treatment Interaction

When method was included in the model in order to test method × management treatment interaction, the highest order interaction involving method that occurred was a three-way interaction of method × Period × FS ($P = 0.044$). An examination of the interaction means suggests that the interaction was probably due in part to the pulse-dose marker method generally having greater estimates of forage DMI than the herbage disappearance or animal performance methods, regardless of period or FS, except on the grass-legume system during Period 3, in which all three techniques gave similar estimates (Figure 1). Holmes et al. (1992) also reported a trend of higher values of forage DMI estimated by a chromium marker method compared to the herbage disappearance method. Among techniques, the herbage disappearance and animal performance methods gave similar patterns of estimates of forage intake between FS during all three periods, but the pulse-dose marker method showed a difference between FS during Period 3 (Figure 1). There is no clear explanation why forage DMI would be different between FS. During Pe-

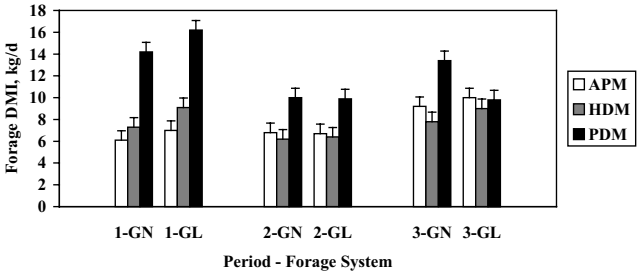

Figure 1. Method (APM, animal performance method; HDM, herbage disappearance method; and PDM, pulse-dose marker method) × period × forage system (GN, N-fertilized grass system; GL, grass-legume system) effect on forage DMI estimates (SE = 0.87, represented by error bars, to compare interaction means).

riod 3, herbage mass was greater on the grass-legume system (1,350 kg/ha) compared to the N-fertilized grass system (1,085 kg/ha), and herbage allowance and animal performance responses (milk production, body condition score, and body weight change) were the same between FS (Table 2; our unpublished data), so greater forage DMI on the N-fertilized grass system seems unlikely. At the same animal performance, it is expected that FS effect on forage intake (averaged across grain supplement intake) will be similar. Thus, these data suggest that these estimates of forage DMI by the pulse-dose marker method may be inaccurate in this situation. Reeves et al. (1996) reported that estimates of pasture DMI by the herbage disappearance method were 2.3 to 2.7 kg/(cow·d) higher compared to calculation based on energy requirements for animal performance.

Method × SR ($P = 0.007$; Table 2) was the only other meaningful interaction found in the estimate of method × management treatment interactions. Forage DMI was estimated to be greater at the low than at the high SR by both the animal performance (8.7 vs. 6.5 kg/d) and the herbage disappearance (9.3 vs. 5.9 kg/d) methods, but estimates by the pulse-dose marker method were similar between low (12.7 kg/d) and high (12.2 kg/d) SR (SE for comparing method × SR interaction means = 0.48). Herbage allowance, milk production, and increase in animal live weight were all greater on low- compared to high-SR treatments (Table 2; our unpublished data); therefore, it is logical that forage DMI should reflect this. Similar estimates of forage DMI between SR treatments by the pulse-dose marker method again suggest that estimates of forage intake obtained using this technique are potentially incorrect. Burns et al. (1994) cautioned that there are a number of difficulties associated with each technique of estimating forage intake. They stated that chances of errors may be greater with the pulse-dose marker method given the likelihood of dosing errors, the high frequency of disturbing animals, numerous laboratory analyses, and

