Technical note: Evaluation of markers for estimating duodenal digesta flow and ruminal digestibility: Acid detergent fiber, sulfuric acid detergent lignin, and *n*-alkanes

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ABSTRACT

The amount of digesta flowing to the duodenum is a relevant measurement for the evaluation of nutrient supply to ruminants, which is usually estimated in animals fitted with a duodenal T-type cannula using internal or external markers. This study evaluated acid detergent fiber (ADF) compared with external (C_{32} *n*-alkane) and internal [sulfuric acid lignin (ADL) and *n*-alkanes C_{31} and C_{33}] markers for estimating duodenal flow and (or) ruminal digestibility of dry matter (DM) in cattle and sheep. In the first assay, 4 duodenally cannulated Holstein steers housed in metabolism cages, dosed with C_{32} *n*-alkane, and fed *Avena strigosa* plus concentrate and increasing levels of tannin extract to reduce ruminal digestibility, were used in a Latin square design. The mobile-bag technique was used to measure the intestinal disappearance of ADL and ADF from forage (*Avena strigosa*, *Pennisetum purpureum*, *Cynodon dactylon*, and *Medicago sativa*) and concentrate (corn grain, soybean meal, and sunflower meal) samples that were previously incubated in the rumen of additional fistulated steer for 12, 24, 36, or 48 h. The ADF concentration in residues recovered in the feces was strongly related to the ADF concentration in residues at the duodenum ($R^2 = 0.93$, standard deviation = 30.0, $n = 901$). This relationship showed a lower precision for ADL fraction ($R^2 = 0.88$, standard deviation = 12.6, $n = 590$). In a second assay, duodenal flow and ruminal DM digestibility were calculated from the duodenal and fecal concentration of either marker. We observed a significant effect of marker type on ruminal DM digestibility values, and the effect of tannin treatments was observed only when ADF or ADL was used as the marker. The lowest residual error was obtained for ADF. Ruminal DM digestibility was, on average,

higher for C_{31} and C_{33} *n*-alkanes, and the use of dosed C_{32} *n*-alkane resulted in a negative value. In the third assay, a data set of 235 individual observations was compiled from digestibility trials to compare ADF and ADL as markers for estimating duodenal digesta flow in wethers ($n = 204$) and cattle ($n = 31$). We observed a strong relationship between markers ($R^2 = 0.84$ in sheep and $R^2 = 0.88$ in cattle), but variance analysis within trials indicated that ADF was more precise than ADL. In conclusion, in digestibility trials in which fecal output was measured and spot samples of the duodenal digesta were obtained, duodenal flow and ruminal digestibility of the DM may be estimated from the relationship between the ADF concentration in feces and that in the duodenal digesta of ruminants.

Key words: cattle, digesta marker, methodology, sheep

Technical Note

Most published studies conducted to evaluate the duodenal flow of nutrients or ruminal digestibility have used animals fitted with simple T-type cannulas and an external or internal marker. Compared with external markers, internal markers have the advantage of being an intrinsic part of the diet and, consequently, they flow intimately associated with the digesta (Titgemeyer, 1997). Porter and Singleton (1971a) conducted a study using sheep fitted with a re-entrant duodenal cannula and showed that lignin degradation occurred entirely in the stomach. Thus, in digestibility studies in which total fecal output is measured, the duodenal digesta flow may be estimated on the basis of fecal output and the ratio of lignin concentration in feces and duodenal digesta. However, when ADL is present in low concentrations in the duodenal digesta, the precision of duodenal flow estimates is usually compromised. Because cellulose and ADF digestibility in the lower gastrointestinal tract is also insignificant (Porter and

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Singleton, 1971b; Charmley and Veira, 1990; Ivan et al., 1996), the ADF fraction of the digesta could also be used as an internal marker, with an analytical advantage over the ADL fraction.

