SOLUBLE LIGNIN AND ITS RELATION TO KLASON LIGNIN, ACID-DETERGENT LIGNIN AND DIGESTIBILITY OF NDF

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INTRODUCTION

Measurements of lignin are among the most used to evaluate forages. The most popular is acid detergent lignin with sulfuric acid (ADL)(Van Soest, 1963) and is currently used in the Cornell Net Carbohydrate and Protein System (CNCPS) (Tedeschi and Fox, 2016), followed by Klason lignin (KL)(Theander et al., 1995). These methods produce disparate values (Hatfield et al., 1994). The ADL values are lower than KL, and are criticized for low recovery of lignin (McSweeney et al., 1994, Lowry et al., 2002). Soluble lignin (ΔL) is the difference between KL and ADL, called dispersible lignin in the Australian papers. The difference between values obtained by these methods is substantial, and has been ignored as a biological variable. NDFD48 is a measure of in vitro digestibility of NDF at a time point of 48 hours, expressed as percent of NDF. This paper investigates the ΔL and its relation to KL, ADL, and NDFD48.

Soluble lignin was found in the rumen fluid of a cow fed Australian Spear grass (Gaillard and Richards, 1975), and has also been observed from the action of fungal cellulases upon cell walls of grasses, (Grabber et al., 2002, Mc Sweeney et al., 1994). The soluble lignin found in the rumen by acid precipitation at pH 3 from clarified rumen fluid is indigestible (Neilson and Richards, 1978), but is not known to have negative effects on fiber digestion (untested). The rumen residue also contains small amounts of protein and hemicellulosic carbohydrates (Gaillard and Richards, 1975).

DATA SETS AND ANALYSIS

The results presented here are based on two data sets: one by J.B. Robertson in the Cornell Laboratory consisting of 7 grass forages, 13 maize plants, 4 corn silages and 15 alfalfa hays. The second set is from Jung et al. 1997, table 1 in their paper, and digestibilities of NDF (NDFD48) from their figures, decoded by Van Soest (2015). The data set of Jung et al. consists of 16 C_3 grasses, 8 C_4 grasses, and 12 legumes, including 6 alfalfas and 6 other legume species and was conducted with the purpose of discrediting ADL. The NDFD48 of one C_3 grass could not be read from their figures. Forages were analyzed for in vitro digestibility (NDFD48), acid-detergent lignin (ADL) (Van Soest 2015) and Klason Lignin (KL) by the method of Theander et al. (1995). Soluble lignin (ΔL) was calculated as the arithmetic difference between KL and ADL, as percent of dry matter, and also as a percent of Klason Lignin (ΔL/KL).

RESULTS AND DISCUSSION

Correlations of ∆L with NDFD48, KL and ADL are shown in table 1. Correlations of NDFD48 with ∆L on a dry matter bases are insignificant, and vary from the negative to the positive. This result is surprising in view of the high negative correlations of ADL and KL with digestibility (Jung et al. 1997). Correlations with KL are variable and positive, as expected, because ∆L is a part of KL. Correlations with ADL are low and mostly insignificant.

Table 1: Correlations of ∆L on a dry matter basis with NDFD48, KL and ADL.

Component	**N**	**NDFD48**	**KL**		**ADL**	
Cornell Data						
Grasses	7	-0.27	0.57		0.15	
Maize Plants[a]	17	-0.34	0.85	**	0.27	
Alfalfas	15	-0.22	0.93	**	0.62	*
Jung et al. (1997)						
C_3 Grasses	16	0.12	0.71	**	0.00	
C_4 Grasses	8	0.39	0.89	**	0.17	
Legumes	12	0.29	0.41		-0.45	

[a] includes silages;
* $P < 0.05$; **$P < 0.01$

The data are shown in a different way in table 2, as mean values of concentrations of dry matter for ADL and KL. The ranges of ∆L are presented as percentages of KL. These amounts are large and variable and average about 50 percent of the KL. Values of ∆L/KL vary to over 80 percent of KL (table 2), probably in the most immature grasses of lowest ADL. The Ranges of ∆L observed by Gaillard and Richards (1974) and McSweeney et al., (1994) are similar. Values of ∆L on a dry matter basis are obtained by subtracting column 3 from column 2 in table 2. The associated decline in ∆L/KL with increasing ADL is seen in all groups (table 3). Correlations of ∆L/KL in table 3 are negative with ADL and positive with NDFD48. The decline in ∆L/KL with increasing ADL suggests the conversion of ∆L to ADL as forages mature, a matter of need for further study. Unfortunately, the dates of cutting and description of forage stages are lacking.

