RUMINANT NUTRITION

Comparison of in situ techniques to evaluate the recovery of indigestible components and the accuracy of digestibility estimates

Jordan M. Adams,¹ Aaron B. Norris,² Luiz F. Dias Batista, Madeline E. Rivera, and Luis O. Tedeschi³

Department of Animal Science, Texas A&M University, College Station, TX 77843-2471

¹Present address: Department of Animal and Food Sciences, Oklahoma State University, Stillwater, OK 74078.

²Present address: Department of Natural Resources Management, Texas Tech University, Lubbock, TX 79409.

³Corresponding author: luis.tedeschi@tamu.edu

ORCID numbers: 0000-0002-7746-6445 (J. M. Adams); 0000-0003-1883-4911 (L. O. Tedeschi); 0000-0002-7345-4703 Luiz F. Dias Batista; 0000-0002-6816-7590 Aaron B. Norris; 0000-0003-3568-4070 Madeline E. Rivera.

Abstract

Indigestible components, including indigestible dry matter (iDM) and indigestible neutral detergent fiber (iNDF), play an integral role as internal markers for determining ruminal kinetics and digestibility estimations. However, the accuracy of internal markers is dependent upon the incubation technique utilized as bag type (BT) and incubation length (IL) can be significant sources of error. Previous studies have primarily focused on iDM and iNDF as digestibility markers, but few studies have compared digestibility estimates to those of acid detergent insoluble ash (ADIA). Therefore, our objective was to investigate the effect of BT (F57, F58, and Dacron) and IL (288 and 576 h) on iDM and iNDF residues, DM and NDF digestibilities, and fecal recoveries when using in situ incubations. Additionally, we evaluated the accuracy of digestibility estimates when using iDM, iNDF, and ADIA. For iDM and iNDF, feed residues demonstrated a BT × IL interaction ($P < 0.01$). However, fecal residues were only influenced by the main effects of BT and IL ($P < 0.01$), with the F58 BT and 288-h IL having the greatest residues for both iDM and iNDF. The variation in residues was greatly reduced when using iNDF compared with iDM. Fecal recovery estimates most closely approximated 100% recovery when utilizing ADIA and iDM using the F57 × 576 h incubation method ($P < 0.01$), although recovery was overestimated for all incubation combinations. Fecal NDF recovery estimates better represented the excretion profiles when the F57 × 576 h combination was used with iDM as the internal marker ($P < 0.01$). Estimates of DM and NDF digestibility were the most accurate when utilizing ADIA ($P < 0.01$) relative to all other treatments. Our results indicate that the proper methodological application is specific to the purpose of the inferences. When evaluating fecal recoveries and digestibility, ADIA or iDM with F57 at 576-h in situ incubation provides the greatest accuracy.

Key words: acid detergent insoluble ash, indigestible DM, indigestible NDF, internal markers

Introduction

The neutral detergent fiber (NDF) content of feedstuffs directly influences digestibility and feed intake and is a primary focus

when formulating ruminant diets (Harper and McNeill, 2015). However, using NDF as a measure of feed quality is complicated due to its chemically heterogeneous nature with potentially

Abbreviations

AD	acid detergent
ADF	AD fiber
ADFD	ADF digestibility
ADIA	acid detergent insoluble ash
aNDF	neutral detergent fiber with the addition of alpha amylase and sodium sulfite
BT	bag type
CP	crude protein
DM	dry matter
DMD	DM digestibility
iDM	indigestible DM
IL	incubation length
iNDF	indigestible neutral detergent fiber
ND	neutral detergent
NDF	ND fiber
NDFD	NDF digestibility
NFC	nonfiber carbohydrates
OM	organic matter
pdNDF	potentially digestible NDF

digestible (pdNDF) and indigestible fractions (iNDF) (Mertens, 2003; Huhtanen et al., 2006). Although not chemically defined, iNDF is the fraction of NDF remaining following prolonged (>288 h) in situ incubation (Harper and McNeill, 2015). Because the digestibility is assumed zero, iNDF provides an ideal nutritional entity that describes the inherent properties of the cell wall (Mertens, 1993; Van Soest, 1994). This makes iNDF essential to nutritional models (Tedeschi and Fox, 2020) and an ideal internal marker (Sampaio et al., 2011; Palmonari et al., 2016).

