Correlation of Acid Detergent Lignin and Klason Lignin with Digestibility of Forage Dry Matter and Neutral Detergent Fiber

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ABSTRACT

The acid detergent lignin and Klason lignin methods were compared for their correlation with forage digestibility. Thirty-six forages, including C_3 legumes and C_3 and C_4 grasses, were analyzed for sulfuric acid detergent lignin, Klason lignin, and in vitro digestibilities of dry matter (DM) and neutral detergent fiber (NDF). Twenty of these forages were also fed to lambs at restricted intake for measurement of DM and NDF digestibilities. Lignin concentrations determined by the two lignin methods were positively correlated, and the Klason lignin value was always greater than the acid detergent lignin concentration. The largest differences were observed for grass forages. Digestibilities of forage DM and NDF were negatively correlated with both lignin methods for the in vitro system and the lamb digestibility trials. The degree of correlation for the two lignin methods with digestibility was generally similar across all forages and within forage classes. Slopes of linear regressions of digestibility on lignin concentration did not differ between legumes and grasses. Although the sulfuric acid detergent lignin and Klason lignin procedures gave very different estimates of forage lignin concentration, they were similarly correlated with digestibility and should yield predictions of forage digestibility that have similar accuracy.
(**Key words**: acid detergent lignin, Klason lignin, digestibility, neutral detergent fiber)

Abbreviation key: **ADL** = acid detergent lignin, **DMD** = DM digestibility, **IVDMD** = in vitro DMD, **IVNDFD** = in vitro NDFD, **NDFD** = NDF digestibility.

INTRODUCTION

Lignification of the plant cell wall has long been considered to be the primary impediment to forage digestibility (8). Abundant data are available that show negative correlations between lignin concentration and both DM and NDF digestibilities using a variety of methods to determine lignin content (7, 17, 21). However, the various methods of lignin determination often give quite different estimates of lignin concentration (3, 4, 6, 14, 26). One major class of methods for lignin determination utilizes strong mineral acids to hydrolyze the other cell-wall components, leaving lignin as a residue to be measured gravimetrically. The other major class of methods employs oxidizing agents to remove the lignin selectively. In the second class of methods, lignin is estimated either by loss in mass of the sample or through a photometric assay for lignin oxidation products. The acid detergent lignin (**ADL**) procedure of Van Soest (23) is most commonly employed by animal scientists and agronomists for analysis of forages. There are both hydrolytic (H_2SO_4) and oxidative ($KMnO_4$) versions of the ADL method; the sulfuric acid variant of ADL is the most popular.

Recently, Hatfield et al. (5) published data suggesting that the Klason lignin procedure does not suffer from protein contamination as was previously assumed (11, 23). This lignin method is part of a two-stage sulfuric acid hydrolysis that is commonly used to determine the neutral sugar components of cell-wall polysaccharides (19). Klason lignin values are typically two to four times greater for grasses than the sulfuric ADL estimates are for lignin concentrations of the same samples. Klason lignin values are approximately 30% higher than ADL values in legumes. The reason for the differences between grasses and legumes in lignin yield caused by method

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of analysis is not known, but presumably relates to structural characteristics of the lignins in these two forage classes. The high content of free phenolic hydroxyl groups in grass lignins is thought to account for their high solubility in alkali (12). Hatfield et al. (5) concluded that Klason lignin is a more accurate estimate of plant cell-wall lignin content than is ADL. Other evidence suggests that an acid-soluble lignin fraction is lost in the ADF step of the ADL procedure, thereby resulting in underestimates of lignin content by the ADL method (10, 13).

Because the ADL and Klason lignin methods yield such vastly different estimates of lignin concentration in forages, we were concerned that they may not be of equal utility for prediction of forage digestibility. Our objective in this study was to compare the relationship of lignin concentration estimated by the sulfuric ADL and Klason lignin methods with both in vitro and in vivo measurements of DM and NDF digestibilities on a large and diverse set of forages.

MATERIALS AND METHODS

A set of 36 forage samples was utilized in this study (Table 1). All forages were grown at Prairie-du-Sac, Wisconsin over a series of years and were preserved as hay, except for the corn silage sample. Most of the forage species consisted of two samples that differed in maturity when harvested. All forage samples were analyzed for NDF, ADF, and sulfuric ADL (hereafter referred to simply as ADL) by the sequential method of Van Soest and Robertson (24). Klason lignin was determined as the unhydrolyzed residue remaining after the two-stage sulfuric acid hydrolysis for the determination of total dietary fiber according to the method of Theander and Westerlund (19). Both ADL and Klason lignin are corrected for ash by these procedures. All data are presented on a DM basis.

