Fiber and Tannins of Some Agricultural and Forest Byproducts. Inclusion of These Parameters in the Prediction of *in Vitro* Digestibility

Almudena Rebolé

Departamento de Producción Animal, Facultad de Veterinaria, Universidad Complutense, Ciudad Universitaria, 28040 Madrid, Spain

The summative equation of prediction of digestibility based on lignin and fiber contents of plant material, gave a good estimation of the *in vitro* value in byproduct feeds such as oak and olive browses and vine branches when the sulfuric lignin content of fiber was used, better than with permanganate lignin. In both cases, the lignin content of the neutral-acid-detergent fiber was more highly correlated with *in vitro* digestibility than the acid-detergent fiber of the acid-neutral-detergent fiber. The inclusion of condensed tannins into the predictive equation, as components that may cause a decline of digestibility, improved the prediction of the *in vitro* value from chemical composition.

INTRODUCTION

Browse species (oak, olive) as well as vine branches are eaten by grazing sheep and cattle in Spain. The nutritive value of these products appear to be limited by fiber and tannins. The detergent system of analysis, originally developed for forages, has been shown to be adequate for the fiber fraction in browse species (McCammon-Feldman, 1980; Horvath, 1981; Reed, 1986; Conklin, 1987; Van Soest *et al.*, 1987; Woodward, 1988). However, tannins have not been incorporated into standard nutritive evaluations of these species by laboratory analyses, and difficulty in evaluating tanniniferous species has been encountered.

Condensed tannins tend to be recovered in crude lignin, therefore elevating the value. It appears that the influence of tannins upon digestibility of fiber is less than that of lignin (Austin *et al.*, 1989). There is a problem in distinguishing tannins from lignin by gravimetric assays because tannins are largely unextractable in dried feeds. Most tannin assays are dependent upon extraction. Consequently, unextractable forms have often been overlooked (Reed *et al.*, 1985). Other deficiencies of methods are described by Marquardt (1989). The extraction is incomplete owing to the varying nature of the polyphenols and their associations with other plant components (Horvath, 1981). Acetone has been the favored solvent for extracting tannins since it inhibits the interactions that can take place between tannins and protein (Hagerman, 1988). The colorimetric measurement suffers from the lack of a suitable compound that can be used as a primary standard (Mueller-Harvey *et al.*, 1988).

Since the order of extractions with neutral- and acid-detergent can be varied, it seemed worth while to apply varied sequential analyses to some agricultural and forest residues and apply the results to the prediction of digestibility in the summative system. Also, condensed tannins measured by 1-butanol-HCl were included into the summative system to determine if such an inclusion would improve the prediction of digestibility from chemical composition.

MATERIALS AND METHODS

Materials. Samples of oak (*Quercus ilex*) were taken from pruned oaks in Sartajada (Toledo, Spain) in March 1985, and samples of olive (*Olea europea*) were taken from olive trees in Valdeolivas (Cuenca, Spain) in March and September 1988; the samples from these two collections were mixed to make a single sample. In addition to the whole foliage, the leaves and stems of oak and olive were analyzed separately. Vine branches (*Vitis vinifera*) were taken from vineyards in Moral de Calatrava (Ciudad Real, Spain) in October 1983 after harvesting of grapes and immediately ensiled. Samples from oak and olive were oven-dried at 55 °C; samples of vine branches were freeze-dried. All samples were ground to pass a 1-mm screen and stored in the dry form in sealed plastic bottles.

Determination of Dry Matter. Dry matter was determined by drying a 1–1.5-g sample at 105 °C for at least 8 h and hot weighing (Goering and Van Soest, 1970; Van Soest and Robertson, 1985). For the hot weighing procedure, a single-pan automatic macroanalytical balance with electronical readout to 0.1 mg was used. To heat up the balance, three weighing sequences were completed, using blank beakers (or crucibles) in a set sequence before the analytical weighing. The balance stabilized for a short period of time while the hot beaker was on the pan.

Determination of the Amount of Fiber and Lignin. The fiber and lignin contents of the samples were determined via the sequential detergent system of analysis (Goering and Van Soest, 1970; Horvath, 1981; Van Soest, 1987). Sequential extractions with neutral-detergent (ND) followed by acid-detergent (AD) and of AD followed by ND were done to measure fiber content. The residue obtained after neutral-detergent extraction followed by acid-detergent treatment was termed NADF. The residue obtained after acid-detergent extraction followed by neutral-detergent treatment was termed ANDF (Figure 1).