Indigestible components, including indigestible dry matter (iDM) and iNDF, play an integral role as internal markers for rumen kinetics, digestibility estimates, and nutritional models. However, the accuracy of internal markers is dependent upon the incubation technique used, with bag type (BT) and incubation length (IL) acting as significant sources of error, particularly when incubating heterogeneous particle sizes for a prolonged period (Huhtanen et al., 1994). Krizsan and Huhtanen (2013) and Krizsan et al. (2015) proposed that the combination of 288-h IL and 6- to 12- μ m bag porosity yielded acceptable iNDF estimates and fecal recoveries. In contrast, recent evaluations suggested lower indigestible residues in samples incubated for 576 vs. 288 h (Norris et al., 2019), though fecal recoveries were not performed. The objective of this study was to evaluate the effect of BT and IL on indigestible component residues, digestibility estimates, and fecal recoveries when using in situ incubations. In addition, the accuracy of digestibility estimates from iDM, iNDF, and acid detergent insoluble ash (ADIA) was evaluated.

Materials and Methods

The animals used in this experiment were registered and cared for according to guidelines approved by the Institutional Animal Care and Use Committee (AUP 2017-0306) of Texas A&M University.

Sample collections

The feed and fecal samples used in this study were obtained from animals during an independent trial that performed total fecal collection over a 4-d period. For this trial, two animals were selected based on the criteria that they received the control diet

and readily consumed all feed with no residual orts. Batch samples (250 g as-fed basis) of the basal diet were collected daily for the last 10 d of each period. Individual animal feces were weighed, homogenized, and subsampled daily before feeding the animals and were stored in a -20 °C freezer. Feed and fecal samples were dried at 55 °C for 72 h (or until weight loss ceased), then ground to pass through a 2-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ) and composited by animal and period based upon the proportion of daily fecal DM output. A 50-g subsample of each composite was shipped to Cumberland Valley Analytical Services (Waynesboro, PA) for chemical analysis of DM (Goering and Van Soest, 1970), NDF with the addition of amylase and sodium sulfite (Van Soest et al., 1991), acid detergent fiber (ADF; Method # 973.18; AOAC, 2000), crude protein (CP; Method# 990.03; AOAC, 2000) using a Leco FP-528 Nitrogen Combustion Analyzer (Leco Corporation, St. Joseph, MO), soluble CP (Krishnamoorthy et al., 1982), a complete mineral panel (Method # 985.01; AOAC, 2000; using a Perkin Elmer 5300 DV ICP, Perkin Elmer, Shelton, CT), and calculation of nonfiber carbohydrates, as shown in Table 1.

Experimental design

A randomized complete block design using a 3 × 2 factorial treatment arrangement was used to evaluate the effects of BT and IL combinations on iDM and iNDF residues, digestibility estimates, and fecal recoveries. Indigestible components were determined using F57, F58, or Dacron-type bags (ANKOM

Table 1. Ingredient and chemical composition of feed and feces utilized for the determination of indigestible content, fecal recovery, and digestibility accuracy

Items ¹	Feed	Feces ²	
Ingredient composition, % DM			
Cottonseed hulls	37.00	—	—
Cracked corn	33.00	—	—
Alfalfa pellets	11.50	—	—
Bermudagrass hay	8.00	—	—
Molasses	7.00	—	—
Mineral	2.50	—	—
Urea	1.00	—	—
Chemical composition ³			
DM, %	86.60		
CP, % DM	12.40	—	—
aNDF, % DM	48.10	—	—
ADF, % DM	33.40	—	—
NFC, % DM	34.20	—	—
Ash, % DM	6.52	—	—
Chemical composition			
Feces, kg	—	7.37	7.26
DM, %	—	22.65	24.76
OM, %	—	8.44	7.43
aNDF, %	—	61.55	62.51
ADF, %	—	49.82	51.18
N, %	—	2.48	2.12
DMD, %	—	63.85	64.21
NDFD, %	—	59.31	55.58
ADFD, %	—	48.78	45.31

¹Items are feed ingredients, and chemical composition of feed and feces evaluated by Cumberland Valley Analytical Services (Waynesboro, PA).

