リグニン含有率と消化率の関係に及ぼすリグニン定量法の影響

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Influence of Lignin Determination Procedures on Relationship between Lignin Content and Digestibility in Forages

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Synopsis

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The lignin content of three grasses and alfalfa samples of known *in vivo* dry matter digestibility was determined by two different methods ; the acid detergent method (successive treatment with acid detergent and 72% sulfuric acid) and the pronase method (successive treatment with pronase and 72% sulfuric acid). The lignin content determined by the acid detergent method was lower than that determined by the pronase method for all the samples. The difference in the lignin content determined by the two methods was much greater in the grass samples than in the alfalfa samples. It was concluded that the acid detergent treatment overestimated the difference in the lignin level between grasses and alfalfa with the same digestibility.

Key Words : Acid detergent, Alfalfa, Digestibility, Grass, Lignin, Pronase.

Introduction

Lignin is considered as a major component disturbing the digestion of forages by ruminants. The lignin content of plant samples is most reliably evaluated by the 72% sulfuric acid method. Forage samples usually contain high levels of protein, which causes a positive error by condensing with lignin during treatment with strong acid. VAN SOEST[9] applied the acid detergent treatment to removal of proteins and proposed the successive treatment with the acid detergent and 72% sulfuric acid for lignin determination, which is now commonly used for forage lignin evaluation. He examined the relationships between lignin content and dry matter digestibility in several forage species and found that alfalfa had a higher lignin content than grasses of the same digestibility[10]. This difference, however, might be caused by the procedure for removal of proteins because the acid detergent reduces the yield of 72% sulfuric acid lignin more markedly from grasses than from legumes[6].

The aim of this study is to elucidate the influence of acid detergent on the relation of lignin content to *in vivo* dry matter digestibility (DMD) in grasses and alfalfa.

Materials and Methods

Forage samples

Forage samples used in the experiments were composed of 8 Italian ryegrass : 4 varieties

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each cut at booting and heading stage, 8 orchardgrass : 4 varieties at booting and heading, 6 perennial ryegrass : 3 varieties at heading and flowering, and 4 alfalfa : 2 varieties at vegetative and bud-appearing. These forages were artificially dried and their DMD was measured with sheep. Samples for analyses were ground to pass a 1 mm screen and then stored at −15°C.

Lignin determination

The lignin content of forage samples was measured by the following two methods :

(a) Acid detergent method

The modification by HORII and ABE[5] of the method of VAN SOEST[9] was used. The sample was refluxed with the acid detergent for 1 hr, filtered on a glass crucible (G 2), and washed with water and acetone. The dried residue was digested with 72% sulfuric acid at room temperature for 4 hr. The mixture was diluted to 3.6% sulfuric acid, boiled for 5 min, and then filtered on grass fiber fieter paper (TOYO GA 200). The residue was washed with water and dried at 105°C for 4 hr. The lignin content was determined by weighing the loss on ashing at 550°C. Nitrogen content in lignin preparations was determined by the Kjeldahl method. The protein content was calculated by multiplying the nitrogen content by the factor of 6.25.

(b) Pronase method

The sample was digested with 0.02% w/v pronase (Actinase-E, from Kakenseiyaku) for 16 hr as described by ABE *et al.*[1] The pronase-digested and dried residue was treated with 72 % sulfuric acid at 20°C for 4 hr. The mixture was diluted to 3% sulfuric acid, boiled for 2 hr under reflux, and filtered on grass fiber filter paper (TOYO GA 200). The residue was washed with water. The lignin content was determined by the same method as the acid detergent method. Nitrogen content was also estimated by the same method as mentioned above.

Results

The lignin content determined by the pronase method was higher than that determined by the acid detergent for each of the samples ; especially for the grasses, the former was beyond two times as high as the latter (Table 1). The pronase method yielded lignin preparations with lower nitrogen contents than the acid detergent method, and the lignin preparations from the alfalfa samples contained nitrogen to a larger extent than those from the grass samples irrespective of the used method (Table 2).

