J. Sci. Food Agric., 65(1), 51-58 (1994)

A Comparison of the Insoluble Residues Produced by the Klason Lignin and Acid Detergent Lignin Procedures

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Abstract: Two methods—Klason lignin (KL) and acid detergent lignin (ADL)—for determining lignin concentration in plants were compared using stem material from lucerne (*Medicago sativa* L), cocksfoot (*Dactylis glomerata* L) and switchgrass (*Panicum virgatum* L), at three stages of maturity, and leaf samples from lucerne and cocksfoot. For all forages, KL values were higher than ADL values. Lucerne samples, which had crude protein levels twice that of the grass species, had KL values that were only 30–40% higher than ADL values; in grasses, KL values were 200–300% greater than ADL values. The addition of nitrogenous materials (bovine serum albumin, lysine, and ammonium sulfate) to commercial xylan and cellulose did not result in additional KL residue. Pyrolysis–GC–MS revealed that both residues appeared to be similar to the orginal plant lignin and did not appear to be contaminated with carbohydrate or protein. The higher values for grass KL residues were not due to protein contamination or incomplete hydrolysis of carbohydrates, but were more likely due to the solubilization of lignin components by the ADL treatment. KL values may give a more accurate quantification of the total lignin within forage plants.

Key words: Klason lignin, acid detergent lignin, forages, crude protein, pyrolysis, lucerne, cocksfoot, switchgrass.

INTRODUCTION

Forages play an important role in providing nutrients to ruminants. The efficiency with which the potential energy of forages is utilized depends upon the type and maturity of the forage. Lignification of plant cell walls has long been correlated with decreased digestibility of the structural polysaccharides (Kamstra *et al* 1958; Terry and Tilley 1964; Van Soest *et al* 1966). Although the mechanism underlying this observation has not been elucidated, there is speculation that the mode of action is due to a physical protection of wall polysaccharides (Jung and Deetz 1993). Defining the role of lignin in limiting wall degradation is complicated by the insolubility of this polymer, leading to difficulties in isolation and quantification.

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A variety of analytical methods have been developed over the years to estimate lignin, all of which have some limitations (Van Soest 1967; Lai and Sarkanen 1971; Kirk and Obst 1988). The available methods for the quantitative determination of lignin fall into two basic groups—analytical procedures that remove all cell-wall constituents except lignin, and procedures that oxidize the lignin polymer out of the cell-wall matrix. The most commonly used methods that result in an insoluble lignin residue utilize H_2SO_4 for hydrolysis of the cell-wall polysaccharides after the removal of potential interfering substances. The Klason lignin (KL) procedure is one such H_2SO_4-utilizing hydrolytic method that was developed for use with woody species and is still used for this type of sample today (Kirk and Obst 1988). It is generally thought that the KL procedure is not appropriate for forages, especially legumes, due to the higher levels of proten found in these plants (Van Soest 1967; Lai and Sarkanen 1971). Early work indicated that the addition of protein to wood meal resulted in an increase in KL concentration and that the KL residue had elevated levels of N (Norman and Jenkins 1934). However, it was found that this increase quickly reached a saturation point after which additional protein did not result in further increases in the KL residue. It is not clear whether the N present in the KL residues represents intact protein, protein fragments, modified protein, or nucleic acids. It is also possible that a portion of the N in KL residues arises from cell-wall proteins that serve a structural role within the wall matrix and have been suggested to be cross-linked to lignin (Whitmore 1978, 1982).

Van Soest (1967) developed the acid detergent lignin (ADL) procedure as an alternative to the KL method. The ADL procedure also utilizes H_2SO_4 to hydrolyze cell-wall polysaccharides to leave an insoluble lignin residue. It should be noted that a major difference between the KL and ADL procedures is in the sequence in which acid concentration and temperature are utilized to effect the hydrolysis of polysaccharides. In the KL method, high concentrations of H_2SO_4 are used at relatively low temperatures, followed by dilution of the acid and the application of heat. For ADL analysis, dilute H_2SO_4 is used initially at high temperature, followed by more concentrated acid at low temperature. Since its development, the ADL procedure has become very widely used, and commonly accepted, for the analysis of lignin concentration in forages.