²Feces used in this study were obtained from two animals during an independent trial that performed total fecal collection.

³aNDF, neutral detergent fiber with the addition of alpha amylase and sodium sulfite; NFC, nonfiber carbohydrates; OM, organic matter; N, nitrogen; ADFD, acid detergent fiber digestibility.

Technology, Macedon, NY), with reported porosity of 25, 10, and 50 μm , respectively. The surface area of BT was calculated to be 6.3 cm^2 for both the F57 and F58 bags and 10 cm^2 for the Dacron bags, which was used to determine the appropriate sample size. Bags were filled with a sample size-to-surface area ratio of 20 mg/cm^2 and incubated for 288 or 576 h in three English-cross steers (incubation animal; 230 ± 10.0 kg) equipped with rumen cannulas (Norris et al., 2019). Animals were maintained in a dry lot and provided ad libitum access to bermudagrass (*Cynodon dactylon*) hay with the supplementation of dried distillers' grains every other day during the incubation period.

Within each incubation animal ($n = 3$), feed and feces from sample animals ($n = 2$) were replicated five times for each F57 and F58 combination (30 reps per BT $\times$ IL) and three times for Dacron combinations (18 reps per BT $\times$ IL). Additionally, to account for error, empty (blank) bags were incubated for each treatment combination and processed in the same manner as sample bags. All bags were incubated in 36 $\times$ 42 cm polyester bags with 50- μm porosity to prevent the fibrous particle accumulation on the outside of the F58 bags as observed when incubated in commercial laundry bags (Norris et al., 2019). A timed bag placement technique was used to ease their removal. In total, two polyester bags were placed within the rumen of each cannulated incubation animal, each containing 52 sample bags. As bags were removed from the rumen, they were immediately soaked in water plus ice to cease fermentation (Dewhurst et al., 1995) and subsequently rinsed in cold water using the delicate cycle of a household washing machine (Krizsan and Huhtanen, 2013) until the water became clear, approx. 1 h.

All bags were dried at 55 $^{\circ}\text{C}$ for 48 h using a forced-air oven, followed by 24 h at 105 $^{\circ}\text{C}$. Upon removal from the oven, bags were placed into desiccators to equilibrate and then weighed. Residue following the initial wash was considered iDM. Bags were subsequently washed in 100 mL/g DM of neutral detergent (ND) solution with the addition of 4 mL of heat-stable amylase using an ANKOM²⁰⁰ fiber analyzer (ANKOM Technology, Macedon, NY). Sodium sulfite was omitted to reduce the potential for residue recoveries to be altered (Van Soest, 1994). Due to the larger dimensions of the Dacron BT, bags were folded in half before being placed in the trays of the ANKOM²⁰⁰ fiber analyzer. Following each ND wash, bags were soaked in acetone before being dried at 105 $^{\circ}\text{C}$ for 24 h, and the resulting residue was considered iNDF. The calculation of iDM and iNDF was performed using the following equations :

$$\text{iDM, \% DM} = \frac{W3 - (W1 \times C1)}{W2} \times 100 \quad (1)$$

$$\text{iNDF, \% DM} = \frac{W4 - (W1 \times C2)}{W2} \times 100 \quad (2)$$

Where W1 is the initial bag weight (g), W2 is the initial sample weight (g), W3 is the weight of the dried bag and sample residue following the initial water rinse (g), W4 is the weight of the dried bag and sample residue following the ND wash (g), C1 is the blank correction factor following the initial water rinse (average weight of dry bag following cold water rinse divided by the initial weight of the bag), and C2 is the blank correction factor following the ND wash (average weight of dry bag following ND wash divided by the initial weight of the bag). The ADIA of feed and feces samples was determined independently using the ANKOM²⁰⁰ fiber analyzer. The ADIA was considered as the remaining DM following the combustion of the ADF residue at 525 $^{\circ}\text{C}$ for 4 h (Van Soest et al., 1991).