Table 1. Lignin content evaluated by two different methods

Forage	No. of samples	Acid detergent method			Pronase method		
		Mean	Max.	Min.	Mean	Max.	Min.
Italian ryegrass	8	4.3	5.7	3.2	9.5	11.4	7.3
Orchardgrass	8	3.8	5.1	2.2	9.1	11.2	7.1
Perennial ryegrass	6	3.8	4.8	2.3	9.1	10.5	6.2
All grasses	22	4.0	5.7	2.2	9.3	11.4	6.2
Alfalfa	4	8.5	9.7	7.6	10.6	11.7	9.2
All forages	26	4.7	9.7	2.2	9.5	11.7	6.2

% of dry matter.

Table 2. Nitrogen content of lignin preparations yielded by two different methods

Forage	Acid detergent method		Pronase method	
	Mean	S.D.	Mean	S.D.
Italian ryegrass	1.8	0.3	1.5	0.3
Orchardgrass	2.0	0.6	1.8	0.6
Perennial ryegrass	1.5	0.2	1.1	0.2
All grasses	1.8	0.5	1.5	0.5
Alfalfa	3.0	0.6	2.7	0.3
All forages	2.0	0.7	1.7	0.6

% of lignin preparation.

Table 3. Correlation coefficients between *in vivo* dry matter digestibility and lignin content

Forage	Acid detergent method		Pronase method	
	Without protein correction	With protein correction	Without protein correction	With protein correction
Italian ryegrass	−0.977***	−0.972***	−0.977***	−0.978***
Orchardgrass	−0.966***	−0.967***	−0.956***	−0.958***
Perennial ryegrass	−0.975***	−0.970**	−0.967**	−0.972**
All grasses	−0.971***	−0.966***	−0.951***	−0.953***
Alfalfa	−0.983*	−0.960*	−0.953*	−0.983*
All forages	−0.271	−0.380	−0.792***	−0.889***

*, **, *** : significant at the 5%, 1%, and 0.1% level, respectively.

A high negative correlation was obtained between the DMD and the lignin content within each forage species and within the three grasses regardless of the used method and the correction for protein content (Table 3). However, when the grasses and alfalfa were combined, the acid detergent method gave the very low correlation coefficient no matter whether the lignin content was corrected for protein content or not, while the pronase method gave the significant coefficient.

The linear regressions of the lignin content on the DMD are shown in Table 4. For all the regressions the coefficient of determination amounted to more than 90%. No significant differences were found both in regression coefficient and in regression constant between three grass species in the all determination methods, but alfalfa had the significantly higher regression contents than each grass species or the all grasses.

The grasses always showed a lower lignin level than alfalfa of similar DMD (Fig. 1). The extent of difference in the lignin level observed between the grasses and alfalfa varied with the lignin determination method ; the difference obtained by the acid detergent method was much more than that obtained by the pronase metod (A and B in Fig. 1), and the protein correction decreased the difference between the grasses and alfalfa in both cases of the acid detergent and the pronase methods (C and D in Fig. 1) as the lignin preparations from the alfalfa were more contaminated with nitrogeneous compounds than those from the grass samples.

Table 4. Linear regressions of lignin content on dry matter digetibility in different forage species

Forage	Regression coefficient	Regression constant	Coefficient of determination (%)	Standard deviation of residuals
	·····Acid detergent method without protein correction·····			
Italian ryegrass	−5.49	85.0	95	1.15
Orchardgrass	−4.87	83.3	93	1.54
Perennial ryegrass	−5.52	85.3	95	1.41
All grasses	−5.29	84.5	94	1.31
Alfalfa	−6.20	119	97	1.24
	·····Acid detergent method with protein correction·····			
Italian ryegrass	−5.70	83.3	95	1.26
Orchardgrass	−4.87	81.2	94	1.51
Perennial ryegrass	−5.94	84.7	94	1.52
All grasses	−5.47	82.9	93	1.40
Alfalfa	−6.00	108	92	1.88
	·····Pronase method without protein correction·····			
Italian ryegrass	−3.48	94.4	95	1.16
Orchardgrass	−3.55	97.1	92	1.73
Perennial ryegrass	−3.21	93.4	94	1.59
All grasses	−3.50	95.7	91	1.68
Alfalfa	−5.19	122	91	2.04
	·····Pronase method with protein correction·····			
Italian ryegrass	−3.44	91.1	96	1.13
Orchardgrass	−3.29	91.5	92	1.69
Perennial ryegrass	−3.26	91.8	95	1.46
All grasses	−3.39	91.9	91	1.64
Alfalfa	−5.85	119	98	0.78