The amount of lignin was estimated using two procedures: the procedure for Klason lignin and the procedure for permanganate lignin (Figure 2). Klason lignin or "sulfuric lignin" (Ls) is the residue insoluble in 72% sulfuric acid. The Klason lignin residue was treated with potassium permanganate, and the loss in weight termed "permanganate lignin" (Lk).

The hot weighing procedure was applied to all fiber and lignin determinations. Two replications were made in all analyses. Values were expressed on a organic matter basis (ash-free values).

Determination of Crude Protein. Crude protein was determined following the macro-Kjeldahl method (AOAC, 1980). Neutral-, acid-, neutral-acid-, and acid-neutral-detergent fiber-bound proteins were determined and termed NDFCP, ADFCP, NADFCP, and ANDFCP, respectively. Protein-free fiber fractions were calculated by subtracting fiber-bound protein from fiber residues.

Determination of Condensed Tannins. Condensed tannins (soluble proanthocyanidins) were estimated via the procedure of Reed *et al.* (1982) by placing 10–30 mg of the original sample into a test tube, adding 10 mL of 5% HCl in 1-butanol, and heating, covered by a marble, for 30 min on a steam bath at 98 °C. The absorbance of solution was read at 550 nm. The amount of condensed tannins was calculated using a standard curve. To prepare the standard curve, 2 g of oak leaves was extracted five times with 20 mL of 70% aqueous acetone. The acetone solution was separated into an aqueous and acetone phase in a separatory

0021-8561/94/1442-0739$04.50/0

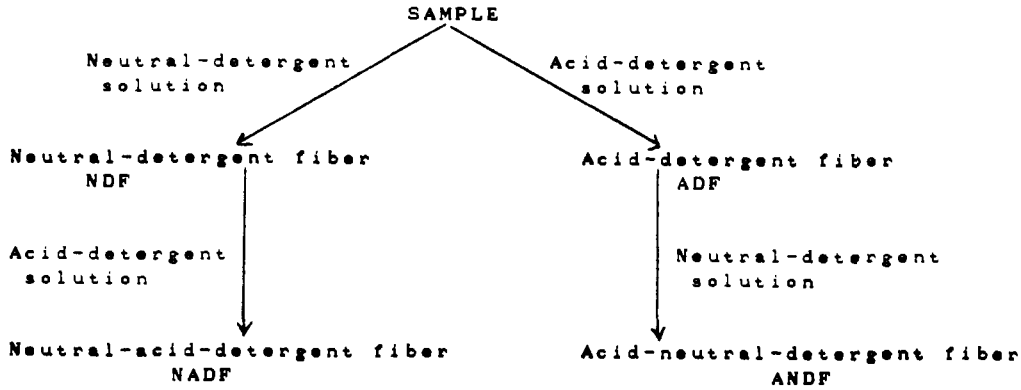

Figure 1. Sequences of analysis of fiber following the sequential detergent system (Horvath, 1981; Van Soest, 1987).

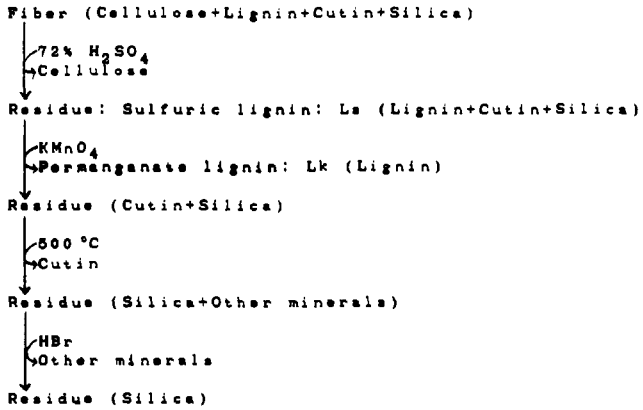

Figure 2. Sequences of analysis of lignin following the detergent system of analysis (modified from Van Soest and Robertson, 1985).

funnel by addition of excess sodium chloride. The lower aqueous phase was removed, and 1:5 ethanol–diethyl ether was added to precipitate tannins. The precipitate was dissolved in 50% aqueous methanol, dialyzed three times against water, and freeze-dried. Dialysis was made against 4 L of distilled water, in dialysis tubing (2000 molecular weight cutoff). Of the precipitate obtained, 1–6 mg was placed in test tubes, adding 10 mL of 5% HCl in 1-butanol, and heating for 30 min in a steam bath at 98 °C. The absorbance was read at 550 nm.