Since the first proposal of their use by Mayes and Lamb (1984), *n*-alkanes have been used both as external (dotriacontane, C₃₂) and internal markers (odd-chain *n*-alkanes) for estimating the fecal output and total apparent DM digestibility in ruminants, respectively, with an accuracy advantage over conventional markers (Dove and Mayes, 1996). However, the use of *n*-alkanes for estimating duodenal digesta flow has not been consistently reported.

Three assays were performed to evaluate (1) the disappearance of ADF and ADL in the lower gastrointestinal tract using the mobile-bag technique; and (2) the use of ADF compared with the ADL and *n*-alkanes as an internal marker to estimate the duodenal digesta flow and ruminal digestibility in cattle and sheep. Research protocols followed the guidelines recommended by the Animal Care and Ethical Committee of the Universidade Federal de Santa Maria.

Assay 1: Mobile-Bag Evaluation of Intestinal Disappearance of ADF and ADL

This assay was conducted as a 4 × 4 Latin square experiment with 15-d periods using 4 Holstein steers (156 ± 33 kg of BW) fitted with duodenal T-type cannulas, housed in metabolism cages, and fed *Avena strigosa* (60%), concentrate (40%), and 1 of 4 levels (0, 0.8, 1.6 or 2.4% of DM) of *Acacia mearnsii* tannin extract (Weibull Black, Tanac S. A., Montenegro, Brazil) to reduce ruminal digestibility. The chemical composition of the diet is shown in Table 1. Feed was offered twice daily (0800 and 1700 h) and was restricted at a rate of 2% of BW/d. Steers had free access to water and to a commercial mineral salt. An additional steer (Devon steer, 650 kg of BW), fitted with a ruminal cannula and kept in a ryegrass pasture, was used for rumen in situ incubations. The mobile-bag test was performed from d 10 to 15 of each Latin square experimental period using forage (*Avena strigosa*, *Pennisetum purpureum*, *Cynodon dactylon*, and *Medicago sativa*) and concentrate (corn grain, soybean meal, and sunflower meal) samples. Approximately 1.5 g of dried and ground (2-mm screen) of each forage and concentrate sample was weighed in a polyester filter bag (7 × 5 cm; 25 µm porosity) and incubated in the rumen of the Devon steer for 12, 24, 36, or 48 h. Although standard bags for evaluating DM digestion in situ have around 50 µm porosity, a smaller pore bag was used to reduce the risk of losing indigested material from the bags. For each sample type, incubation time, experimental period, and

Holstein steer, 4 bags were incubated (i.e., a total of 256 bags per sample type). After rumen incubation, 2 of the 4 bags were introduced into the duodenum of Holstein steers and recovered in feces (i.e., 128 bags per sample type). All bags were washed in tap water and dried in a forced-air oven (55°C) for analysis. The DM content was determined by drying at 105°C for at least 24 h. The ADF concentration was analyzed according to AOAC International (1997; method 973.18) except that samples were weighed into polyester filter bags (porosity of 25 µm) and treated with neutral or acid detergent solution in an autoclave at 110°C for 40 min (Senger et al., 2008). The ADL concentration was analyzed by soaking the bags containing the ADF residue in 12 M H₂SO₄ for 3 h (AOAC International, 1997; method 973.18). All ADL and ADF concentrations included ash and were expressed as grams per kilogram of sample DM incubated in the rumen. The residual ADF or ADL concentration values in bags not inserted in the duodenum were compared using linear regression with those of the respective paired bags inserted in the duodenum and recovered in feces. A confidence interval (95%) of the equation parameters was generated to evaluate the deviation of the slope from 1 or of the intercept from 0.