Discussion

Besides the acid detergent method, another method, the pepsin method[3], has been adopted as the method for determining lignin in the Official Methods of Analysis of the A.O.A.C., but its method includes 1 N acid hydrolysis in the procedures of pretreatment. The 1 N acid hydrolysis is proved to deprive some portion of lignin of grass fibers[8]. According to MORRISON[7], the pronase digestion of forage plants can yield cell walls with low nitrogen contents without dissolving lignin from plant tissues. Thus in our experiments the pronase – 72% sufuric acid method was employed as a standard method though this method is not the official one, and then the lignin contents evaluated by the acid detergent method were compared with those by the standard method.

The two determination methods both gave the high correlation coeffiecients between the lignin content and the DMD within the grasses and within alfalfa. These high coefficients indicate that lignin is a good indicator for predicting the digestibility of forage. The acid detergent method, however, estimated the lignin content of the forage samples low, compared with the pronase method ; the marked discrepancy between the two methods was found in the

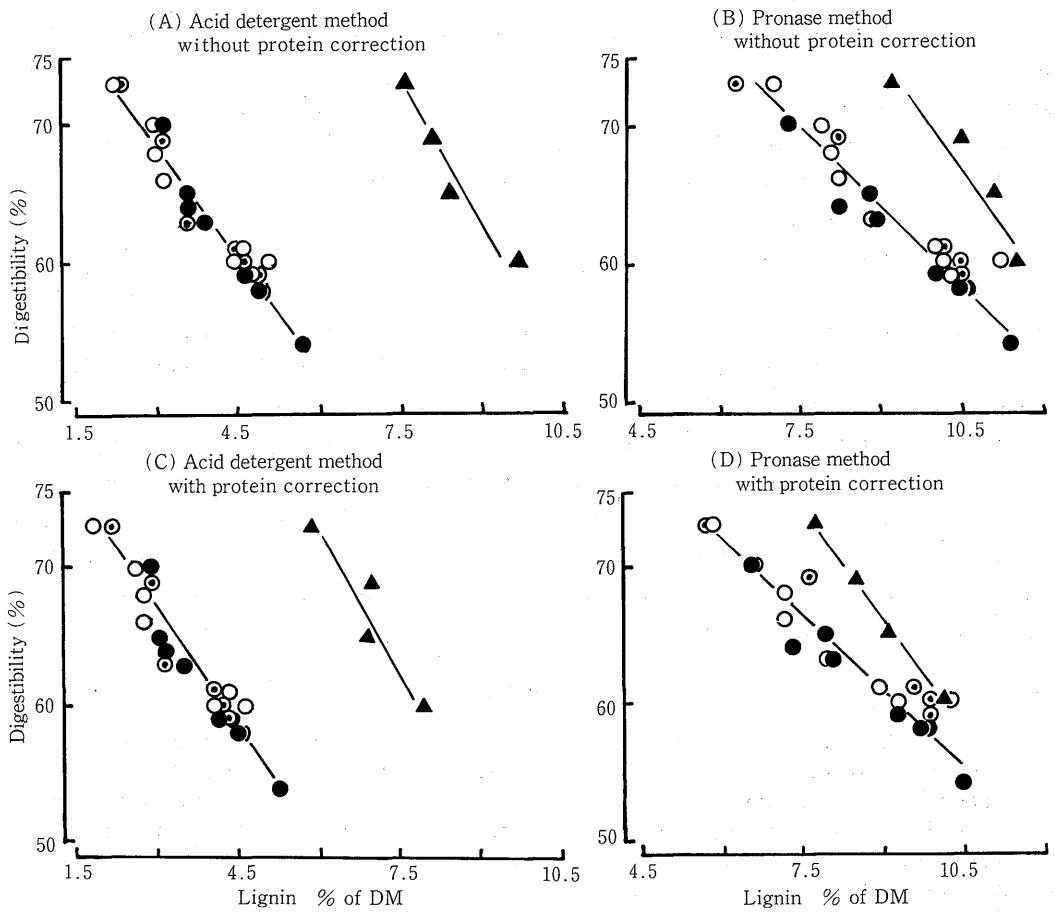

Fig. 1. Relationships between *in vivo* dry matter digestibility and lignin content evaluated by two different methods.
● : Italian ryegrass, ○ : orchardgrass, ⊙ : perennial ryegrass, ▲ : alfalfa.

grass samples. In this connection, it has been recently reported that Klason lignin content (19.8%) of straw is 2.4 times higher than its lignin content (8.3%) determined by the acid detergent method[2].

As described above, the acid detergent treatment is known to remove effectively proteins from forage samples and to leave fibrous residues. But its treatment has been revealed to cause a greater lignin loss in grasses than in legumes[6]. The lower lignin values obtained for the grasses with the acid detergent method must be due to this differential effect of the acid detergent. From the comparison of the relations of the lignin content to the DMD measured by the two different method (Fig. 1), it is concluded that the acid detergent treatment overestimates the difference in the lignin level between grasses and alfalfa of the same DMD.