Determination of Digestibility. Digestibility was determined experimentally via the *in vitro* technique of Goering and Van Soest (1970). The sample (0.5 g, ground to pass a 1-mm screen) was weighed in a 125-mL Erlenmeyer flask, placed in a water bath at 40 °C, and treated with 40 mL of a medium prepared with 2 g of trypticase, 400 mL of water, 0.1 mL of micromineral solution (calcium chloride, manganese chloride, cobalt chloride, ferric chloride, and distilled water), 200 mL of buffer solution (ammonium bicarbonate, sodium bicarbonate, and distilled water), 200 mL of macromineral solution (disodium phosphate, monopotassium phosphate, magnesium sulfate, and distilled water), and 1 mL of resazurin. After the carbon dioxide pressure was removed, 2 mL of reducing solution (cysteine hydrochloric acid, distilled water, sodium hydroxide, and sodium sulfide) was added. The sample was then fermentated for 48 h with 10 mL of rumen inoculum from a fistulated Jersey cow fed with timothy hay; after collecting, the inoculum was blended for 2 min under carbon dioxide and filtrated through glass wool. At the end of fermentation, the residue was treated with 100 mL of the neutral-detergent solution via the same procedure as in the neutral-detergent fiber determination to eliminate bacterial residues. Digestibility was calculated as 100 minus the percent of the ash-free neutral-detergent residue. Two replications of *in vitro* digestibility per sample were made.

Also, organic matter digestibility (SEOMD) was calculated from the summative equation of Goering and Van Soest (1970): SEOMD = 0.98(100 − NDF) + (CWD/100)NDF, where 0.98 is the average digestibility of cellular contents; NDF is the percent of the neutral-detergent fiber (cell wall); 100 − NDF is the percent of cellular contents; and CWD is the cell wall digestibility, which can be calculated from the sulfuric lignin content of fiber: CWD = 147.3 − 78.9 log[(Ls/fiber)100], or from the permanganate lignin content of fiber: CWD = 180.8 − 96.6 log[(Lk/fiber)100]. The proportion of condensed tannins (CT) was subtracted from SEOMD (SEOMD − CT) to include the correction owing to tannins into the equation.

Statistical Analysis. Analysis of variance was performed to study the variation among analytical procedures for measuring fiber and lignin contents and to study separately each plant material. Simple linear regression (expressed as R^2) was applied to study the relationships among experimental and *in vitro* data.

RESULTS AND DISCUSSION

In each plant material, the neutral-detergent fiber was always the fraction that represented the highest proportion of fiber, while ANDF gave the lowest estimate (Table 1). NDF was the fraction that showed the greatest variation among samples within a plant, while ANDF showed the lowest variation in oak and vine branches and NADF the lowest variation in olive. In oak, NADF and ANDF gave nonsignificantly different estimations of fiber and different estimations of NDF and ADF. In olive, the four procedures to estimate the amount of fiber gave results not significantly different ($p > 0.05$). In vine branches, ADF and NADF gave nonsignificantly different results of fiber and different results of NDF and ANDF.

In oak samples, the highest estimate of lignin was obtained from the ADF residue, with NADF giving the maximum value in the rest of plants, with the exception of olive stems, where ANDF gave the highest value. Lignin content obtained by means of the 72% sulfuric acid procedure was higher than that obtained by means of the permanganate method. Any nonlignin substances that are insoluble in 72% sulfuric acid increase lignin Klason value (Van Soest and Robertson, 1985). In all plants the sulfuric lignin value obtained from the ANDF residue gave the highest variation among samples within a plant, while NADF gave the lowest. The highest variation among the three analytical routes to estimate permanganate lignin in oak was obtained with ADF and NADF and with NADF in olive and vine branches. In all plants, the three fiber fractions gave not significantly different ($p > 0.05$) sulfuric lignin content. In vine branch material, the three estimations of permanganate lignin were significantly different, while differences were not found in oak and olive.