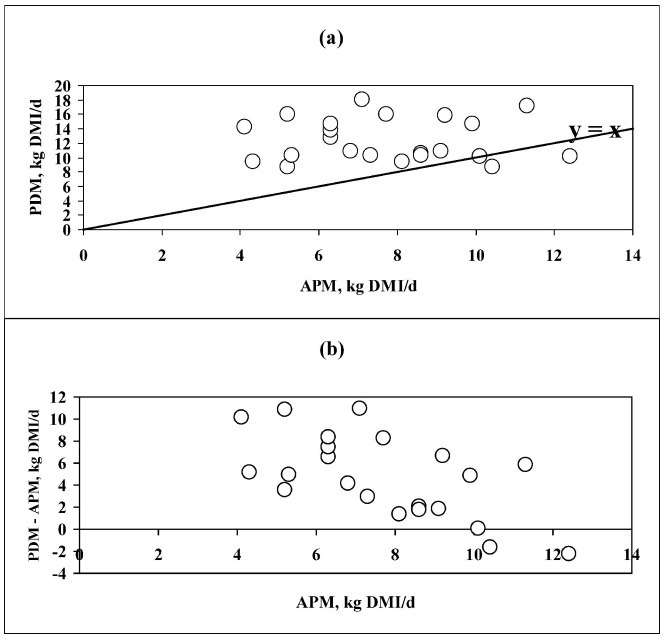

Figure 2. Relationship between a) estimates of forage DMI by the pulse-dose marker method (PDM) and the animal performance method (APM) and b) the difference between PDM and APM relative to APM.

the complexity of modeling marker flow and calculating the parameters.

Comparison Among Methods

There was lack of correlation between estimates of the pulse-dose marker and the animal performance methods ($P = 0.681$; r = 0.06) (Figure 2) and between the pulse-dose marker and the herbage disappearance methods ($P = 0.253$; r = 0.09) (Figure 3). Analysis of data subsets at fixed levels of period, SR, or CS did not change this inference. There was a positive correlation ($P < 0.001$; r = 0.57) between forage DMI estimates of the animal performance and the herbage disappearance methods (Figure 4). Analysis of subsets of the data at fixed levels of period, SR, or CS did not change this inference; most were significant ($P < 0.05$) or showed a trend ($P < 0.10$). The lack of a quantitative relationship between the pulse-dose marker and the other two methods supports the observation that the pulse-dose marker method was not estimating forage DMI in the same pattern as the other methods.

A plot of estimates by the pulse-dose method against the animal performance method showed that most data points occur above the line for the slope of unity (Figure 2a), suggesting there generally was overestimation with the pulse-dose method compared with the animal performance method. This amount of overestimation generally depended on management treatment and period, and ranged from 70 to 250% in Period 1, 17 to 94% in Period 2, and 0 to 62% in Period 3. Examination of the raw data suggests that the largest overestima-

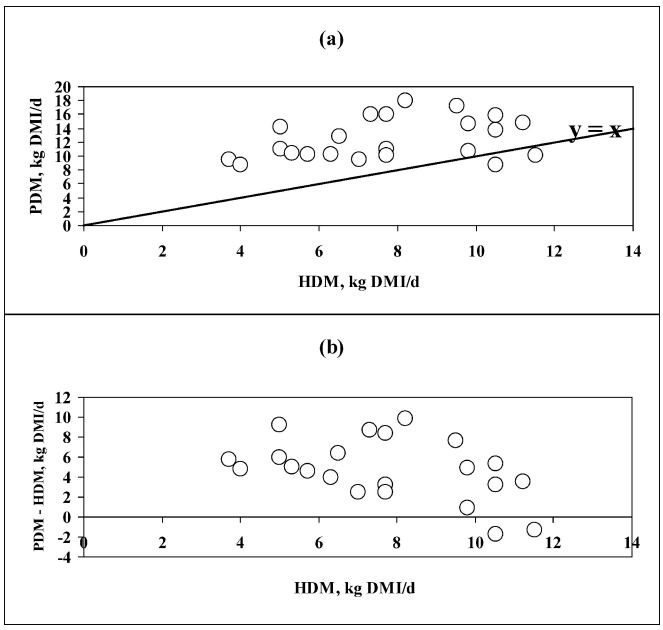

Figure 3. Relationship between a) estimates of forage DMI by the pulse-dose marker method (PDM) and the herbage disappearance method (HDM) and b) the difference between PDM and HDM relative to HDM.