Assay 2: Comparison Between *n*-Alkanes, ADF, and ADL as Markers for Estimating Ruminal Digestibility in Cattle

This assay was conducted as a Latin square experiment with 4 Holstein steers as previously described. From d 5 to 15 of each experimental period, animals were orally dosed twice daily with 125 mg of C₃₂ *n*-alkane, which was impregnated in 2-g cellulose pellets. Feed, eventual refusals, and feces were weighed and sampled daily from d 10 to 15 of each experimental period. From d 10 to 14 of experimental periods, duo-

Table 1. Chemical composition of dietary components

Item	<i>Avena strigosa</i> ¹	Concentrate ²
DM (%)	11.2	86.3
Chemical composition (% of DM)		
OM	87.5	91.3
Nitrogen	2.8	3.2
NDF	47.7	22.0
ADF	28.5	10.7
ADL	1.8	5.0
NFC	21.6	46.4
Ether extract	4.9	2.6

¹Cut 5 cm above the ground from swards at vegetative stage of growth and stored frozen (−20°C).

²Composed of soybean meal (30%), rice bran (35%), and corn cracked grain (35%).

Figure 1. Relationship between the concentrations (g/kg of incubated DM) of ADF (A, $n = 901$, $SD = 30.0$; $P < 0.05$) or ADL (B, $n = 590$, $SD = 12.6$; $P < 0.05$) in the residue of forage and concentrate samples incubated in the rumen for 12, 24, 36, or 48 h and not inserted in the duodenum (X) with those obtained after passage of residual feed samples through the lower gastrointestinal tract (Y) of steers. For both chemical components, the intercept was not different from 0, nor was the slope different from 1.

denal digesta samples (200 mL) were collected at 8-h intervals, advancing 2 h each day to obtain samples at 2-h intervals over a 24-h period. All samples were oven-dried at 55°C for at least 72 h, ground to pass a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA), and pooled by animal and period for analysis. The DM, ADF, and ADL analysis was performed as previously described. The concentrations of *n*-alkanes were analyzed in the feed, duodenal digesta, and fecal samples according to the protocols of extraction and

Table 2. Descriptive variables (range) of digestibility trials¹ carried out with sheep and cattle fitted with duodenal T-type cannula

Trial	n	Diet	Species	BW (kg)	DMI (g/d)	DM digestibility (%)
1	16	<i>Cynodon dactylon</i> fresh	Sheep	23–31	294–784	47–74
2	15	<i>Sorghum sudanense</i> frozen plus cracked corn grain and canola meal	Sheep	28–34	330–963	50–86
3	14	<i>Lolium multiflorum</i> fresh plus <i>Acacia mearnsii</i> tannin extract (intraruminally infused)	Sheep	28–33	245–879	62–81
4	24	<i>Lolium multiflorum</i> fresh	Sheep	18–28	221–926	63–91
5	28	<i>Lolium multiflorum</i> silage plus maize silage	Sheep	22–38	390–1,413	63–75
6	66	<i>Lolium multiflorum</i> fresh or <i>Cynodon</i> sp. hay plus levels of crude glycerine (intraruminally infused)	Sheep	15–36	105–711	43–80
7	25	<i>Pennisetum purpureum</i> plus <i>Arachis pintoi</i> hay (0.7:0.3)	Sheep	31–44	575–1,258	58–69
8	16	<i>Pennisetum purpureum</i> plus levels of <i>Arachis pintoi</i> hay	Sheep	35–46	915–1,542	47–63
9	15	<i>Avena strigosa</i> fresh plus soybean meal, rice bran, corn cracked grain, and levels of <i>Acacia mearnsii</i> tannin extract	Cattle	110–205	2,176–4,144	73–83
10	16	Maize silage plus soybean meal or canola meal, rice bran, corn cracked grain, and levels of <i>Acacia mearnsii</i> tannin extract	Cattle	190–395	4,926–9,868	67–75

¹Trials were conducted between 2003 and 2011 at the Universidade Federal de Santa Maria, Santa Maria, RS, and at the Universidade do Estado de Santa Catarina, Lages, SC, in southern Brazil (29°4'S, 53°4'W).