Neutral-detergent extraction dissolves pectin, silica, and tannins, while acid-detergent extraction recovers silica, tannin–protein complexes, and to a partial degree, pectins (Van Soest, 1987). The differences found among all fiber estimations (NDF, ADF, NADF, and ANDF) can be attributed to the cited components. Silica was not detected in the samples studied. Pectins, if present, are in ADF. That means that cell wall protein (fiber-bound protein)

Table 1. Fiber, Lignin, and Condensed Tannins (Percent of Dry Matter) in Different Samples[a]

	fiber fraction				sulfuric lignin Ls[h]			permanganate lignin Lk[i]			
	NDF[d]	ADF[e]	NADF[f]	ANDF[g]	ADF	NADF	ANDF	ADF	NADF	ANDF	CT[j]
oak leaves	48.5	38.1	32.0	31.4	14.2	11.5	12.1	9.8	7.9	7.5	8.7
oak stems	67.3	54.2	47.6	46.2	24.3	21.2	21.9	14.3	12.9	9.8	4.7
oak foliage	57.5	44.2	36.7	35.7	17.4	13.8	13.7	8.5	7.4	6.5	8.3
mean	57.8A	45.6B	38.8C	37.8C	18.6A	15.5A	15.9A	10.9A	9.4A	7.9A	7.2
SE	4.43	3.91	3.77	3.59	2.43	2.39	2.48	1.43	1.43	0.80	1.04
olive leaves	44.7	37.5	33.5	31.3	19.9	21.1	20.1	9.2	10.9	8.2	1.5
olive stems	59.9	46.1	40.7	39.9	13.2	13.9	14.3	8.1	9.7	7.0	0.7
olive foliage	38.1	26.2	28.0	21.3	11.2	15.2	9.7	2.4	4.3	2.0	1.7
mean	47.6A	36.6A	34.1A	30.8A	14.8A	16.7A	14.7A	6.6A	8.3A	5.7A	1.3
SE	5.27	4.70	3.00	4.39	2.15	1.81	2.46	1.72	1.66	1.55	0.25
vine branches											
fresh	53.9	35.6	34.8	29.8	9.4	10.8	7.8	5.2	6.9	4.4	6.0
silage	66.3	43.0	42.6	36.2	12.2	14.5	10.3	7.3	9.7	6.2	11.1
silage FA[b]	57.0	40.7	39.5	34.6	11.4	13.8	11.4	8.6	10.5	6.7	10.4
silage FSA[c]	56.7	43.9	41.3	35.8	14.5	15.7	13.3	11.1	13.0	6.2	8.2
mean	58.5A	40.8B	39.6B	34.1C	11.9A	13.7A	10.7A	8.1A	10.0B	5.9C	8.9
SE	2.34	1.61	1.48	1.28	0.91	0.90	0.99	1.07	1.09	0.44	1.00

[a] Means within a row with different uppercase letters are significantly different at $p < 0.05$. [b] Treated with formic acid. [c] Treated with formaldehyde and sulfuric acid. [d] Neutral-detergent fiber. [e] Acid-detergent fiber. [f] Neutral-acid-detergent fiber. [g] Acid-neutral-detergent fiber. [h] Klason lignin or sulfuric lignin (Ls), obtained by treatment of the different fiber fractions with 72% sulfuric acid. [i] Permanganate lignin (Lk), obtained by treatment of the different Klason lignin residues with potassium permanganate. [j] Condensed tannins were measured by the acid-butanol assay (Reed *et al.*, 1982).

Table 2. Fiber-Bound Protein and Protein-Free Fiber Residues (Percent of DM)[a]

