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Effect of lignin type on extent and rate of neutral detergent fibre digestion and potential energy yield

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Abstract

The objective of this work was to study the effects of both Klason lignin (KL) and acid detergent lignin (ADL) on *in vitro* neutral detergent fibre (NDF) digestion (IVNDFd) in an effort to assess if acid labile phenolic compounds affect the rate of degradation (k_d). Eighty five forages (lucerne, maize silages and grasses) were analyzed for NDF, ADL, KL and IVNDFd (6, 12, 24, 30, 36, 48, and 96 h fermentations were used for k_d estimations). Correlations were estimated among lignin types (KL *vs*. ADL), lignin and extent of IVNDFd, and lignin type and NDF k_d and tested for significance. Within and among all forage types, the correlation between ADL and KL was in general positive when on NDF basis and high and positive when on DM basis (0.77 to 0.90). Within and among all forages, only ADL was consistently negatively correlated with IVNDFd at all time points (-0.54 to -0.94). Correlations among forages for NDF k_d and lignin type were not consistent. Among all forages, KL was negatively correlated with IVNDFd and NDF k_d. The correlation between IVNDFd and ADL increased as fermentation length increased among all forages. However, the correlation of KL and IVNDFd was greatest up to 48 h of fermentation suggesting that the soluble phenolics affected both the rate and extent of IVNDFd. Unlike ADL, KL disappeared during IVNDFd, and in most forages there was a high negative correlation associated with the difference between KL and ADL (ΔL) and IVNDFd, except for brown midrib hybrids maize and early cut grasses that had a different behaviour. Among forages, a one unit increase in ΔL corresponded to an average 18% decrease in the k_d demonstrating that the greater the difference between KL and ADL the lower the rate of digestion.

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Introduction

The rate (k_d) and extent of NDF digestion is important because it impacts on the energy available from fibre, the passage of particles out of the rumen and the physical fill of the rumen (Mertens, 1993). The k_d and the extent of digestion of NDF are affected by lignin and the impact of KL and ADL, however, the difference among these two measures for NDF digestion remains obscure. Since KL appears to represent less polymerized phenolic compounds, the potential to negatively impact the ND solubles by either dilution or hindering digestion appears to be a possible hypothesis.

The lignin methods differentiate the phenolic acids primarily by acid solubility. The two lignin methods of interest are Klason lignin (KL) (Theander & Westerlund, 1986) and the acid-detergent lignin (ADL) (Van Soest *et al*., 1991). The KL results in higher values and represents total phenolic acids and some acid dispersible phenolics while ADL represents more polymerized phenolics that appear to behave in a more nutritionally uniform manner and impact the extent of NDF digestion in anaerobic conditions.

Correlation coefficients observed between KL and ADL and digestibility did not indicate any advantage of either method in predicting rate or extent of digestion (Jung *et al.*, 1997). Fukushima & Hatfield (2004) presented contrasting results for the relationship between *in vitro* NDF digestibility (IVNDFd) and KL with low correlations between the two measures and even lower correlations between ADL and IVNDFd.

Acid detergent lignin has been related to the ultimate extent of NDF digestion in the study of Chandler *et al.* (1980) who fermented waste residues for 60 or 90 days in methane fermenters. However, previous work has suggested that soluble phenolic-carbohydrate complexes are released during fibre degradation and might affect microbial degradation and subsequent energy yield by diluting the neutral detergent solubles (Gaillard & Richards, 1975; Lowry *et al.*, 1994), thereby implicating un-polymerized phenolics and not just ADL in digestibility. Since the ND soluble fraction of forage material contains the greatest energy content, anything that dilutes this fraction will reduce the energy content of the forage.

The objective of this work was to study the relationship between KL and ADL with *in vitro* NDF digestion in an effort to assess if acid labile phenolic compounds might affect the rate (k_d) of NDF degradation.

Materials and Methods

Eighty-five forages of various species and stages of maturity were harvested, dried in a forced air oven at 60 °C for 24 h and then ground through a 1 mm screen in a Wiley Mill (Thomas Scientific, Swedesboro NJ). The samples consisted of different maturities (early vegetative to boot stage) of lucerne, silages and grasses (*Lolium*, *Phleum pratense*, *Dactylis glomerata* L, *Panicum coloratum* L) and varieties of maize silage (standard hybrids and brown midrib hybrids (BMR, which are low lignin hybrids with a visible marker)) were analyzed for NDF (Mertens, 2002), ADL (Van Soest, 1973), KL (Theander & Westerlund, 1986) and IVNDFd. *In vitro* NDF fermentations were conducted in Erlenmeyer flasks following the procedures of Goering & Van Soest (1970) on samples ground through a 1 mm sieve. After digestion, residues were analyzed for NDF with 0.250 mL amylase (3 000 U/mL, Megazyme International, Ireland) without sodium sulphite and filtered in crucibles fitted with Whatman 934-AH glass microfibre filters (Whatman International Ltd., Maidstone, UK). Residues were also analyzed for ADL and KL using the modification of glass filters in the crucible as a filtering aid. Residues from 6, 12, 24, 30, 36, 48, and 96 h fermentations were used for k_d estimations (Van Amburgh *et al.*, 2003).