Figure 2. Relationship between the duodenal flow of DM estimated using the marker ADF or ADL, which were both measured in the duodenal digesta and feces of sheep (A, 8 trials, $n = 204$, $SD = 70.5$; $P < 0.05$) and cattle (B, 2 trials, $n = 31$, $SD = 465.5$; $P < 0.05$) fitted with duodenal cannula. For both animal groups, the slope of the linear regressions was different from 1 ($P < 0.05$). In trials with sheep, but not with cattle, the intercept was different from 0 ($P < 0.05$).

analysis by GC as previously described by Dove and Mayes (2006). The recovery of n -alkanes C_{31} and C_{33} was calculated as follows: [feces DM (g/d) $\times$ marker in feces (mg/g of DM)]/[feed DM (g/d) $\times$ marker in feed (mg/g of DM)]. The recovery of n -alkane C_{32} was calculated as [feces DM (g/d) $\times$ marker in feces (mg/g of DM)]/orally dosed C_{32} (mg/d). The duodenal flow (g/d) of DM was calculated from C_{32} n -alkane as fol-

Figure 3. Average coefficient of variation of rumen DM digestibility estimated within trials using the marker ADF or ADL in trials with sheep (8 trials, $n = 204$) and cattle (2 trials, $n = 31$). The lines above the bars indicate the standard deviation of the means. $P < 0.05$, $n = 10$.

lows: orally dosed C_{32} (mg/d)/ C_{32} in duodenal digesta (mg/g of DM), and from duodenal and fecal concentration of marker (i.e., ADL, ADF, or one of the n -alkanes C_{31} , C_{32} , and C_{33}) as follows: [feces DM (g/d) $\times$ marker in feces (mg/g of DM)]/[marker in duodenal digesta (mg/g of DM)]. Rumen DM digestibility was calculated for each marker as [DMI (g/d) – duodenal DM (g/d)]/DMI (g/d). Ruminal digestibility data were analyzed using PROC MIXED (SAS Institute, 2002). The statistical model included the fixed effect of the level of tannin extract, marker type, and their interaction, and the random effects of animal and period. The least squares means of ruminal DM digestibility estimated with each marker, as average of all tannin treatments, were compared using Student's t -test.

Assay 3: Comparison Between ADF and ADL as Markers for Estimating the Duodenal Digesta Flow and Ruminal Digestibility in Sheep and Cattle

A data set of 235 individual observations was compiled from digestibility trials testing several diet sources to compare ADF and ADL as markers for estimating the duodenal digesta flow. These trials were conducted between 2003 and 2011 at the Universidade Federal de Santa Maria, Santa Maria, RS, and at the Universidade do Estado de Santa Catarina, Lages, SC, in southern Brazil (29°4'S, 53°4'W) using wethers (8 trials, $n = 204$, ranging from $n = 14$ to 66 each) and cattle (2 trials, $n = 15$ and 16 each) fitted with a duodenal T-type

Table 3. Ruminal DM digestibility in steers fed *Avena strigosa* (60%), concentrate (40%), and levels of *Acacia mearnsii* tannin extract (0, 0.8, 1.6, or 2.4% of DM), estimated through different markers of duodenal digesta flux¹

Marker ²	LSM	SD	Probability of tannin effect
Sulfuric acid lignin	34 ^c	11.0	0.01
ADF	49 ^{bc}	5.1	0.001
<i>n</i> -alkane C ₃₁	73 ^a	11.6	0.20
<i>n</i> -alkane C ₃₂	52 ^{bc}	12.9	0.08
<i>n</i> -alkane C ₃₃	63 ^{ab}	14.5	0.27
<i>n</i> -alkane C ₃₂ dosed-based	−4 ^d	26.7	0.25

^{a-d}Means followed by different letters differ by Student's *t*-test ($P < 0.05$).

¹Values are least squares means of all tannin treatments ($n = 16$).