	CP[b]	NDFCP[c]	NDF*[g]	ADFCP[d]	ADF*[g]	NADFCP[e]	NADF*[g]	ANDFCP[f]	ANDF*[g]
oak leaves	8.0	0.9	47.6	1.2	36.9	1.8	30.2	0.8	30.6
oak stems	4.9	1.4	65.9	2.4	51.8	3.1	44.5	0.8	45.4
oak foliage	6.9	1.9	55.6	2.3	41.9	1.2	35.5	0.9	34.8
mean	6.6	1.4	56.4A	2.0	43.5A	2.0	36.7A	0.8	36.9A
SE	0.74	0.24	4.32	0.31	3.57	0.46	3.41	0.03	36.0
olive leaves	10.2	6.6	38.1	5.6	31.9	7.1	26.4	4.9	26.4
olive stems	4.1	1.1	58.8	1.4	44.7	2.3	38.4	0.6	39.3
olive foliage	8.8	3.0	35.1	0.9	25.7	1.6	26.4	0.4	20.9
mean	7.7	3.6	44.0A	2.6	34.0A	3.7	30.4A	2.0	28.9A
SE	1.50	1.32	6.08	1.22	4.65	1.41	3.27	1.20	4.45
vine branches									
fresh	6.0	2.4	51.5	1.0	34.6	0.7	34.1	0.4	29.4
silage	5.0	2.5	63.8	1.8	41.2	1.6	41.0	0.7	35.5
silage FA	4.7	2.5	54.5	0.8	39.9	2.2	37.7	0.6	34.0
silage FSA	5.4	3.7	53.0	2.0	41.9	3.8	37.5	0.5	35.3
mean	5.3	2.8	55.7A	1.4	39.4B	2.1	37.5B	0.6	33.6C
SE	0.23	0.27	2.40	0.25	1.43	0.56	1.22	0.06	1.23

[a] Means within a row with different uppercase letters are significantly different at $p < 0.05$. [b] CP: crude protein (nitrogen Kjeldahl × 6.25) measured in the whole sample. [c] NDFCP: crude protein measured in neutral-detergent fiber, expressed on a dry matter basis. [d] ADFCP: crude protein measured in acid-detergent fiber, expressed on a dry matter basis. [e] NADFCP: crude protein measured in neutral-acid-detergent fiber, expressed on a dry matter basis. [f] ANDFCP: crude protein measured in acid-neutral-detergent fiber, expressed on a dry matter basis. [g] Protein-free fiber, obtained by subtracting fiber-bound protein from fiber residue.

and tannins may be components which make all fiber estimations different. Fiber-bound protein was measured in the four residues, and protein-free fiber was calculated by subtracting protein–fiber bound from the corresponding fiber fraction (Table 2). The protein-free fiber estimations were not significantly different in oak, so fiber-bound protein could be a component which made the fiber estimations of oak different. In vine branches, the protein-free fiber residues still were significantly different, so this material may have tannins which may be largely responsible for the disparity found among fiber estimations. Moreover, from sequential detergent analysis of fiber, it could be said that proanthocyanidins are present in samples from oak and vine branches, since they showed a red color when heated in acid-detergent solution.

Condensed tannin content (Table 1) was highest in samples from oak and vine branches, while olive samples showed the lowest value. Stems had less tannins than leaves (oak and olive), as observed in other browse species (Conklin, 1987). The condensed tannins content of byproducts studied was not significantly correlated ($p > 0.05$) with the amount of fiber or lignin (data not shown). Reed (1986, 1987) found a significant correlation among insoluble proanthocyanidins and NDF, NDFCP, and lignin.

Table 3 shows *in vitro* organic matter digestibility (IVOMD). Rebolé *et al.* (1988) found values of 33.2 and 39.9, respectively, for the *in vivo* (sheep) and *in vitro* dry matter digestibilities of vine branches. Boza and Guerrero (1981) found values of 49.3%, 60.4%, and 55.2% for the *in vitro* organic matter digestibility (goats) of oak foliage, olive leaves, and olive foliage, respectively. Olive presented the highest variation of *in vitro* digestibility among samples, while vine branches presented the lowest.

Digestibility calculated from the summative equation using the analytical data (SEOMD) is in Table 3. Permanganate lignin gave the highest variation of digestibility among samples of olive and vine branches, while sulfuric lignin gave the highest variation among samples of oak. In oak and olive the three analytical sequences gave not

Table 3. *In vitro* (IVOMD) and Predicted (SEOMD) Organic Matter Digestibilities (Percent of DM)[a]

