Use of Calorimetry to Verify Lignin Concentration Estimates

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Introduction

Lignin is a cell-wall polymer derived from free-radical reactions of hydroxycinnamyl alcohols and other cell-wall constituents. Lignin has been implicated as the primary component of cell walls that limits forage cell-wall digestibility by ruminants. A major difficulty in studying the role of lignin in cell-wall digestibility has been that a definitive molecular structure cannot be drawn for lignin, and all lignin concentration estimates are purely empirical, based on the particular method of analysis chosen. As a result, lignin concentration estimates vary widely among methods. Acid detergent lignin (ADL) employing 12 *M* H_2SO_4 is the most commonly used method in animal science and agronomy. We have previously shown that Klason lignin, which gives lignin concentration estimates two to five times higher than ADL, is not significantly contaminated with protein or carbohydrate and is similar to ADL in molecular composition. We believe the ADL method underestimates lignin concentration due to loss of acid-soluble lignin in the acid detergent step of the procedure. Calorimetry data are presented which verify that ADL does not account for all of the plant lignin and Klason lignin does not over-estimate lignin concentration.

Materials and Methods

A diverse group of 10 forage samples was used in this study. All forages had been dried and ground to pass a 1-mm screen in a cyclone mill. These forages were analyzed for total carbohydrate by a two-stage acid hydrolysis with neutral sugars quantified by GLC and uronic acids with colorimetry. Protein was determined as total N x 6.25. Lipids were analyzed by extraction with ether. Ash content of all samples was determined by combustion at 450 °C. Klason lignin was determined as the ash-free residue from the two-stage acid hydrolysis. Acid detergent lignin was determined by sequential detergent analysis. Bomb calorimetry was used to determine the gross energy (GE) of all forage samples. A calculated GE value was derived by applying published GE values for the forage chemical components to the measured concentrations of those components:

calculated GE = (protein x 5700 kcal/kg) + (carbohydrate x 4000 kcal/kg) + (lipid x 9500 kcal/kg) + (lignin x 8000 kcal/kg).

These calculations were done for ADL and Klason lignin concentration estimates. The calculated GE values were then compared to the corresponding measured GE of the forages.

Results and Discussion

The forages varied widely in chemical composition and Klason lignin values were, as expected, much greater than corresponding ADL values (Table 1). Measured concentrations of protein, lipid, and ash were similar to published values for these types of forages. Gross energy values of these forages determined by bomb calorimetry (Table 2) were also similar to expected levels. When GE was calculated from the analyzed composition of the forages, use of Klason lignin resulted in GE recoveries (as a percent of the measured GE) averaging 93.1% whereas a similar calculation of GE based on ADL resulted in an average recovery of only 75.8% (Table 2).

Because lignin concentration estimates were the only element of the calculation that changed, this difference in recovery between lignin methods was expected. However, if Klason lignin indeed overestimates lignin concentration, then the calculated GE should have been greater than 100% of measured GE. The facts that Klason lignin allowed a better accounting for measured GE than ADL and that this GE recovery was less than 100%, indicate Klason lignin is a more accurate quantitative measure of lignin concentration. The lack of complete recovery of

the measured GE in the calculation can be attributed to the incomplete recovery of sample dry matter by the component analyses. Using Klason lignin, dry matter recoveries ranged from 84 to 93%. If we assume all non-recovered GE by the component calculation is carbohydrate (the least energy dense forage component), then dry matter recoveries using Klason lignin increase (94 to 100%). We have reason to believe carbohydrate recoveries were incomplete because there were unidentified peaks on the neutral sugar chromatograms. We are investigating the source of these peaks.

Table 1. Chemical composition of the forage samples.

Sample	Lignin Protein	Carbohydrate	Lipid	Ash	ADL	Klason
			% DM			
Alfalfa	21.3	44.5	1.8	8.8	5.8	15.0
Alfalfa	16.2	46.1	2.3	10.6	5.9	13.6
Kura clover	21.1	41.2	4.0	8.9	2.1	8.4
Annual Medic	25.9	38.5	2.9	14.3	2.6	9.3
Corn silage	7.1	63.3	3.6	5.0	1.4	12.2
Corn silage	11.1	59.6	4.2	7.5	1.4	10.0
Orchardgrass	14.2	42.9	4.4	11.4	2.5	12.6
Bromegrass	11.6	52.9	1.6	9.0	3.8	15.5
Switchgrass	9.3	51.2	1.3	8.3	2.4	14.2
Oat straw	4.2	60.4	0.6	10.7	4.7	16.6

Table 2. Percent of measured forage gross energy accounted for by calculated gross energy using ADL and Klason lignin.

Sample	Measured Gross Energy	Calculated Gross Energy	
		ADL	Klason lignin
	kcal/kg	%	
Alfalfa	4493	80.8	97.1
Alfalfa	4371	79.1	93.1
Kura clover	4460	76.2	87.6
Annual Medic	4308	81.3	93.7
Corn silage	4392	77.2	96.9
Corn silage	4497	78.4	93.7
Orchardgrass	4485	70.1	88.1
Bromegrass	4323	74.8	96.5
Switchgrass	4377	66.1	87.8
Oat straw	4182	73.8	96.7