The digestibility and fecal excretion of DM and NDF were calculated using the following marker equations:

$$\text{DMD, \%} = 100 - \left[100 \times \left[\frac{M_i}{M_e} \right] \right] \quad (3)$$

$$\text{NDFD, \%} = 100 - \left[100 \times \left[\frac{M_i}{M_e} \times \frac{\text{NDF}_e}{\text{NDF}_i} \right] \right] \quad (4)$$

$$\text{Fecal DM, kg} = \text{DMI} \times [1 - \text{DMD}] \quad (5)$$

$$\text{Fecal NDF, kg} = \text{NDF}_i \times [1 - \text{NDFD}] \quad (6)$$

Where DMI is DM intake (kg), M_i being the marker concentration of the diet (%), M_e is the marker concentration of the feces (%), NDF_i is the NDF intake (kg), and NDF_e is the NDF concentration of the feces (%). The accuracy of digestibility estimates and fecal recoveries was calculated as estimated values divided by observed values from the independent metabolism trial.

Statistical analyses

All statistical procedures were performed using SAS software (SAS Institute Inc., Cary, NC). Residuals of indigestible fractions and calculated digestibilities were checked for normality and outliers. Because preliminary analysis indicated that variances were not homogeneous, treatments were clustered by k-means using PROC FASTCLUS for each dependent variable, and such information was taken into account in the subsequent analyses. Indigestible fractions were evaluated by sample type (feed and feces), considering bag as the experimental unit, following a 3 $\times$ 2 factorial arrangement using PROC GLIMMIX with incubation animal serving as a random factor in the model. The denominator degrees of freedom were adjusted using the SATTERTHWAITTE option, and variances were estimated separately using the GROUP option in the REPEATED statement. Differences were deemed significant at $P \leq 0.05$, and tendencies were assumed at $0.10 \geq P > 0.05$. Digestibility estimations were assessed using the previous model with the sample animal (animal the sample was collected from) as a random factor, and incubation animals considered the experimental units. Comparisons of digestibility accuracy and fecal recoveries to ADIA were performed using a treatment arrangement with contrast statements being used for a priori comparisons. Statistical model selections were based upon the corrected Akaike's information criterion. Mean comparisons were performed using LSMEANS and the Tukey-Kramer adjustment for all significant effects ($P \leq 0.05$).

Results

Indigestible matter

Dry matter

Indigestible DM residue of the feed displayed an interaction of BT $\times$ IL ($P < 0.01$) with the F58 $\times$ 288 h combination having the greatest residue (Table 2). There was no effect of BT $\times$ IL ($P = 0.29$) for iDM residue of the feces, but the main effects of BT and IL were influential ($P < 0.01$) with the F58 BT and 288-h IL having greater fecal iDM (Table 3). Resultant DM digestibility (DMD) demonstrated an interaction for BT $\times$ IL ($P < 0.01$) with