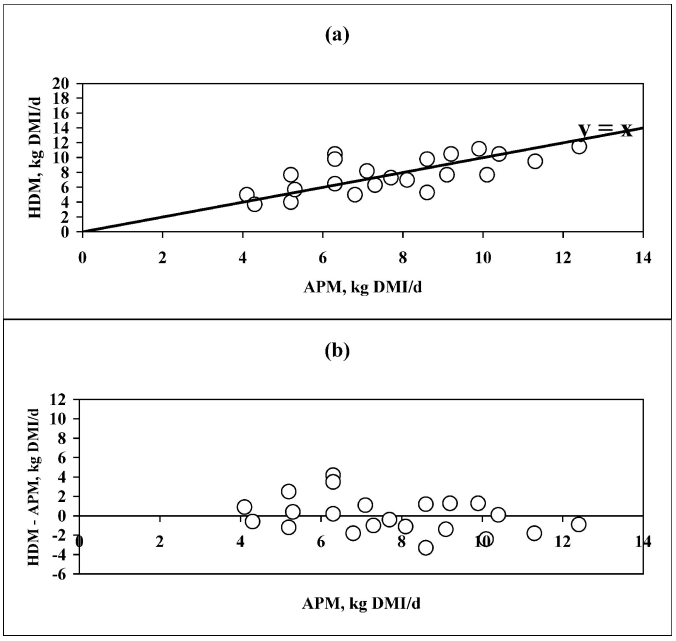

Figure 4. Relationship between a) estimates of forage DMI by the herbage disappearance method (HDM) and the animal performance method (APM) and b) the difference between HDM and APM relative to APM.

tions tended to occur during Period 1 (also see Figure 1), but there is no clear explanation for this. Similarly, plotting pulse-dose marker estimates against estimates obtained by the herbage disappearance method (Figure 3a), and then plotting the magnitude of difference between the two methods, with estimates by the herbage disappearance as the independent variable (Figure 3b) also suggest that, compared to the herbage disappearance method, there generally was a pattern of overestimation by the pulse-dose marker method. Plotting estimates by the herbage disappearance method against estimates by the animal performance method indicate that most data points were within close proximity of the slope of unity line (Figure 4a), supporting the observation that these two methods gave similar estimates ($P > 0.10$) of forage DMI. This was further confirmed when the magnitude of difference between estimates of the two methods were plotted against estimates of the animal performance method as the independent variable (Figure 4b). Examination of the data points indicates that there was about as much overestimation as there was underestimation (average deviation of all data points was 0.04 kg/d).

Several factors may be responsible for the observed large magnitude of difference of estimates by the pulse-dose marker method from the estimates obtained by the other methods, and, perhaps more importantly, for the lack of an empirical relationship between the pulse-dose marker estimates and the others. Moore (1996) cautioned that acceptable estimates of DMI by the pulse-dose marker method are provided for only those periods of time when samples were obtained. It is possible that conditions may have varied at the time of sampling on some management treatments in the present study to an extent that extrapolation across the experimental period provided inaccurate estimates. Another possible cause for overestimation may be dosing errors. As was indicated in the description of the procedure, pieces of gelatin capsules with mordanted fiber were observed infrequently on the floor of the walkways leading away from the animal handling areas, indicating that one or two animals may have chewed and/or spit out some of the mordanted fiber. Although this was not widespread, it points out that there likely were instances, for individual animals (impossible to identify unless observed), when the amount of marker dosed could be lower than used in computation. The lower marker dosage results in lower fecal marker concentration and thus overestimation of fecal output leading to overestimation of intake. In our study, two animals were identified during the first dosing cycle that possibly spitted out small amounts of mordanted fiber. Subsequently, it was found that the fecal chromium concentration data from these animals did not adequately fit the marker appearance curve, and their intake data were omitted from statistical analyses. At the second dosing time, it was suspected that rejected mordanted fiber may have come from only one animal, but this animal was not identified. Marker concentration data from two animals in this period were also considered as not giving adequate fit to the marker appearance curve and were therefore omitted from statistical analyses. It is possible that one of these animals was the one that rejected some mordanted fiber. Omission of the

data from these animals in the statistical analysis may help reduce the potential for invalid data. The fact that rejected pieces of capsules and fiber were observed was because cows were in a confined walkway. If cows were dosed on pasture, it was unlikely that rejection could have been observed at all. Thus, this observation demonstrated a possible inherent fault of the pulse-dose technique that may have been missed in other research.