More recently, a question has been raised as to whether ADL methods are underestimating the true lignin content of forage species. Kondo *et al* (1987) found large discrepancies between KL and ADL residues, particularly for grass forages. Their results indicated that ADL treatment of samples solubilized some of the lignin. This solubilization appeared to be extensive in grass samples. They concluded that major differences between concentrations of KL-generated residues and ADL residues were due to the loss of lignin as a result of ADL treatment rather than a large incorporation of proteins into the KL residue.

An advantage of the KL procedure is that it can be easily adapted to cell-wall hydrolysis schemes to obtain values for total carbohydrates, total uronic acids, and neutral sugar composition of forage samples, as well as estimating lignin content. The question remains, however, as to how accurately it estimates forage lignin content. The objective was to investigate the chemical differences in the insoluble residues created by these two lignin methods, which use H_2SO_4 to remove the cell-wall polysaccharides. Many modifications to the original KL and ADL procedures have been developed. In this work, a single, commonly employed version of each lignin method was chosen (Theander and Westerlund (1986) for KL, and Van Soest and Robertson (1980) for ADL) which has been used previously in the present authors' research.

EXPERIMENTAL

Forage samples

Lucerne (*Medicatgo sativa* L 'Arrow'), cocksfoot (*Dactylis glomerata* L 'Bromex'), and switchgrass (*Panicum virgatum* L 'Trailblazer') were grown at Ames, IA, USA, with plots established in the spring of 1986 as four field replicates in a randomized complete block design. Soil type was a Canisto silty clay loam (fine-loamy, mixed, (calcareous) mesic Typic Haplaquolls), with an organic matter content of approximately 3·5% and a pH of 7·5. Commercial P, K and N were applied at 4·4, 23·2, and 9·0 g m^{-2}, respectively, in the spring before growth started. All forages were harvested in the spring and summer of 1988 at three stages of maturity. The growth stages for the grasses are given in brackets below and are based on the staging system of Moore *et al* (1991).

Maturity stage 1. Lucerne and cocksfoot samples were harvested on 17 May. Lucerne was 50 cm tall and at mid-bud stage, while cocksfoot was 40 cm tall with early heads half emerged (R1). Switchgrass was harvested on 28 June when 65 cm tall and with two palpable nodes (E2).

Maturity stage 2. The second harvests of lucerne and cocksfoot were taken on 7 June. Lucerne plants were 80 cm tall and at about 75% bloom, while cocksfoot was 65 cm tall and in full anthesis (R4). The switchgrass second harvest was completed on 19 July when it was 65 cm tall at leaf tips and plants were in the boot stage (R0).

Maturity stage 3. The third harvest was on 28 June when lucerne was 115 cm tall and in the green pod stage with seed in the soft dough stage, and cocksfoot

was 60 cm tall with heads and peduncles turning brown with some seeds maturing and beginning to be shed (S4). Switchgrass plants were harvested for the third time on 9 August when plants were 83 cm tall and the heads were in early anthesis (R4).

All leaves, including the sheaths, were removed and stems frozen immediately following harvest and stored at −20°C until lyophilized. Individual forage samples were ground in a cyclone mill to pass a 0·5-mm screen.

In addition, lucerne and cocksfoot leaf samples from previous experiments (Buxton *et al* 1985; Buxton and Marten 1989) were utilized. Three field replicate samples of 55°C oven-dried material were available for each of these leaf materials. The leaf samples were ground in a cyclone mill to pass a 1·0-mm screen.