Table 2. Two-way interactions for residues and digestibilities of iDM and iNDF

		BT × IL ¹							
		F57		F58		Dacron			P-value
Marker	Item	288 h	576 h	288 h	576 h	288 h	576 h	SEM ² , %	BT × IL
iDM	Feed residue, %DM	28.02 ^B	24.21 ^C	33.29 ^A	28.79 ^B	18.60 ^D	17.90 ^D	0.732	<0.01
	Fecal residue, %DM	63.74	62.03	63.68	62.79	40.88	37.78	0.889	0.29
	DMD, %	56.01 ^B	60.98 ^A	47.73 ^C	54.11 ^B	54.61 ^B	52.52 ^{BC}	0.761	<0.01
	NDFD, %	47.61	53.62	37.75	45.36	45.99	43.36	3.109	0.10
iNDF	Feed residue, %DM	21.84 ^B	19.26 ^C	26.57 ^A	22.89 ^B	15.09 ^D	14.43 ^D	0.743	<0.01
	Fecal residue, %DM	48.70	47.46	49.54	49.27	32.37	30.37	0.738	0.22
	DMD, %	55.10 ^B	59.41 ^A	46.27 ^B	53.57 ^B	53.46 ^B	52.50 ^B	2.908	<0.01
	NDFD, %	46.50	51.78	35.98	44.74	44.65	43.43	4.183	0.12

¹BT: F57 = 25 µm, F58 = 10 µm, and Dacron = 50 µm (ANKOM technology, Macedon, NY); IL: 288 and 576 h.²Group variances estimated separately, the largest SEM is reported.^{A-D}Means followed by different superscripts within rows differ ($P \leq 0.05$).**Table 3.** Main effects for residues and digestibilities of iDM and iNDF

Main effects ¹										
Marker	Item	BT			SEM ² , %	IL		SEM, %	P-value	
		F57	F58	Dacron		288 h	576 h		BT	IL
iDM	Feed residue, %DM*	26.12 ^B	31.04 ^A	18.23 ^C	0.427	26.63 ^A	20.52 ^B	0.283	<0.01	<0.01
	Fecal residue, %DM	62.88 ^A	63.24 ^A	39.33 ^B	0.540	56.10 ^A	54.20 ^B	0.406	<0.01	<0.01
	DMD, %*	58.50 ^A	50.92 ^B	53.56 ^B	0.923	52.79 ^B	55.87 ^A	0.732	<0.01	<0.01
	NDFD, %	50.62 ^A	41.56 ^B	44.67 ^B	1.957	43.78 ^A	47.45 ^A	1.440	<0.01	0.07
iNDF	Feed residue, %DM*	20.55 ^B	24.73 ^A	14.76 ^C	0.418	21.17 ^A	18.86 ^B	0.265	<0.01	<0.01
	Fecal residue, %DM	48.08 ^B	49.41 ^A	31.37 ^C	0.404	43.54 ^A	42.37 ^B	0.303	<0.01	<0.01
	DMD, %*	57.26 ^A	49.92 ^B	52.98 ^B	1.561	51.61 ^B	55.16 ^A	1.065	<0.01	0.01
	NDFD, %	49.14 ^A	40.36 ^B	44.04 ^B	2.357	42.38 ^B	46.65 ^A	1.678	0.01	0.05

¹BT: F57 = 25 µm, F58 = 10 µm, and Dacron = 50 µm (ANKOM technology, Macedon, NY); IL: 288 and 576 h.²Group variances estimated separately, the largest SEM is reported.^{A-C}Means followed by different superscripts within rows differ ($P \leq 0.05$).

*Displayed a significant interaction of BT × IL.

the F57 × 576 h combination resulting in an average increase in digestibility of 15.4% relative to all other combinations (Table 2). However, there was no interaction of BT × IL for NDF digestibility (NDFD). The NDFD was affected by BT ($P < 0.01$), as the F57 BT displayed a 17.5% increase in digestibility, on average, with the 576-h IL having a tendency ($P = 0.07$) to increase NDFD (Table 3).

Neutral detergent fiber

Similarly, iNDF residue of the feed demonstrated an interaction of BT × IL ($P < 0.01$) with the F58 × 288 h combination having the highest residue (Table 2). There was no interaction of BT × IL for iNDF residue of the feces. As with iDM, the main effects were significant ($P < 0.01$) with the F58 BT and 288-h IL having greater fecal iNDF (Table 3). The F58 and 288 h treatments had, on average, 30.1% and 2.7% greater fecal iNDF values, respectively. An interaction of BT × IL was present for DMD ($P < 0.01$), as the F57 × 576 h combination resulted in a 14.3% increase in DMD, on average (Table 2). Estimates of NDFD were influenced by the main effects ($P \leq 0.05$) with the F57 and 576 h treatments, increasing NDFD 16.6% and 10%, on average, respectively (Table 3).