All forage samples were subjected to in vitro ruminal digestibility determinations of DM and NDF. Ruminal fluid was collected from a nonlactating Holstein cow, fed a mixed alfalfa grass hay, after 12 h without feed. Separate forage subsamples, ground to pass a 1-mm screen, were used to determine DM and NDF digestibilities. In vitro DM digestibility (**IVDMD**) was determined after a 48-h ruminal fermentation followed by 48-h digestion with acid and pepsin (20). In vitro NDF digestibility (**IVNDFD**) was determined by extracting the residue from a 48-h ruminal fermentation with neutral detergent (9). Both sets of fermentations were run concurrently with the same batch of ruminal fluid.

In vivo digestibility data were available for 20 of the forages (Table 1) from a series of digestibility trials using lambs. The trials were conducted according to the protocol of Cherney et al. (2) and approved by the US Dairy Forage Research Center Animal Care Committee. Briefly, wether lambs were housed in metabolism crates and fitted with canvas fecal collection bags. The lambs were fed the forages once daily, and fecal bags were emptied twice daily. Experimental periods consisted of 42 d, and all lambs received a standard alfalfa hay for the first 10 d. The test forages were fed for the next 32 d in the following sequence: 1) ad libitum intake for 17 d, 2) 100% of ad

TABLE 1. Chemical composition of forage samples.

Forage species	NDF	ADF	Lignin	
			ADL[1]	Klason
	(% of DM)			
Legumes				
Alfalfa	49.8	33.3	8.1	10.4
	50.5	34.7	7.7	12.1
	51.4	35.7	7.7	11.6
	51.5	36.7	8.4	11.8
	54.6	40.1	9.1	12.7
	57.4	39.7	9.4	13.5
Birdsfoot trefoil[2]	48.5	36.7	8.8	12.2
	50.0	37.3	9.4	14.3
Ladino clover[2]	35.2	24.7	5.2	10.3
	43.0	30.0	4.9	9.5
Red clover[2]	51.2	37.4	6.3	13.7
	63.5	48.1	7.9	13.4
C_3 Grasses				
Barley[2]	61.1	27.7	2.1	6.6
	64.4	28.4	2.8	8.7
Oats[2]	63.4	30.7	2.9	9.5
	70.0	37.7	4.1	10.7
Orchardgrass[2]	60.7	31.7	2.7	11.6
	66.0	30.7	3.9	12.5
Reed canarygrass	58.1	22.0	1.6	9.6
	64.3	28.9	2.2	10.1
Perennial ryegrass	65.0	34.2	3.4	11.7
	65.5	33.0	2.8	8.4
Smooth bromegrass[2]	74.0	41.0	5.9	12.8
	74.3	35.1	6.1	12.0
Tall fescue	51.6	23.5	1.7	6.4
	59.8	29.0	2.5	8.4
Timothy[2]	67.3	36.3	3.3	9.6
	68.0	35.6	4.8	10.1
C_4 Grasses				
Big bluestem	70.1	31.5	3.9	7.2
	71.6	35.3	4.8	10.3
Corn silage	36.3	13.6	2.5	4.8
Stalklage	66.4	37.7	4.3	10.5
Pearl millet[2]	62.4	26.3	3.1	8.4
	62.7	28.7	2.7	9.1
Sorghum sudangrass[2]	63.2	25.4	3.3	9.0
	64.9	28.0	2.6	8.5

[1]Acid detergent lignin.

[2]Forage samples for which in vivo digestibility data from lambs were available.

libitum intake for 7 d, and 3) 1.8% BW for 8 d. Intake at 1.8% of BW was chosen to represent a maintenance intake for lambs used in these trials (growing from 30 to 50 kg of BW). Total fecal collections were conducted during the last 7 d that lambs were fed for ad libitum intake and during the last 5 d that lambs were fed for 100% of ad libitum intake and at 1.8% of BW. Dry matter (**DMD**) and NDF (**NDFD**) digestibilities were determined. Only digestibility data from the period during which lambs were fed at 1.8% of BW were utilized in the current study. Each digestibility measurement for an individual forage was the mean from four lambs. No adjustments were made for variation among lambs.