The peculiar nature of the ADL-KL-∆L relationships is shown in in figure 1. Soluble lignin and KL are plotted against ADL on a dry matter basis. The ∆L relationship with ADL is flat and statistically insignificant and relatively constant as a proportion of dry matter. The KL rises with a unity slope relative to ADL (1.14-0.14). The intercepts of the two regressions are the same at 6.3 percent of dry matter. The rise in values of KL against almost constant ∆L leads to a decline in proportion of ∆L as a part of KL, (∆L/KL). This is a large statistical interaction due to increasing ADL, and contributes to negative associations between ∆L/KL and ADL. The declining relations of ∆L/KL with ADL are shown in in figures 2 and 3. The variability is greater in mature forages (Figure 3) than in growing forages (Figure 2). However, considerable association occurs in mature forages, like maize and other C_4 plants grown for their seed. The decline in ∆L/KL with increasing ADL, gives a strong statistical interaction with positive correlations with NDFD48, which occur in all groups (Table 3 and Figure 4). The positive relations between ∆L/KL and NDFD48 indicate that ∆L has no negative effect on NDFD48, and refutes claim that KL represents the main negative factor affecting NDFD48 and forage quality.

Table 2: Mean values of Klason, acid detergent lignin, ∆L/KL and their ranges.

Component	N	KL	ADL	∆L/KL (%)	Range ∆L/KL
Cornell Data					
Grasses	7	13.8	6.4	55	42 – 73
Maize Plants	13	6.4	2.8	56	47 – 70
Maize Silage	4	7.7	3.2	59	53 – 65
Alfalfa	15	11.3	6.6	41	25 – 52
Jung et al. (1997)					
C_3 Grasses	16	9.9	3.3	67	49 – 83
C_4 Grasses	8	8.5	3.4	59	46 – 70
Alfalfas	6	12.0	8.4	30	22 – 36
Other Legumes	6	12.2	7.1	43	28 - 54

KL – Klason lignin
ADL – Acid detergent lignin
∆L/KL – Acid detergent soluble lignin divided by Klason lignin (%)

Table 3: Correlation between acid detergent soluble lignin expressed as percentage of Klason Lignin (∆L/KL) with acid detergent lignin (ADL) on a dry matter basis and with in-vitro digestibility of neutral detergent fiber (NDFD48).

Forage Class	N	∆L/KL * ADL		∆L/KL * NDFD48		ADL * NDFD48	
Cornell							
Grasses	7	-0.85	*	0.76	*	-0.95	**
Maize Plants	17	-0.64	**	0.44		-0.78	**
Alfalfas	15	0.07		0.12		-0.55	*
Jung							
C_3 Grasses	16	-0.90	**	0.83	**	-0.79	**
C_4 Grasses	8	-0.41		0.73	*	-0.59	
Legumes	12	-0.81	**	0.58	*	-0.69	*
All Legumes Combined	27	-0.48	*	0.34		-0.53	**