The evaluation of the coefficient of variation (CV) within sample types (feed and feces) demonstrated that iNDF tended to have less variation relative to iDM (Figure 1). However, for the feed samples, iNDF displayed increased variation when using F58 treatments. As for the fecal samples, indigestible components

were somewhat similar across BT and IL combinations, with the exception to iDM when using Dacron × 576 h. The average CV of feed was 7.6% and 7.2% for iDM and iNDF, respectively, whereas the CV of the feces was 6.1% and 5.2%.

Recovery and accuracy

Dry matter

Recovery of fecal DM was overestimated for all incubation combinations with estimates ranging from 108% to 149% recovery (Table 4). Of the methods investigated, ADIA and iDM using the F57 × 576 h incubation method most closely approximated 100% recovery, with 97.8% and 108.5% recovery, respectively ($P < 0.01$). The contrast analyses indicated that ADIA was significantly different from all incubation combinations and the F57 × 576 h methods ($P < 0.01$). Relative to all other treatments, ADIA provided the most accurate and highest estimate of DMD, 101.19% and 64.79%, respectively.

Neutral detergent fiber

The fecal NDF recoveries of all treatments exceeded 100% (Table 4). However, in contrast to DM recovery, fecal NDF recovery of ADIA was 38.9% greater than the F57 × 576 h combination with iDM as the internal marker ($P < 0.01$). Although there was no difference in NDFD estimates when using ADIA or the F57 × 576 h methods ($P = 0.13$), ADIA had the highest digestibility

Figure 1. CV for indigestible residues of BT for feeds (A) and feces (B): F57 = 25 μ m, F58 = 10 μ m, and Dacron = 50 μ m and ILs: 288 and 576 h of feed and feces for iDM (solid bars) and iNDF (empty bars).

estimate when compared with all other treatments ($P < 0.01$). Accuracy of NDFD was highest for F57 $\times$ 576 h using iDM as the marker and ADIA, 93.62% and 101.28%, respectively ($P < 0.01$). However, the contrast comparison indicated that ADIA was more accurate than the F57 $\times$ 576 h methods ($P = 0.02$).

Discussion

The use of iDM as an internal marker is preferred to iNDF due to the reduced analytics required (Detmann et al., 2001), but multiple reports have demonstrated that the use of iDM results in increased variation due to microbial and inert contamination during incubations (Huhtanen et al., 1994; Van Soest, 1994; Valente et al., 2011). In general, washing in ND solution reduced the contamination of feed and fecal samples with iNDF residues displaying an average reduction in CV of 0.4% and 0.9% for feed and feces, respectively, when compared with those of iDM. However, samples incubated in the F58 bags demonstrated an increase in CV compared with samples incubated in the F57 and Dacron bags. This difference may have been the result of particle adhesion to the bag during the washing process. It was reported previously that F58 bags tended to sequester particles due to the filter bag material (i.e., fabric), which differs from the F57 and Dacron bags (Norris et al., 2019). Overall, the observed CV are less than the 3.5% and 4.8% difference in feed and feces samples, respectively, reported by Norris et al. (2019), but the differences in diet type and the inclusion of secondary

compounds likely account for much of the discrepancy in CV. Similar to the present study, Valente et al. (2011) observed a general reduction in variation for different sample types when using iNDF compared with iDM.