All laboratory analyses were done in duplicate. Estimates of lignin concentration determined by the ADL and Klason lignin methods were compared by a paired *t* test of samples grouped by forage class. Data were analyzed by the correlation and regression routines in PC-SAS (15). Slopes of the regression lines for the combined grass and legume regression were compared with the separate forage class regressions by *F* tests using the sums of squares for deviations from regression (18). Fit of the regression models to the data was compared by a homogeneity of variance test using the model error mean squares.

RESULTS AND DISCUSSION

The ADL and Klason lignin procedures are similar in concept, but differ in order of reaction conditions. In the ADL method, the sample is first subjected to dilute acid treatment (1 *M* H_2SO_4) at an elevated temperature (100°C) during the ADF step and then to concentrated acid (12 *M* H_2SO_4) at a lower temperature (approximately 25°C); solubilized matter is removed by filtration between the two acid steps. In contrast, in the Klason lignin procedure, the sample is first treated with concentrated acid (12 *M* H_2SO_4) at a low temperature (39°C) followed by dilute acid (0.4 *M* H_2SO_4) at a high temperature (125°C in an autoclave) without a filtration step in between. These differences in the order of acid strength used, inclusion of detergent in the ADF step, and addition of the filtration step to the ADL procedure account for the difference in lignin values as measured by the ADL and Klason lignin methods (10, 13).

In agreement with results of previous research (5), Klason lignin concentrations were higher ($P < 0.05$) than corresponding ADL measurements for all forage classes in the present study (Table 1). Klason lignin concentrations ranged from 4.8 to 14.3%, and ADL values ranged from 1.6 to 9.4%. The correlation between the two lignin methods was significant across all forages (Figure 1) and was of similar magnitude within legumes and C_3 grasses ($r = 0.63$ and 0.70 for legumes and C_3 grasses, respectively; $P < 0.05$), but this relationship between lignin methods was not significant ($P > 0.10$) for C_4 grasses. A similar correlation ($r = 0.79$; $P < 0.01$) between ADL and Klason lignin concentrations was found in the data of Hatfield et al. (5) for 11 forage samples. The slopes for the linear regressions of Klason lignin on ADL concentration for legumes and grasses, shown in Figure 1, were not different ($P > 0.10$). Hydrolytic and oxidative results of lignin analysis are generally positively correlated, as was found for a set of 12 forage samples analyzed by Iiyama and Wallis (6), who compared two versions of the acetyl bromide method with ADL ($r = 0.86$ and 0.79; $P < 0.01$). However, lignin methods are not always correlated. Two oxidative methods, $NaClO_3$ and $KMnO_4$, were not correlated ($P > 0.10$) for a set of 12 forages (3). Although not rigorously tested, oxidative methods apparently provide higher estimates of lignin concentration than those found by hydrolytic methods across a variety of forages (6, 25).

Table 2 presents the correlation coefficients for digestibility measures with ADL and Klason lignin