Chemical analyses

All forage samples were analyzed for neutral detergent-fibre (NDF), acid detergent fibre (ADF), ADL, KL, and total N concentration. The NDF and ADL contents were determined by the sequential detergent method of Van Soest and Robertson (1980). Briefly, ADL was measured by extraction of a 0·5-g sample with 100 ml of boiling neutral detergent for 60 min. The residue was collected by filtration through a coarse-porosity Gooch crucible, washed with hot water, rinsed with acetone, and dried at 100°C overnight. After weighing to determine the NDF content, the crucible and residue were extracted with 200 ml of boiling acid detergent solution for 60 min. The crucible was then removed from the beaker, the outside rinsed with hot water into the beaker, and the ADF collected by filtration through the crucible. The ADF, after washing with hot water and rinsing with acetone, was dried overnight at 100°C. ADL was isolated by filling the crucible containing the ADF with 12 M H_2SO_4 and allowing it to drain from the crucible. Additional acid was added periodically over the 3-h room-temperature hydrolysis. The acid-insoluble residue was collected by filtration and extensively washed with hot water. A final acetone rinse was used prior to drying the sample overnight at 100°C. The ADL content was determined as the difference in weight of the residue before and after ashing at 450°C.

KL was determined as the residue remaining after total hydrolysis of cell-wall polysaccharides by the method of Theander and Westerlund (1986). In brief, 0·1-g samples were treated with α-amylase (Sigma, A3403)† and amyloglucosidase (Boehringer Mannheim, #102857) to remove starch, and solubilized wall polymers were recovered by precipitation in 80% (v/v) ethanol. Starch-free, alcohol-insoluble residues were solubilized in 1·5 ml of 12 M H_2SO_4 at 30°C for 60 min. The samples were then diluted with 43·5 ml of water to 0·4 M H_2SO_4 prior to secondary hydrolysis in an autoclave (121°C) for 60 min. The nonhydrolyzed residue was collected by filtration through a coarse-porosity Gooch crucible, extensively washed with hot water and dried overnight at 100°C. KL was determined as the difference in weight of the residue before and after ashing at 450°C for 6 h. All analyses were done in duplicate and component concentrations were corrected to a 100°C dry matter basis.

† Mention of a trade name or manufacturer does not constitute a guarantee or warranty of the product by the US Department of Agriculture or an endorsement by the Department over products not mentioned.

Analysis of isolated lignins

One field replicate of stems from each forage species, at each stage of maturity, was used to isolate sufficient KL and ADL for the determination of molecular composition and the N content of isolated lignins residues. Additionally, KL and ADL residues were prepared from all leaf samples for N analysis. The lignins were isolated by the same methods described above, except that they were not ashed. Pyrolysis–GC–MS was used to determine the compositional characteristics of the lignin preparations and the original forage material using the methods of Ralph and Hatfield (1991). The N content of forage samples was determined by the total combustion method of N analysis (Carlo Erba Inst. NA 1500 Nitrogen Analyzer). Crude protein was estimated as N × 6·25.

Sensitivity of lignin methods

The effect of hydrolysis time in the autoclave of the second stage in the KL procedure was investigated. One field replicate of the most immature stage of lucerne and cocksfoot stems and Sigmacel-50 cellulose (Sigma) and oat spelts xylan (Sigma) were used to prepare KL by the standard method, except hydrolysis times in the autoclave were 0·5, 1, 2, or 3 h, This experiment was done in quadruplicate.

The effect of nitrogenous compounds on yield of KL was determined. Sigmacel-50 cellulose and oat spelts xylan were used to prepare KL under five alternate conditions. The standard procedure was that of Theander and Westerlund (1986); the negative control was the same as the standard method, except that α-amylase and amyloglucosidase were not added during the sample preparation steps. The effects of protein and amino acid N on KL recoveries were evaluated by adding 10 mg of bovine serum albumin or lysine to 100 mg of each isolated polysaccharide. Because the amyloglucosidase employed in the KL method contains 3·2 M $(NH_4)_2SO_4$ as a stabilizer, this inorganic form of N was also tested for possible effects on the residue yield by adding 84 mg to 100 mg of polysaccharide.

These substrates were then analyzed for KL yield by the standard procedure, except that the initial preparation of a starch-free, alcohol-insoluble residues was omitted. This experiment was done in quadruplicate.

Statistical analysis

Statistical evaluation was done by analysis of variance for the yield of KL and ADL residues, and the sensitivity analyses for the KL method. Individual treatments were compared by the least-significant difference method when the analysis of variance procedure indicated significance ($P < 0.05$). Linear correlation analysis was done for the relationship of forage N concentration with lignin yield.