The use of the F58 bags resulted in greater iDM and iNDF residue values for both feed and fecal samples. Although it is difficult to separate the source of error, the increased residue values are likely due to the high contamination potential of the F58 bag material, coupled with lower bag porosity that can alter sample degradation within the bag (Nocek, 1988). The lower fecal recoveries of the F57 and Dacron bags relative to the F58 appear to be from sample loss due to increased bag porosity (Huhtanen et al., 1994). Even so, regardless of marker utilized, the 576-h incubations resulted in less feed and fecal residue compared with 288 h. This finding is in agreement with Norris et al. (2019), who reported lower residue estimates and reduced variability for 576-h incubations compared with 288 h. Our results further indicate that the length of in situ incubation required for the determination of truly indigestible constituents exceeds the 288-h IL often used.

In general, digestibility estimates from iDM exceeded those of iNDF by 0.9% and 1.1%, on average, for DMD and NDFD, respectively. This, however, is inconsistent with previous reports that observed higher digestibility estimates using iNDF compared to iDM (Huhtanen et al., 1994; Norris et al., 2019). In the present study, the lower digestibility estimates using iNDF could be indicative of iNDF washout during ruminal incubation or analytical procedures due to the heterogeneous porosities of incubation bags. However, the marginal difference in digestibilities could potentially be just an artifact of the error associated with less than optimal replication within experimental units. The incubation of large numbers of bags may contribute to the greater sample residue, such as that observed with the 288-h IL. However, we are not aware of such limitations given the bag size and numbers used in our trials. We believe that the number of incubated bags is not interfering with the normal ruminal functions as long as they do not alter the rumen motility or cause a decrease in feed intake and water consumption by the animals. We did not observe abnormal rumen movements and feed or water intake during the trials. Furthermore, the evaluation of indigestible components of high-roughage diets or feces requires the use of greater sample replications as a result of the reduced total fermentable substrate (Norris et al., 2019). The use of 576-h incubations has been reported to provide increased digestibility predictions (Norris et al., 2019). In the current study, this appears to hold for the F57 and F58 BT as digestibilities tended to increase when incubated for 576 h. However, the Dacron BT did not demonstrate the differences in digestibilities between incubation times, likely a result of feed particle washout.

The complete recovery of feces is an important factor when establishing an adequate marker for digestibility estimations (Owens and Hanson, 1992). Of the markers investigated, ADIA and the F57 $\times$ 576 h using iDM provided complete fecal DM recoveries (97.78% and 108.5%, respectively), whereas the remaining treatments resulted in a 28.2% overestimation, on average. This result is in agreement with Huhtanen et al. (1994), who reported that fecal recoveries were not significantly different from 100% when utilizing iDM and ADIA methods. However, for NDF recovery, all methods overestimated fecal NDF recovery with F57 $\times$ 576 h using iDM having the most accurate recovery (108.6%) and ADIA exhibiting the least accurate recovery (151%). In this study, F57 $\times$ 576 h using iDM provided accurate recovery of fecal DM and NDF with ADIA having accurate recovery of fecal