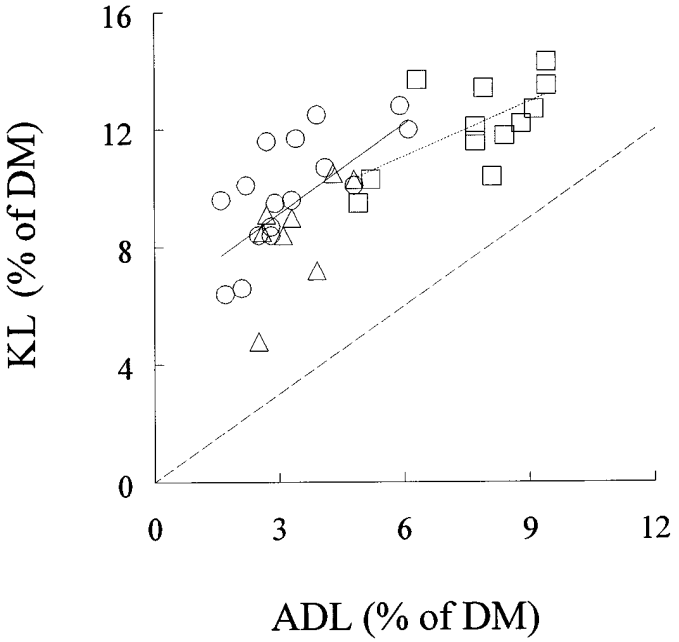

Figure 1. Correlation between lignin methods for 36 forage samples ($r = 0.75$; $P < 0.001$) and the linear regressions for Klason lignin (KL) versus acid detergent lignin (ADL) of the 12 legumes (· · · ; □; $y = 0.62x + 7.31$; $r^2 = 0.40$; $P < 0.05$) and 24 grasses (—; $y = 1.02x + 6.03$; $r^2 = 0.38$; $P < 0.01$) [16 C_3 (O) and 8 C_4 (△) grasses]. The dashed line represents unity between the lignin methods.

concentrations. Across all forages, both lignin methods were negatively correlated with both in vitro and in vivo digestibilities except for DMD on ADL concentration. Results varied when the relationships between digestibility and lignin method were examined within various forage classes. Klason lignin was not correlated with IVNDFD for both legumes and all grasses combined, but the correlation was strong for ADL with IVNDFD in both forage classes. In the C_3 grasses, both lignin methods were negatively correlated with all measures of digestibility, except for DMD with ADL, but very little correlation was observed for lignin methods with digestibility for the C_4 grasses. Of the 20 possible relationships listed in Table 2 between digestibility and either lignin method, the correlations were significant in 75 and 65% of the cases for ADL and Klason lignin, respectively. Goto et al. (4) found similar degrees of correlation for ADL and Klason lignin with IVNDFD in a set of 26 grasses.

Cherney et al. (2) previously showed that 48-h in vitro digestibility was more closely correlated to in vivo digestibility in lambs fed at a restricted intake than to in vivo digestibility in lambs fed for ad libitum intake (r = 0.85 vs. 0.72, respectively). The measurements of in vivo digestibility were highly correlated with the estimates for 48-h in vitro digestibility (r = 0.90 and 0.94 for DM and NDF, respectively) in our study. As a result, across all forages, the correlations of DMD and NDFD with ADL and Klason lignin concentrations were generally similar to those of in vitro digestibility (Table 2).

TABLE 2. Pearson correlation coefficients for acid detergent lignin (ADL) and Klason lignin concentration with in vitro and in vivo digestibility measurements for legumes and C_3 and C_4 grasses by forage classes.

Forage class	Digestibility measurement[1]			
	IVDMD	IVNDFD	DMD	NDFD
All forages				
ADL	–0.47	–0.73	NS[2]	–0.80
Klason lignin	–0.58	–0.48	–0.46	–0.81
Legumes				
ADL	–0.85	–0.70	–0.74	–0.93
Klason lignin	–0.74	NS	NS	–0.84
All grasses				
ADL	–0.81	–0.60	–0.71	–0.57
Klason lignin	–0.67	NS	–0.82	–0.70
C_3 Grasses				
ADL	–0.82	–0.67	–0.66	NS
Klason lignin	–0.71	–0.46	–0.78	–0.63
C_4 Grasses				
ADL	–0.96	NS	–0.96	NS
Klason lignin	NS	NS	NS	NS

[1]IVDMD = In vitro DM digestibility, IVNDFD = in vitro NDF digestibility, DMD = DM digestibility, and NDFD = NDF digestibility.

[2]$P > 0.10$.

Often there is no correlation between digestibility and ADL when grass and legume forages are combined into a single data file, although the individual forage classes have negative correlations for digestibility with ADL (10). Previous reports (10, 21, 22) on the correlation of ADL with digestibility have found that grasses and legumes have different slopes for this relationship. Grasses contain less ADL than do legumes at similar stages of maturity, and the slope of the negative relationship with digestibility is steeper for grasses (21). Based on similar observations, Buxton and Russell (1) concluded that permanganate ADL was more detrimental to the NDF digestibility of grass than to the NDF digestibility of legumes.