RESULTS AND DISCUSSION

Forage leaf samples contained higher concentrations of crude protein and less NDF than stem samples (Table 1). Ranges in forage maturity were reflected in the general increased NDF and decreased crude protein concentration of stems as maturity advanced. Switchgrass was an exception that did not show an increase in NDF, although there was a decrease in the crude protein content of these stems with maturation. The two methods of determining lignin content resulted in different amounts of residue, with KL values consistently higher ($P < 0.05$) than ADL values for all forage samples (Table 2). For the grass species, the KL residue concentrations were on the order of 2 to 3·5 times greater than the ADL residues. Both methods reflected maturity trends, although the magnitude of the change was different and varied with species. Switchgrass showed only a 14% change based on KL compared to a 39% change based on ADL, while cocksfoot showed a 200% increase by ADL and 150% by KL, and lucerne stems increased 43% by ADL and 37% by KL with maturation.

TABLE 1
Neutral detergent fibre (NDF) and crude protein (CP) concentrations (g kg^{-1} DM) of forage samples

Forage	*Component*	*Leaves*	*Stems—maturity stage*		
			Early	*Mid*	*Late*
Lucerne	NDF	180	525	635	660
	CP	292	107	86	75
Cocksfoot	NDF	485	541	657	610
	CP	202	58	33	23
Switchgrass	NDF	—	751	743	760
	CP	—	27	18	14

TABLE 2
Klason lignin (KL) and acid detergent lignin (ADL) concentration (g kg^{-1} DM) of forage samples

Forage	*Lignin method*[a]	*Leaves*	*Stems—maturity stage*		
			Early	*Mid*	*Late*
Lucerne	KL	32	110	148	152
	ADL	29	82	107	117
Cocksfoot	KL	41	58	111	132
	ADL	27	15	41	45
Switchgrass	KL	—	127	144	145
	ADL	—	54	70	75
SEM		3	8	8	8

[a] For all forage samples, KL concentrations were greater than ADL concentrations ($P < 0.05$).

It is generally considered that the major limitation of the KL method for forage samples is the inclusion of protein in the insoluble residue, resulting in artificially high lignin values (Van Soest 1967; Lai and Sarkanen 1971). Cetyl trimethylammonium bromide (CTAB) is included in the acid detergent solution specifically for protein removal in the ADL method (Van Soest 1967), whereas the KL method does not contain a protein-removal step. With the exception of lucerne leaves, the N content of the KL residues was always greater than the N content of ADL residues in the present study (Table 3). However, KL yield and the difference between the KL and ADL concentrations of individual samples were negatively correlated with the N content of the original forage samples ($r = -0.77$, $P < 0.05$ and $r = -0.90$, $P < 0.05$, respectively) rather than positively correlated, as would be expected if protein was a major contaminant of KL. ADL yield was not correlated with forage N content ($P > 0.05$). Also, the proportion of N from the original forage sample retained in both lignin residues was lower in the high N samples, leaves and lucerne stems than in the low N grass stem samples. The proportion of sample N retained in the lignin residues increased as the N content of the original forage materials declined (Table 3). This suggests that the N content in the lignin residues reaches a maximal level that is roughly proportional to the total cell-wall mass. Earlier work by Norman and Jenkins (1934) using oat (*Avena sativa* L) straw had shown similar results from the addition of exogenous protein to samples before solubilizing in 72% H_2SO_4. They found that N in the acid-insoluble residue reached a maximum level after relatively small additions of protein. The similarity of the N content in all the KL residues may be a reflection of a general mechanism for binding N (protein or modified protein) to lignin that saturates regardless of the plant source. The present authors' observations do not indicate that forage protein content is related to lignin yield by either method examined.