Table 4. Comparison of internal marker recoveries and estimate accuracy

	F57						F58						Dacron						SEM ¹	P-value ²	Contrast	
	288 h			576 h			288 h			576 h			288 h			576 h					1	2
	iDM	iNDF	iDM	iNDF	iDM	iNDF	iDM	iNDF	iDM	iNDF	iDM	iNDF	iDM	iNDF	ADIA	ADIA	ADIA	ADIA				
Feces DM recovery, %	122.50 ^{BC}	124.83 ^B	108.50 ^D	112.83 ^{CD}	145.33 ^A	149.33 ^{AB}	127.50 ^{AB}	129.00 ^{AB}	126.00 ^{AB}	129.50 ^{AB}	132.00 ^{AB}	131.83 ^{AB}	97.78 ^D	8.237	<0.01	<0.01	<0.01	<0.01				
DMD, %	56.01 ^C	55.10 ^C	60.98 ^{AB}	59.41 ^B	47.73 ^D	46.27 ^{CD}	54.11 ^{CD}	53.57 ^{CD}	54.61 ^C	53.46 ^{CD}	52.52 ^{CD}	52.50 ^{CD}	64.79 ^A	2.908	<0.01	<0.01	<0.01	<0.01				
DMD accuracy, %	87.48 ^C	86.06 ^C	95.24 ^{AB}	92.79 ^B	74.56 ^D	72.27 ^{CD}	84.51 ^{CD}	83.66 ^{CD}	85.29 ^C	83.49 ^{CD}	82.03 ^{CD}	82.01 ^{CD}	101.19 ^A	4.578	<0.01	<0.01	<0.01	<0.01				
Feces NDF recovery, %	122.50 ^{CD}	125.00 ^C	108.67 ^E	112.83 ^{DE}	145.50 ^{AB}	149.67 ^{ABC}	127.67 ^{BC}	129.33 ^{BC}	126.50 ^{BC}	129.50 ^{BC}	132.00 ^{ABC}	132.00 ^{BC}	151.00 ^A	8.172	<0.01	<0.01	<0.01	<0.01				
NDFD, %	47.61 ^{A,BCD}	46.50 ^{ABCD}	53.62 ^{AB}	51.78 ^{ABC}	37.75 ^D	35.98 ^{CD}	45.36 ^{ABCD}	44.75 ^{ABCD}	45.99 ^{ABCD}	44.65 ^{BCD}	43.43 ^{ABCD}	43.36 ^{CD}	58.05 ^A	4.183	<0.01	<0.01	<0.01	0.13				
NDFD accuracy, %	82.97 ^{BC}	81.00 ^{BCD}	93.62 ^A	90.42 ^{AB}	65.68 ^D	62.55 ^{CD}	79.04 ^{BCD}	78.01 ^{BCD}	80.18 ^{BCD}	77.87 ^{CD}	75.41 ^{BCD}	75.64 ^{CD}	101.28 ^A	6.666	<0.01	<0.01	<0.01	0.02				

¹Group variances estimated separately, the largest SEM is reported.²Method = incubation combination.³1 = ADIA vs. all other methods; 2 = ADIA vs. F57 × 576 h methods.A-E Means followed by different superscripts within rows differ ($p \leq 0.05$).

DM. However, since ADIA does not require ruminal incubation, this method expedites the analytical processes when fecal DM is of interest.

As for the accuracy of digestibility, ADIA more accurately estimated DMD compared with all other treatments with the accuracy of digestibility approximating 100%. Relative to all other methods, the DMD estimate using ADIA was 10.9% higher. These results are in agreement with Huhtanen et al. (1994), where the authors reported that digestibility was more accurately predicted when utilizing ADIA. Similarly, NDFD was more accurately predicted using ADIA, with estimates being increased 22.7% relative to all other methods and 7.6% greater than the estimates of the F57 × 576 h combinations. This finding is in concordance with the increased NDFD using ADIA relative to iNDF observed by Lee and Hristov (2013). Collectively, ADIA provided a better estimate of DM and NDFD.

Conclusions

Based on our results, using iNDF as an internal marker provides greater precision in the determination of indigestible constituents. Variation in feed and fecal residues due to contamination is greatly reduced when using iNDF compared with iDM. The use of 576-h incubations resulted in lower indigestible residues compared with those of 288 h, thus indicating the availability of pdNDF in the rumen exceeds 288 h. Although bags with a porosity between 6 and 12 µm are recommended for the determination of indigestible residues (Krizsan et al., 2015), our results indicate greater accuracy in fecal recovery when using the F57 BT with a 25-µm porosity. Overall, fecal recovery was complete with the F57 BT and 576-h IL using iDM, for both DM and NDF. However, when only fecal DM is of interest, ADIA provided accurate fecal DM recovery estimations and may be beneficial as ruminal incubation is not required. Furthermore, the use of ADIA by analytical laboratories allows for the opportunity to maximize data output, as prolonged ruminal incubation is not required when this method is used. In this study, improved accuracy in digestibility estimations for DM and NDF was attained using ADIA. Thus, indicating ADIA is an adequate marker for approximating digestibility. However, further research should investigate the accuracy of ADIA in the determination of digestibilities using different feedstuffs.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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