Unlike those earlier reports, we did not observe dissimilar slopes for the relationships of digestibility with ADL or Klason lignin in grasses and legumes. The linear regressions for digestibility on lignin concentration, as measured by both methods, are shown in Figures 2 and 3. For IVNDFD, the legume and grass forage classes did not have significant ($P > 0.10$) regressions with Klason lignin concentration, but the regression for all forages was significant ($P < 0.05$). However, the regression for DMD on Klason lignin concentration for legumes was not significant ($P > 0.10$). In fact, for all comparisons of legume and grass regressions of digestibility on lignin concentration, we detected no difference in slope ($P > 0.10$) between the two forage classes. For a few relationships, such as NDFD on ADL concentration (Figure 3b), it could be argued that a single regression could describe the relationship for both legumes and grasses. Although we cannot explain why a common relationship accounted for the correlation between ADL and digestibility of both grasses and legumes in our study, grasses and legumes would be expected to be similar for the relationship between Klason lignin and digestibility because the disparity in ADL concentration between these forage classes is markedly reduced in terms of Klason lignin. Compared with legumes, grasses have a much higher increase in estimated lignin concentration when measured as Klason than when measured as ADL. As a result, the lignin concentrations of grasses and legumes of similar maturity and digestibility are more nearly equal when measured as Klason lignin.

In addition, tests of the ADL and Klason lignin regression models with digestibility indicated that these two lignin methods were not different ($P > 0.10$) in their fit to the data. This similar fit suggests that, even though the relationships of these two lig-

nin methods with digestibility yield different regression lines because lignin concentration estimates differ between the methods, the accuracy of the digestibility predictions will be similar.

A question that arises from this study is why ADL and Klason lignin are similarly related to digestibility when the ADL method may result in the loss of a substantial portion of the total forage lignin. This loss may imply that acid-soluble lignin (lignin lost during the ADF step of ADL determination) has an effect on forage digestibility equivalent to the acid-insoluble lignin fraction. Sawai et al. (16) and Lowry et al. (13) have reported negative correlations between in vitro hemicellulose digestion by a mixed population of rumen microbes and increased concentration of acid-soluble lignin. In the study of Sawai et al. (16), the correlation of acid-soluble lignin with hemicellulose digestibility was based on the concentration of acid-soluble lignin in the original plant material, and ADL (acid-insoluble lignin) concentration was similarly correlated with digestibility. Addition of acid-soluble lignin from grasses to mixed ruminal organisms grown on defined media depressed growth rate; however, Lowry et al. (13) stated that their acid-soluble lignin would "not be the same as the molecular species liberated" during ruminal fermentation of