TABLE 3

Nitrogen content (g kg^{-1} lignin) of Klason lignin (KL) and acid detergent lignin (ADL) preparations from forage samples[a]

Forage	*Lignin method*[a]	*Leaves*	*Stems—maturity stage*		
			Early	*Mid*	*Late*
Lucerne	KL	17·6 (1%)	18·4 (12%)	17·2 (18%)	17·4 (22%)
	ADL	22·3 (1%)	11·8 (6%)	13·1 (10%)	12·7 (12%)
Cocksfoot	KL	25·0 (3%)	18·3 (10%)	14·0 (29%)	14·2 (52%)
	ADL	18·5 (2%)	9.3 (2%)	6.7 (5%)	6.6 (8%)
Switchgrass	KL	—	11·8 (35%)	10·4 (52%)	9·2 (58%)
	ADL	—	5·3 (6%)	4·9 (12%)	5·0 (16%)

[a] Values in parentheses are percent of original forage N retained in the residue.

It should be noted that both lignin residues contain significant N (Table 3). This recalcitrant N may reflect cell-wall proteins, particularly those that have structural roles and may be cross-linked into the matrix and are not extractable. Proteins have been proposed to be linked to lignin (Whitmore 1982), although the chemical proof has not been established. Grasses are known to contain less wall protein, with a portion functioning as structural proteins (Darvill *et al* 1980). This is reflected in the generally lower N values in the ADL and KL residues of the grasses compared to lucerne (Table 3).

To test the theory that condensation of proteins with wall carbohydrates during strong acid treatment results in elevated lignin values, three sources of N were combined with two different commercially available polysaccharide sources (Table 4). The addition of protein (bovine serum albumin), an amino acid (lysine), or inorganic N ($[NH_4]_2SO_4$) did not result in an increase in the insoluble residue collected after the KL procedure for either polysaccharide. This clearly indicates that factors within the wall matrix are responsible for the incorporation of N into KL residues, not the simple presence of protein in forage samples.

TABLE 4

Effect of the addition of various nitrogen sources on Klason lignin residues of isolated cellulose and xylan

Nitrogen source	*Sigmacel-50 cellulose* (*g kg^{-1} DM*)	*Oat spelts xylan* (*g kg^{-1} DM*)
Control	29	52
None	21	41
Bovine serum albumin	22	47
Lysine	26	47
$(NH_4)_2SO_4$	23	41
SEM	3	2

It was considered possible that KL residues might contain residual nonhydrolyzed polysaccharide that could account for the higher recoveries of KL residues compared to ADL. A small amount of insoluble residue was collected from each isolated polysaccharide subjected to the KL procedure (Table 4). The control and no-enzyme treatments resulted in larger KL residues for xylan than cellulose. This is not surprising in that oat spelts xylan contains small amounts of lignin as determined by pyrolysis–GC–MS (Ralph and Hatfield 1991). The KL residue from the cellulose (Sigmacel-50) was white in colour, rather than the brown of true lignin residues. This residue was probably nonhydrolyzed cellulose and hydrolysis of a less crystalline cellulose preparation (Sigmacel-100) resulted in a smaller KL residue (4 g kg^{-1}). Cellulose in forages is considered to be relatively noncrystalline (Elofson *et al* 1984; Cyr *et al* 1990). Therefore, nonhydrolyzed cellulose should be of minor importance as a contaminant of KL residues from forages. Data summarized in Fig 1 illustrate the effect of duration of secondary hydrolysis time on the KL residue. For all forage samples and polysaccharide samples, the optimum hydrolysis time was 1 h. Shorter hydrolysis times resulted in an increase ($P < 0·05$) in the residue, indicative of incomplete hydrolysis of polysaccharides. Longer hydrolysis times resulted in an increase ($P < 0·05$) in the residue, indicative of dehydration reactions to furan derivatives followed by condensation to polymeric materials resulting in the accumulation of insoluble material (Kennedy and White 1983).

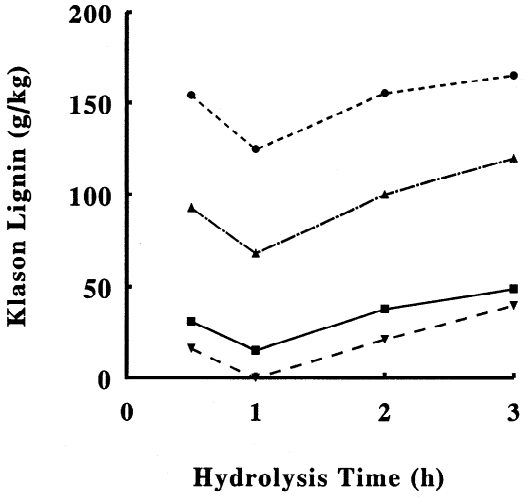

Fig 1. Effect of second-stage hydrolysis time in the autoclave on Klason lignin concentration of ●, lucerne; ▲, cocksfoot; ■, oat spelts xylan; and ▼, Sigmacel-50 cellulose. For all substrates, the Klason lignin yield from the 1-h hydrolysis time point was lower ($P < 0.05$) than all other time points.