These seemingly poor estimates by the pulse-dose marker method may have been caused by any number of factors, but they all point to the need for careful procedure and precision in preparing the mordanted fiber, in dosing, in obtaining samples, in chemical analysis, and in quantifying supplement intake. One possible cause of error in the current study may be grinding of the forage to prepare it for mordanting (J. C. Burns, personal communication). Burns suggests that mordanted fiber needs to move down the gastrointestinal tract like the ingested fiber, but this may be altered by the grinding of the forage before mordanting (i.e., particle size effect). He also believes that consumption of concentrate feed could potentially lead to errors in estimating forage intake based on fecal output from a mixed diet. Our approach, however, attempted to correct for potential changes in digestibility of a diet that included pasture and concentrate feed. Additionally, bearing in mind that the estimates represent the period in which samples were obtained, care should be taken to ensure conditions during the sampling process are representative of the overall conditions of the study. The herbage disappearance method only provides estimates for groups of animals, and neither the herbage disappearance nor the animal performance method describe digesta kinetics. Thus, the pulse-dose method may be more useful than the others where intake estimates are required for individual animals and where digesta kinetics of consumed herbage may be critical to providing explanation of pasture characteristics and its impact on forage intake. This likely will have to be done in a controlled environment and may require the use of animals fitted with rumen cannula to ensure precise and accurate dosing. Results of the current study do not provide strong support for use of the pulse-dose method in situations like those reported.

Estimates of forage DMI obtained using the animal performance method seem to make biological sense. Forage DMI was greater when forage allowance was higher, concurring with the conclusions from several studies (Kristensen, 1988; Dougherty et al., 1992; Hoogendoorn et al., 1992; Fisher et al., 1996). Furthermore, forage DMI estimates were greater with the animal performance method when cows were fed at the low-CS level and vice versa, indicating that cows fed less supplement attempted to meet energy requirements by consuming more forage. Similar findings were reported when dairy cows were managed on grazed pasture systems (Berzaghi and Polan, 1992; Holden et al., 1995; Reeves et al., 1996).

One criticism of the animal performance method is that intakes calculated are derived from generalized equations and this may not represent the intake of individual animals (Reeves et al., 1996). This limitation may be overcome if the inferences are made for pastures with more than one animal representing sampling units as was done in the present study, that is, allowing for reduction of between-animal variation. Additionally, precise estimates of supplement intake as well as forage digestibility (Moore, 1996) are a requirement for successful use of this technique. Procedures to ensure adequate prediction of forage digestibility are well known (Moore, 1996). Another serious limitation is accurate measurement of changes in live BW of animals with time, especially those occurring over short periods. Because it was not possible to obtain shrunk BW measurements (Stuedemann and Matches, 1989) with lactating dairy cows, the BW changes measured may not be accurate, but the impact of this on subsequent intake predictions cannot be determined. The method may be more precise in estimation because it directly relates to animal performance. An advantage of this method is that it integrates intake responses over an entire period of study. Also, this method may be useful for planning efficient feeding management strategies that synchronize nutrient requirements with production levels.

Estimates of forage DMI by the herbage disappearance method more closely approximated the estimates calculated based on NEL requirements than did estimates obtained by the pulse-dose marker method. Moore (1996) suggested that use of the herbage disappearance technique for estimating forage DMI by groups of animals or a pasture was likely to be successful only for rotational stocking and short grazing periods. Burns et al. (1994) defined the "short" grazing period as 1 to 3 d. These authors also indicated that the success of this method depended on using a cutting height that was low enough to include forage that may have been trampled (Burns et al., 1994). These conditions were met by the procedures used in this study.

The herbage disappearance method provided estimates that closely matched those obtained based on energy requirements, suggesting that under the conditions of this experiment, the technique provided acceptable estimates of forage intake. The technique does not allow estimation of intake for individual cows; therefore, it is more appropriate in settings where group estimates are required. Estimates of forage DMI by this method are not hindered by group feeding of concentrate supplement because knowledge of individual animal supplement intake is not required to estimate forage intake. These results support the conclusion that the herbage disappearance technique is a suitable alternative for estimating forage intake for groups of animals on well-managed, rotationally stocked pastures, with short grazing periods.

Implications

Estimation of forage intake based on energy requirements for animal performance is useful when evaluat-

ing intake of lactating dairy cows grazing pastures. Substantial fieldwork is not required but detailed calculations are involved in arriving at estimates. Estimates by the herbage disappearance method were equally suitable. The frequent sampling required often has been a criticism for the use of this method, but these samples are required to characterize pasture responses and to couple pasture characteristics with animal performance, so it should not be viewed as additional work. Estimates by the pulse-dose marker method varied more and seemed less biologically rational, but there is no way to conclusively determine whether they are less acceptable than those of the other methods. The volume of work and the complexity of the procedures involved in using this method are significant, calling into question its use when measures of individual animal intake are not required.

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