* $P < 0.05$; ** $P < 0.01$

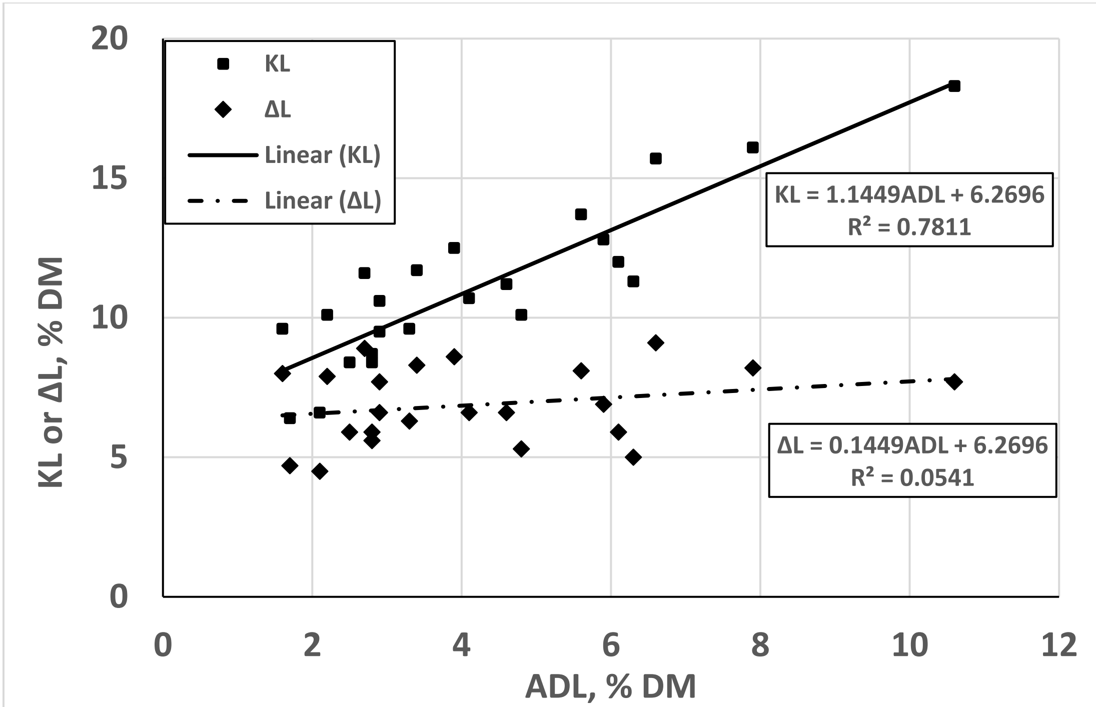

Figure 1: Relationships of KL and ΔL with ADL for C_3 forages from Van Soest and Robertson and Jung et al. (1997).

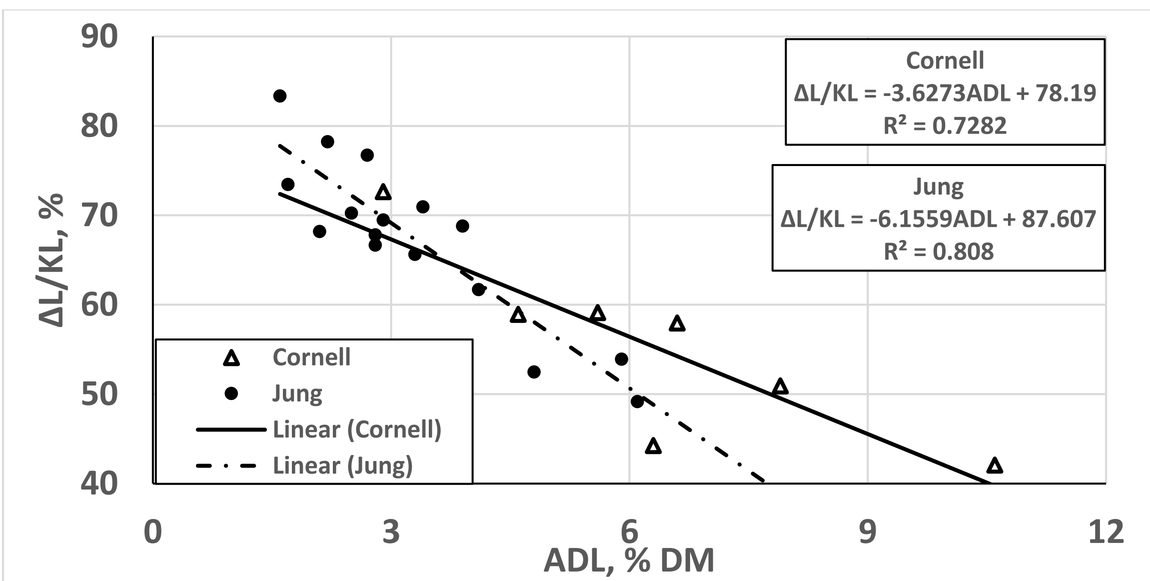

Figure 2: Relation of acid-detergent soluble lignin as a percent of Klason lignin with acid-detergent lignin as a percent of dry matter. Combined data for forage grasses from Robertson (Cornell) and Jung et al 1997.