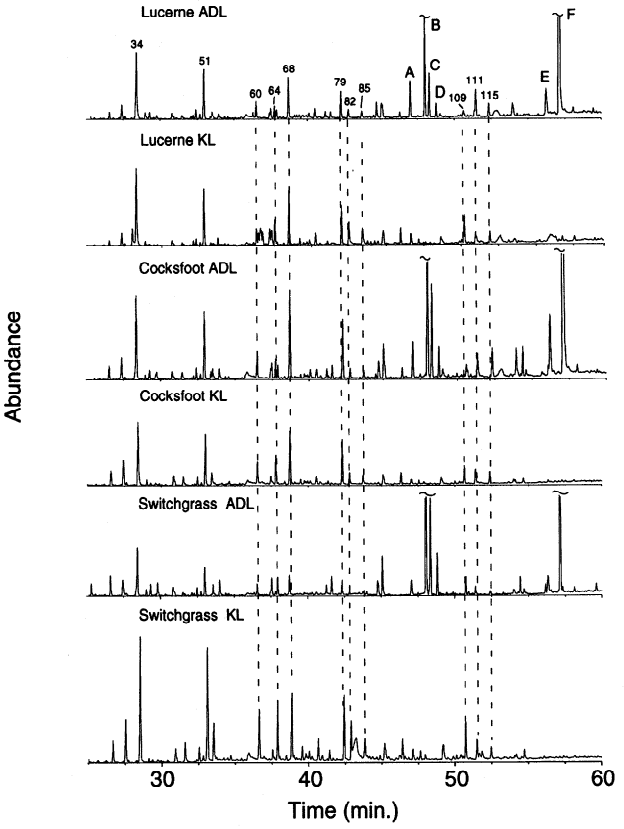

Fig 2. Pyrograms generated from the pyrolysis of Klason lignin (KL) and acid detergent lignin (ADL) residues from lucerne, cocksfoot, and switchgrass stems. Samples shown are from the most mature stage of development. Indicated compounds are guaiacol (34), 4-methylguaiacol (51), 4-ethylguaiacol (60), 4-vinylguaiacol (64), 2,6-dimethoxyphenol (68), 2,6-dimethoxy-4-methylphenol (79), (*E*)-isoeugenol (82), acetovanillone (85), (*E*)-2,6-dimethoxy-4-propenylphenol (109), acetosyringone (111), and syringylacetone (115). Numbering of compounds follows Ralph and Hatfield (1991).

Of particular interest was the investigation of possible chemical differences in the residues produced by the two methods. To compare the two procedures, pyrolysis–GC–MS analysis of lignin residues from the ADL and KL procedures were referenced to the original plant samples for the stem material of the three forages, at three maturity stages. The percentage of sample dry matter pyrolyzed was not different ($P > 0.05$) between the KL and ADL residues (57 and 60%, respectively), but both lignin residues were less ($P < 0.05$) completely pyrolyzed than the original forage samples (81%). Figure 2 represents typical data obtained from this analysis (only the data for the most mature stem material of each forage are shown). It is clear that there was a high similarity among the lignin types for each forage. The major pyrolysis peaks have been indicated on the figure. Absolute differences exist for the different compounds, but the relative abundance of each compound compared to guaiacol was similar when comparing ADL and KL within a forage type. It is interesting to note that several major peaks (A–F) showing up in the ADL residues are due to plant extractives (most likely pigments) and to residual detergent (CTAB, peak F). It is not possible to quantify these materials due to their unknown pyrolytic behavior. For example, CTAB probably has a high pyrolytic efficiency such that relatively minor amounts can result in what appears to be a major contaminant peak (Ralph and Hatfield 1991).