Some of nitrogeneous compounds still remained in the residues pretreated for removal of proteins and were incorporated into the lignin preparations (Table 2). The lignin prepara-

tions from the alfalfa samples contained more nitrogen than those from the grass samples. Similar results were obtained with alkali-lignins by BONDI and MEYER[4]. They showed that the lignins from grasses and from legumes contained 1.2–1.6% and 2.9–3.4% nitrogen, respectively. Because of the different nitrogen contents of the lignin preparations, the correction of the lignin values for protein content (nitrogen % × 6.25) resulted in a decrease in the difference between the lignin values of the grass and alfalfa samples.

Indeed the grasses showed a lower lignin level than alfalfa of the same DMD independently of the determination method, but this does not always mean that the grasses have the significanlty lower content of 'total' lignin than alfalfa of the same DMD, because acid-soluble lignin, which is lost on filtration after boiling with dilute acid, was not estimated in the experiments. In addition, nitrogeneous contaminants interfere with accurate evaluation of the total lignin content. Although the factor 6.25 used to correct the lignin values for protein content is derived from the elementary composition of original protein, its composition may not be the same as that of protein fragments condensed with lignin. There is evidence[11] that protein remaining in lignin preparations isolated by 72% sulfuric acid method is characteristically rich in hydroxyproline. It needs further close experiments to determine whether there is the significant difference in the total lignin content between grasses and legumes of the same DMD or not.

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リグニン含有率と消化率の関係に及ぼすリグニン定量法の影響

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要　約

乾物消化率既知のイネ科牧草およびアルファルファについて，2つの定量法，(1) 酸性デタージェント—72% 硫酸連続処理法，(2) プロナーゼ—72% 硫酸連続処理法，で測定したリグニン含有率を比較した。その結果，酸性デタージェント法によるリグニン含有率はプロナーゼ法に基づく場合より低く，その差はイネ科牧草において特に大きいことが判明した。酸性デタージェント処理は，同じ乾物消化率で比較したときのイネ科牧草とアルファルファ間のリグニン含有率の差を過大評価すると結論された。

キーワード： アルファルファ，イネ科牧草，酸性デタージェント，消化率，プロナーゼ，リグニン．

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