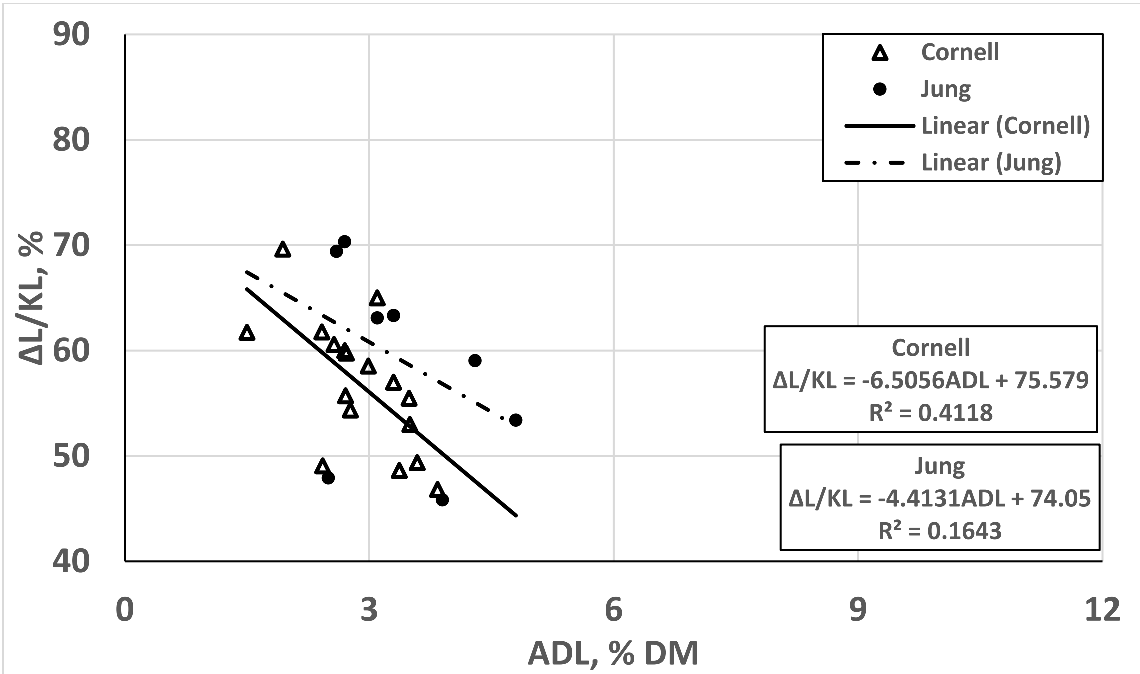

Figure 3: Relation of acid-detergent soluble lignin as a percent of Klason lignin with acid-detergent lignin as a percent of dry matter for C_4 mature plants. Combined data from Robertson (Cornell) and Jung et al 1997 (n=25).