²For all markers except dosed-based C₃₂, duodenal DM flux was calculated as follows: [feces DM (g/d) × marker in feces (mg/g of DM)]/marker in duodenal digesta (mg/g of DM). For dosed-based C₃₂, duodenal DM flux was calculated as follows: orally dosed C₃₂ (mg/d)/C₃₂ in duodenal digesta (mg/g of DM).

cannula, housed in metabolic cages, and fed forage alone or with supplements. The description of diets and relevant variables are shown in Table 2. Experimental periods varied between 15 and 21 d, with 10 to 14 d for adaptation and 5 to 7 d for data and sample collection. The feed offered, refusals, and feces were weighed, recorded, and sampled daily during the collection periods. Duodenal digesta samples were obtained at 2- or 3-h intervals over a 24-h period. All samples were dried at 55°C for at least 72 h in a forced-air oven, ground to pass a 1-mm screen, pooled by animal within the experimental period, and analyzed for ADF and ADL concentrations as previously described. Data analysis was performed using the PROC MIXED of SAS (SAS Institute, 2002). The variance analysis model was $Y_{ij} = \mu + E_i + bC_{ij} + b_iC_{ij} + \varepsilon_{ij}$, where Y is the dependent variable (i.e., duodenal flow estimated using ADF), μ is the overall mean, E is the fixed effect of trial, bC is the slope of the general linear regression between individual duodenal flow values estimated using ADF against those estimated through ADL (X), b_iC_{ij} is the slope of each linear regression within E , and ε is the residual error. The confidence interval (95%) of the equation parameters was generated to evaluate the deviation of either the slope from 1 or the intercept from 0. Data of ruminal DM digestibility estimated within each trial using each marker (i.e., ADL or ADF) were analyzed using a mixed model that included the fixed effect of treatment and random effects of animal and period. A coefficient of variation was generated within each trial and marker, and the set of coefficient of variation values generated with ADL were compared with those generated with ADF through the F test.

Results of the mobile-bag assay indicated that the ADF and ADL concentrations in bags recovered in feces

were strongly related to the ADF and ADL concentrations in the paired bags not inserted in the duodenum, respectively (Figure 1). For both markers, the intercept was not different from 0, and the slope was not different from 1. However, because the coefficient of determination (R^2) was higher and the standard deviation was lower for ADF, the precision of the linear relationship was higher for ADF than for ADL.

Results of the digestibility assay indicated a significant effect of marker type ($P < 0.05$) on ruminal DM digestibility (Table 3), which was higher, on average, for C₃₁ and C₃₃ *n*-alkanes. The recovery of *n*-alkanes in the feces was (mean ± SD) 0.63 ± 0.11 , 0.47 ± 0.09 , and 0.76 ± 0.11 for C₃₁, C₃₂, and C₃₃, respectively. Except for C₃₂, these recovery values were similar to those reported by Morais et al. (2011) and Keli et al. (2013). Because a very low recovery of C₃₂ was obtained, the use of the relationship between the amount of orally dosed C₃₂ and its concentration in the duodenal digesta resulted in a negative ruminal DM digestibility. As suggested by Ohajuruka and Palmquist (1991), these results indicated that considerable disappearance of *n*-alkanes occurred in the stomach, mainly for C₃₂. In addition, a significant and expected effect of tannin treatments on ruminal DM digestibility was observed only for ADF or ADL but not when *n*-alkanes were used as a marker, with the lowest standard deviation of the mean for ADF.

Analysis of the data compiled from digestibility trials in the third assay indicated a strong relationship between the duodenal DM flow estimated using either ADL or ADF, for both sheep and cattle (Figure 2). However, the variance analysis within trials indicated that ADF was more precise than ADL for estimating ruminal DM digestibility in these trials (Figure 3).

In conclusion, in digestibility trials in which fecal output is measured and spot samples of the duodenal digesta are obtained, duodenal flow and ruminal digestibility of DM might be estimated from the relationship between the ADF concentration in feces and digesta of ruminants.

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