For evaluation of the relationships, correlations were estimated simple Pearson correlations for all forage types between lignin measures, lignin and extent of IVNDFd, and lignin type and IVNDFd k_d and tested for significance ($P < 0.05$).

Results and Discussion

The chemical compositions and the *in vitro* digestibility values of the forages used in the study are shown in Table 1. The highest NDF digestibility was for lucerne at six hours, for the grasses harvested during the early vegetative stage, at 48 hours and for the BMR at 96 hours. Correlations among lignin types and extent (IVNDFd) and rate of digestion (k_d) were all statistically significant ($P < 0.05$). Within and among all forage types, the correlations between ADL and KL were high and positive, except for the BMR maize and the early cut grasses. Furthermore, correlations on dry matter basis were larger than on NDF basis for all forages. This shows how the neutral detergent solution might disperse some of the soluble phenolics. Temperate, vegetative grasses had KL measures exceeding 15% and ADL values less than 2%.

Table 1 Mean values (standard deviations in parentheses) of chemical composition (%DM) and *in vitro* NDF digestibility (%NDF) of forages used in this study (Z temperate grasses, ZZ early cut grasses)

	NDF	ADL	KL	IVNDFd 6 h	IVNDFd 48 h	IVNDFd 96 h
Lucerne	36.50	6.35	11.12	20	50	54
	(6.20)	(1.27)	(2.62)	(6.20)	(6.10)	(7.18)
Maize	41.96	2.97	6.85	12	64	77
	(4.69)	(0.71)	(1.17)	(4.15)	(8.01)	(9.14)
Conv.	43.26	3.19	7.19	11	62	72
	(4.24)	(0.60)	(1.00)	(4.10)	(6.01)	(6.14)
BMR	36.79	2.09	5.48	14	76	91
	(2.78)	(0.39)	(0.83)	(2.14)	(4.02)	(5.23)
Z	73.17	6.37	13.75	9	57	67
	(10.21)	(2.46)	(2.88)	(5.12)	(8.12)	(9.12)
ZZ	44.98	2.70	10.50	15	78	81
	(4.80)	(0.47)	(5.19)	(2.12)	(3.10)	(4.01)

Conv. – standard maize hybrids; BMR - brown midrib hybrids maize silage.

Among all forages, the correlation between KL and IVNDFd was greater early in the fermentation period, whereas the correlation between IVNDFd and ADL increased as fermentation length increased, which is consistent with previous findings (Chandler *et al*., 1980). Within and among all forages, both KL and ADL were negatively correlated with IVNDFd at any time point of digestion (Table 2). This suggests a two-fold effect of lignin types on both the extent and rate of NDF digestion. Since KL and ADL are positively correlated in most plants, the observation that KL impacts digestion is not extraordinary, except that the KL is dispersed and less recoverable as digestion progresses. This suggests that soluble phenolics or unpolymerized phenolics are potentially attached to some carbohydrate moieties resulting in less digestion or create some steric hindrance or resistance creating a barrier for bacterial enzyme activity.

Table 2 Correlations between lignin types (NDF basis) and extent (IVNDFd) and rate of digestion (k_d). (X lucerne, Y maize silages, Z temperate grasses, ZZ early cut grasses)

	ADL *vs.* KL	ADL *vs.* IVNDFd 24	ADL *vs.* IVNDFd 96	KL *vs.* IVNDFd 24	KL *vs.* IVNDFd 96	ADL *vs.* k_d	KL *vs.* k_d	ΔL *vs.* k_d
Lucerne	0.57	-0.64	-0.83	-0.44	-0.48	0.04	-0.09	-0.32
Maize	0.51	-0.79	-0.87	-0.67	-0.64	-0.58	-0.52	0.09
Conv.	0.53	-0.82	-0.89	-0.69	-0.55	-0.52	-0.43	-0.52
BMR	0.10	-0.69	-0.84	-0.48	-0.42	-0.23	-0.20	0.57
Z	0.70	-0.85	-0.94	-0.69	-0.63	-0.75	-0.48	-0.40
ZZ	-0.37	-0.54	-0.62	-0.31	-0.29	0.25	-0.48	0.49
All	0.44	-0.59	-0.85	-0.48	-0.40	0.20	0.48	0.44

Conv. – standard maize hybrids; BMR - brown midrib hybrids maize silage.