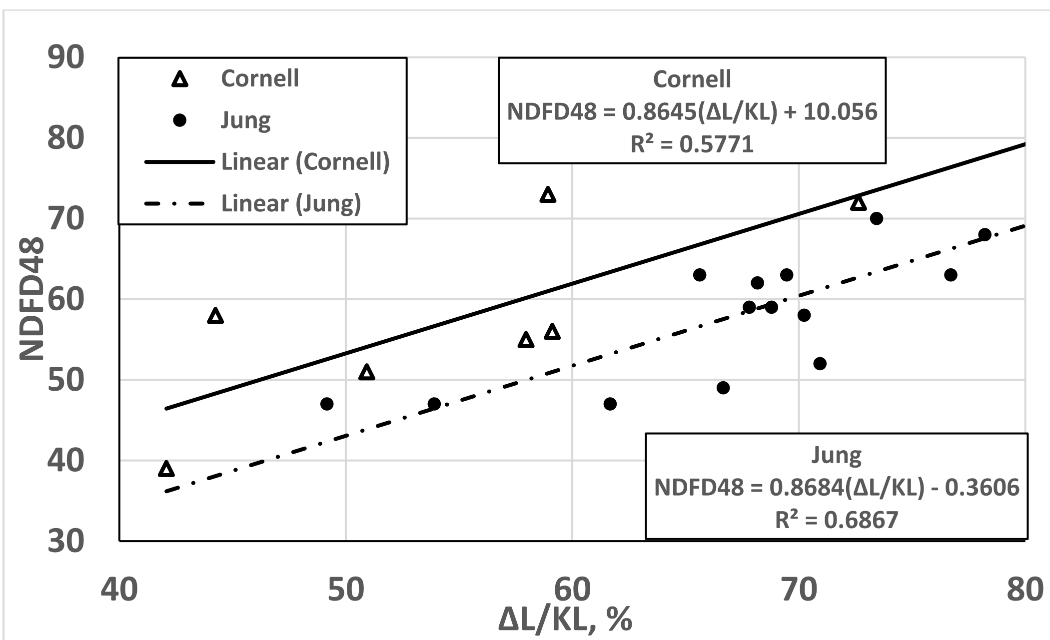

Figure 4: Relation between the in vitro digestibility of NDF (NDFD48) with acid-detergent soluble lignin as a percent of Klason lignin (ΔL/KL). Combined data for forage grasses (n=23).

The variances of ∆L and ADL are the same on a KL basis, because the sum of the two are a constant 100 percent. Thus a correlation of positive sign for ∆L (Figure 4) has the opposite negative value with ADL. Correlations of ∆L and ADL on a KL basis with NDFD48 are in Table 3. The relation of NDFD48 with KL is more variable, because the KL is a composite of the two differing fractions. The high variance of KL was noted by Jung et al., 1997. The KL does not appear to be a uniform substance in a nutritional sense (Van Soest 1994, chapter 22).

The data of Jung et al (1997) included here and reanalyzed as an independent set supports our conclusions. The paper of Jung et al was conducted to discredit ADL and was unsuccessful because their statistical analyses failed to show any advantage of KL, which also has the disadvantage of being a more laborious and time consuming procedure. Jung et al and related literature was used to justify KL, and overlooked the biological role of lignin as a major plant protection factor. Lignin is energetically very expensive (Jung et al, 1999), and is irretrievable in times of plant stress, such as dry conditions and cold (Van Soest and Hall 1998). Corn plants reduce lignification in favor of seed production in unfavorable conditions, as a matter for survival.

Historically at the time of the original publications of the ADL (Van Soest 1963; Van Soest, 1973), it was thought that the precipitate formed on prolonged boiling in acid represented non-lignin matter (Moon and Abou-Raya 1952). This precipitate is however the main component of ∆L. Since that time developing lignin chemistry changed view to regard KL as total lignin. This paper shows the divergent characteristics of ∆L and ADL (Figures 2, 3, 4 and Table 3). The ADL is unanalyzable and cannot be resolved into identifiable phenolics (McSweeney et al 1994), although such phenolics are found in ∆L (Raffrenato 2011).

CONCLUSIONS

Current views of lignin have criticized ADL for a lack of recovery of ∆L, overlooked as an independent anti-quality factor. Soluble lignin differs from KL in its statistical associations, and is variably related to KL, of which it is a part. Klason lignin appears as a heterogeneous entity. However, when ∆L is expressed as a proportion of KL, it has a strong negative association with ADL. This interaction yields positive correlations with NDFD48. The negative association of ∆L/KL with ADL occurs in all forage classes. However, ADL is consistently related to indigestibility of NDF and its use in the CNCPS is validated.

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