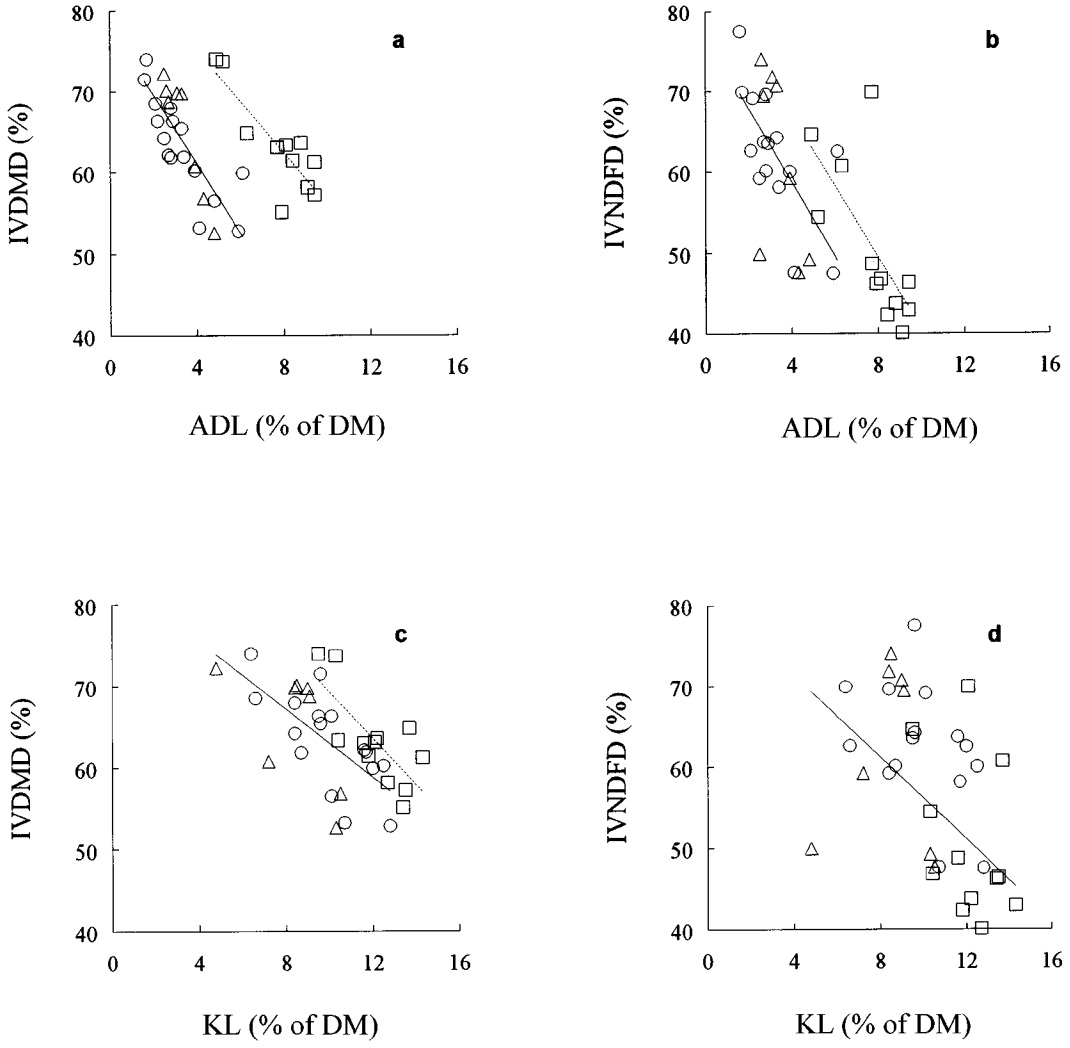

Figure 2. Linear regressions of in vitro DM (IVDMD) and NDF (IVNDFD) digestibilities with acid detergent lignin (ADL) and Klason lignin (KL) concentrations for 12 legumes (· · ·; □) (IVDMD = –3.19ADL + 87.9, r^2 = 0.72, $P < 0.01$; IVNDFD = –4.42ADL + 84.4, r^2 = 0.49, $P < 0.05$; and IVDMD = –2.85KL + 97.8, r^2 = 0.55, $P < 0.01$ for Figure 2, a, b, and c, respectively) and 24 grasses (—) (IVDMD = –4.27ADL + 78.2, r^2 = 0.64, $P < 0.01$; IVNDFD = –4.58ADL + 77.0, r^2 = 0.36, $P < 0.01$; and IVDMD = –2.10KL + 84.0, r^2 = 0.45, $P < 0.01$ for Figure 2, a, b, and c, respectively) [16 C_3 (o) and 8 C_4 (△) grasses]. However, a linear regression for combined forage is shown in Figure 2d (IVNDFD = –2.25KL + 81.4, r^2 = 0.23, $P < 0.001$) because the individual legume and grass regressions were not significant ($P > 0.10$) for IVNDFD on KL.

forages (page 47). The true role of acid-soluble lignin in forage digestion remains to be determined, and how that role relates to the ADL and Klason lignin residues is unclear.

CONCLUSIONS

The Klason lignin method yielded greater estimates of lignin concentration in forages than did the sulfuric acid version of the ADL method. Lignin concentrations determined by these two methods were positively correlated across a variety of legume and grass species. Both lignin methods were generally negatively correlated with DM and NDF digestibilities in both in vitro and in vivo systems. The degree of correlation of ADL and Klason lignin with forage digestibility was generally similar, although ADL tended to be slightly more consistently correlated with digestibility than was Klason lignin. However, Klason lignin may be a more accurate measure of total lignin content of forages than ADL, especially for grasses. The use of either ADL or Klason lignin to predict forage digestibility is acceptable because both methods result in digestibility predictions of similar accuracy.