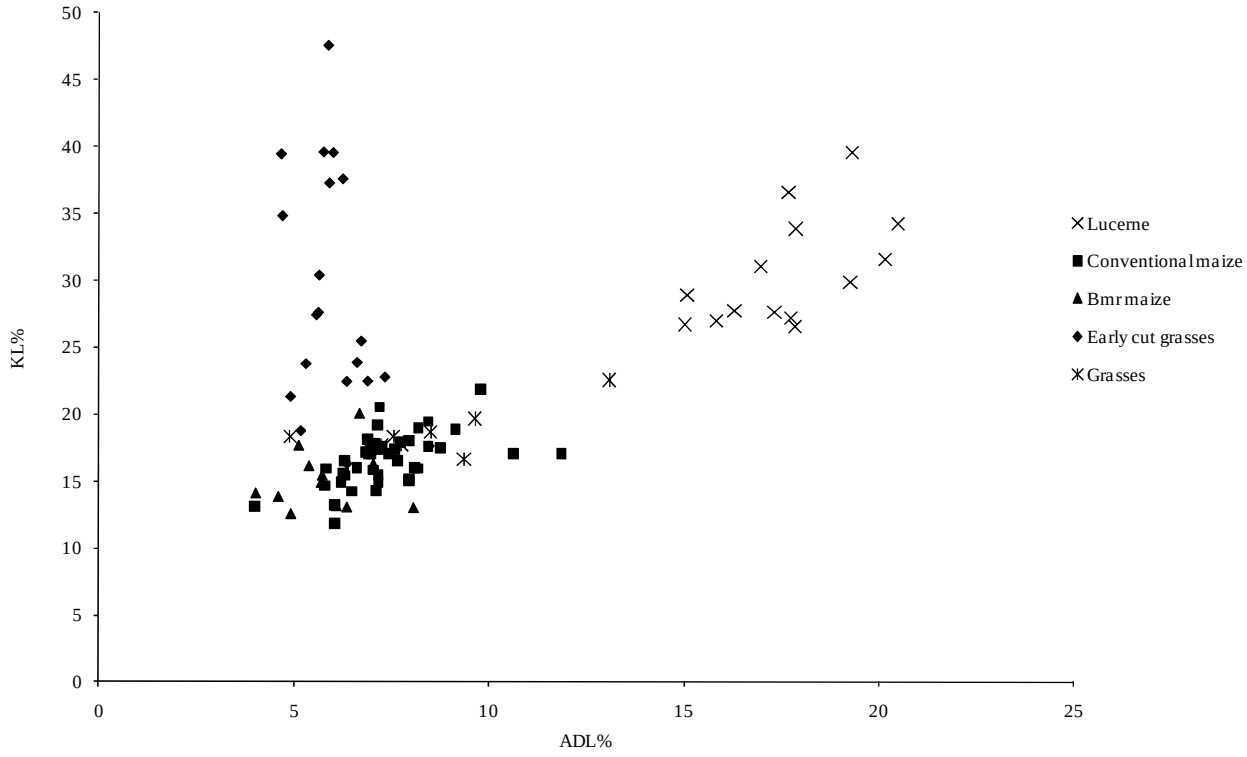

Figure 1 Klason (KL) and ADL values as % of NDF per forage group.

Among all forages, KL was negatively correlated with IVNDFd and k_d, and this was particularly true for conventional maize silages and mature grasses and less for BMR maize, early cut grasses and lucerne. The difference between the KL and ADL (ΔL) and extent and rate of digestion did not behave similarly among forages, especially in BMR maize and early cut grasses. Figure 1 shows the different behaviour of BMR and early cut grasses. This further supports the presence of dispersible lignin or acid-labile phenolic acids that impacted microbial degradation consistent with the concept of steric hindrance resulting in diverse correlations with the respective k_d. The data further suggest that BMR maize, although harvested at maturity, behaves like an immature plant with respect to lignin polymerization and acid-labile phenolic acids.

Overall, these data clearly indicate that both lignin types impact the rate of digestion and imply that as NDF digestion occurs, acid-labile phenolic acids have the ability to modulate the rate of digestion of temperate forages and it appears that these phenolics would be recovered in the ND soluble, reducing the energy available from microbial digestion.

Conclusions

The data presented demonstrates several key factors related to forage digestion by ruminal microbes and the need for improved methods of forage and fibre analyses. Methods for measuring NDF digestibility and lignin need to include steps to improve recovery such as the use of specific filter papers. This enhances our ability to describe the behaviour of fibre degradation in a more quantitative and descriptive manner which will allow both plant breeders and nutritionists to make better decisions about genetic selection and forage selection and feeding behaviour for specific agronomic conditions. The factors within a plant that impact digestibility are different among plant varieties and need to be considered when predicting forage digestion and energy yield.

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