It appeared that, in most cases, both lignin residues contained similar lignin pyrolytic peaks as seen for the original plant material. There did not appear to be major differences in the pyrolytic products produced from either of the lignin residues. All maturity stages gave similar results. Both methods produced residues that were free of major carbohydrate contamination, as indicated by the absence of major carbohydrate derived peaks, such as (5H)-furan-2-one and 5-hydroxymethyl-2-furfuraldehyde, which were present in the pyrograms of the original forage materials. Popoff and Theander (1972, 1976) reported that sugars could be converted to phenolic compounds under acidic conditions and high temperature. These phenolic products could be present in the present pyrograms, but the reported yields from sugars are too low (0·15 and 1·45% for xylose and glucose, respectively) to account for a significant portion of the lignin residues. Also, no pyrolytic peaks diagnostic for protein were detected in either KL or ADL residues, which makes the identification of the N contamination of both lignin residues difficult. The compounds vinylphenol and vinylguaiacol, which are primarily derived from *p*-coumaric and ferulic acids, respectively, were greatly reduced in both lignin residues derived from grasses. This is to be expected, since the majority of these phenolic acid residues are ester-linked within the wall matrix and are relatively easily

hydrolyzed. This would indicate that cell-wall phenolic acids were not selectively retained in the KL residues.

Lignin composition was estimated as the syringyl-to-guaiacyl ratio of lignin components by summation of total ion abundance for five major syringyl-derived (2,6-dimethoxyphenol, 2,6-dimethoxy-4-methylphenol, 2,6-dimethoxy-4-vinylphenol, 2,6-dimethoxy-4-propenylphenol and syringaldehyde) and five major guaiacyl-derived (guaiacol, 4-ethylguaiacol, 4-methylguaiacol, isoeugenol, and vanillin) pyrolysis products. Yields of lignin pyrolysis products were normalized to the total ion abundance for guaiacol in each sample. As expected, the syringyl-to-guaiacyl ratio increased ($P < 0.05$) for the original forage samples of all species across the three maturity stages (0·62, 0·83 and 0·95, respectively), and this ratio increased similarly in both lignin residues. However, the mean syringyl-to-guaiacyl ratios of the KL (0·60) and ADL (0·53) residues were lower ($P < 0.05$) than the original forage materials (0·80). This suggests that syringyl-rich lignins are preferentially lost during H_2SO_4 hydrolysis. Musha and Goring (1974) previously reported that acid solubility of lignin from wood increased and KL yield decreased with increasing syringyl-to-guaiacyl ratios. However, the present pyrolysis data suggest that the differences in recovered amounts of lignin between the KL and ADL methods would not appear to be due to the selective loss of a particular type of lignin by the ADL treatment because their syringyl-to-guaiacyl ratios were not different ($P > 0.05$). Also, the syringyl-to-guaiacyl ratios of the grasses (0·98 and 0·58 for cocksfoot and switchgrass, respectively) were not consistently greater ($P > 0.05$) than for lucerne (0·84), even though the KL and ADL residue differential was always greatest for the grasses (Table 2). The nature of the lignin removed from forages by ADL treatment is under active investigation by authors' group.

CONCLUSIONS

The KL method results in lignin concentrations that are larger than those produced by the ADL procedure. A small part of this difference can be attributed to nitrogenous compounds. The difference between KL and ADL values were of too great a magnitude to be accounted for simply as residual protein condensed in the residues. Acid detergent does appear to remove some of the lignin as suggested by Kondo *et al* (1987), especially in grasses. The remaining lignins in both KL and ADL residues appear to be similar to the original lignin in the plant. The ADL residues appear to be contaminated with plant extractives, although it is not possible from these data to estimate their contribution to the final weight. It is concluded that KL is a better estmate of the total lignin concentration in forages than is ADL, especially for grass species. However, recent work suggests that both KL and ADL are of similar accuracy in predicting forage digestibility *in vitro* and *in vivo* (Jung *et al* 1993).

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