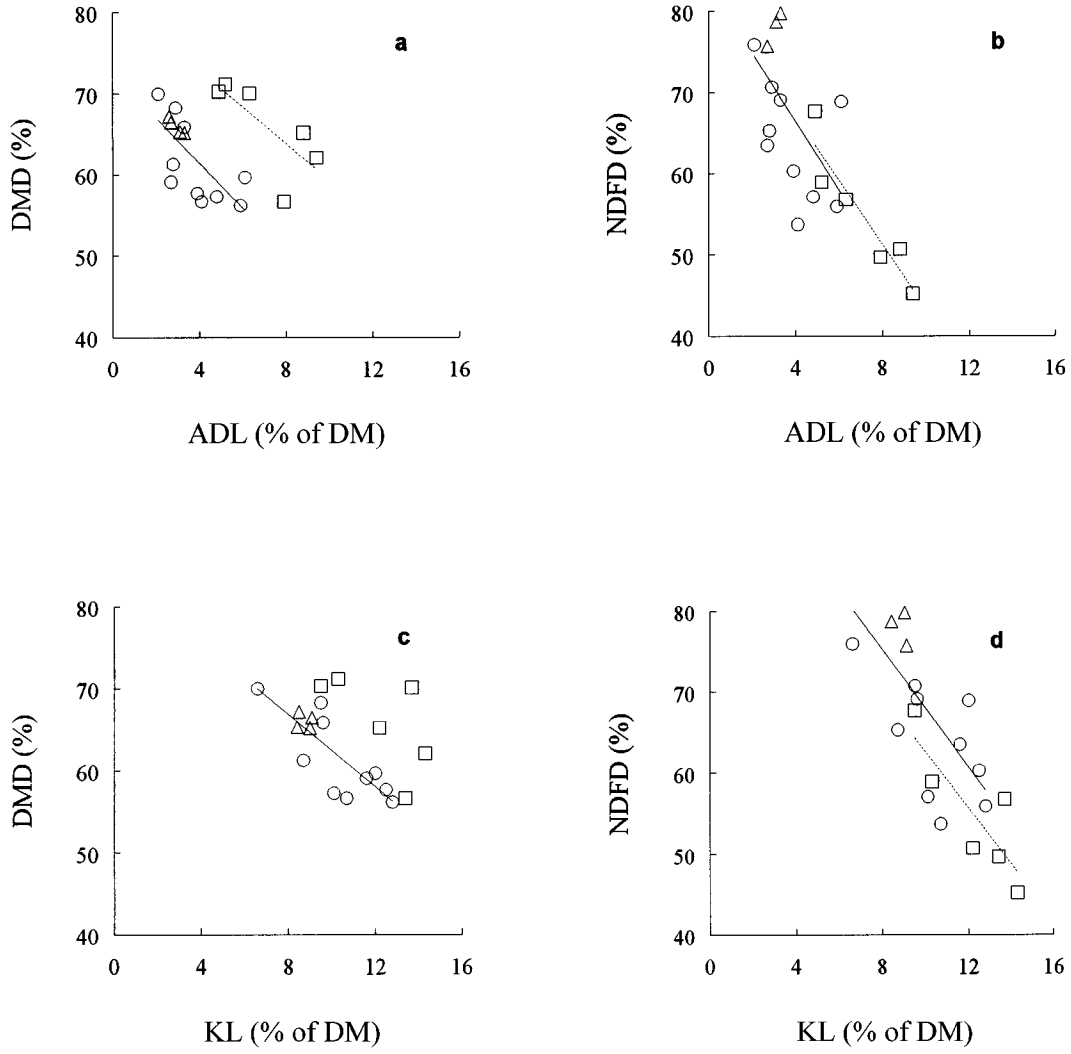

Figure 3. Linear regressions of in vivo DM (DMD) and NDF (NDFD) digestibilities with acid detergent lignin (ADL) and Klason lignin (KL) concentrations for six legumes (· · ·; □) (DMD = –2.26ADL + 81.9, r^2 = 0.55, $P < 0.10$; NDFD = –3.96ADL + 82.9, r^2 = 0.87, $P < 0.01$; and NDFD = –3.47KL + 97.3, r^2 = 0.71, $P < 0.05$ for Figure 3, a, b, and d, respectively) and 14 grasses (—) (DMD = –2.78ADL + 72.6, r^2 = 0.51, $P < 0.01$; NDFD = –4.25ADL + 83.5, r^2 = 0.34, $P < 0.05$; DMD = –2.20KL + 84.5, r^2 = 0.67, $P < 0.01$; and NDFD = –3.59KL + 103.9, r^2 = 0.49, $P < 0.01$ for Figure 3, a, b, c, and d, respectively) [10 C_3 (o) and 4 C_4 (△) grasses]. However, the regression for DMD on KL of legumes is not shown because this relationship was not significant ($P > 0.10$).

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