		SEOMD[b]					
		Ls[c]			Lk[d]		
	IVOMD	ADF	NADF	ANDF	ADF	NADF	ANDF
oak leaves	63.4	61.8	62.4	61.2	72.1	72.9	73.6
oak stems	43.3	43.6	43.6	42.2	61.5	60.6	67.5
oak foliage	52.3	54.0	54.9	54.5	74.3	73.1	75.6
mean	53.0	53.1A	53.6A	52.6A	69.3A	68.9A	72.2A
SE	4.75	4.30	4.46	4.54	3.23	3.38	1.99
olive leaves	68.8	59.2	56.6	56.3	75.0	69.7	73.8
olive stems	53.2	58.7	55.1	54.1	75.6	67.9	75.6
olive foliage	77.8	67.8	64.6	66.9	94.1	85.9	93.7
mean	66.6	61.9A	58.8A	59.1A	81.6A	74.5A	81.0A
SE	5.78	2.41	2.41	3.23	5.12	4.67	5.19
vine branches							
fresh	57.9	64.1	61.1	64.3	82.0	75.1	81.8
silage	49.5	54.7	50.5	54.6	74.1	66.0	73.9
silage FA	55.0	61.0	56.7	57.8	72.2	66.8	74.3
silage FSA	52.8	58.0	55.3	55.7	68.1	62.9	77.1
mean	53.8	59.5A	55.9A	58.1A	74.1A	67.7B	76.8A
SE	1.54	1.74	1.89	1.88	2.53	2.26	1.58

[a] Means within a row with different uppercase letters are significantly different at $p < 0.05$. [b] Organic matter digestibility calculated from the summative equation of Goering and Van Soest (1970); SEOMD = 0.98 (100 − NDF) + (CDW/100)NDF, where CWD is the cell wall digestibility. [c] Using sulfuric lignin value: CWD = 147.3 − 78.9 log[(Ls/fiber)100]. [d] Using permanganate lignin value: CWD = 180.8 − 96.6 log[(Lk/fiber)100].

Table 4. Predicted Organic Matter Digestibility Including the Correction for Condensed Tannins (SEOMD − DT) (Percent of DM)[a]

	SEOMD − CT[b]					
	Ls			Lk		
	ADF	NADF	ANDF	ADF	NADF	ANDF
oak leaves	53.1	53.7	52.5	63.4	64.2	64.9
oak stems	38.9	38.9	37.5	56.8	55.9	62.8
oak foliage	45.7	46.6	46.2	66.0	64.8	67.3
mean	45.9A	46.4A	45.4A	62.1A	61.6A	65.0A
SE	3.35	3.49	3.55	2.24	2.34	1.06
olive leaves	57.7	55.1	54.8	73.5	68.2	72.3
olive stems	58.0	54.4	53.4	74.9	67.2	74.9
olive foliage	66.1	62.9	65.2	92.4	84.2	92.0
mean	60.6A	57.5A	57.8A	80.3A	73.2A	79.7A
SE	2.25	2.22	3.04	4.96	4.50	5.05
vine branches						
fresh	58.1	55.1	58.3	76.0	69.1	75.8
silage	43.6	39.4	43.5	63.0	54.9	62.8
silage FA	50.6	46.3	47.4	61.8	56.4	63.9
silage FSA	49.8	47.1	47.5	59.9	54.7	68.9
mean	50.5A	47.0A	49.2A	65.2A	58.8A	67.9A
SE	2.57	2.78	2.75	3.17	3.00	2.57

[a] Means within a row with different uppercase letters are significantly different at $p < 0.05$. [b] Predicted organic matter digestibility less condensed tannins.

significantly different ($p > 0.05$) values of the digestibility calculated from permanganate lignin as well as from sulfuric lignin. In vine branches, the permanganate lignin from the ADF and ANDF gave not significantly different digestibility, but different from that calculated from NADF.

To see how the detergent system of analysis is able to predict the *in vitro* digestibility from chemical composition, both parameters were compared (Table 4). The sulfuric lignin content of NADF gave the best prediction ($R^2 = 0.683$) of the experimentally determined *in vitro* value, and the permanganate lignin content of ANDF gave the poorest correlation ($R^2 = 0.542$). In some cereal and legume straws studied by Rebolé *et al.* (1990), the best

Table 5. Correlations (R^2) among *in Vitro* and Predicted Organic Matter Digestibilities

			IVOMD, R^2
SEOMD	Ls	ADF	0.639[b]
		NADF	0.683[b]
		ANDF	0.612[b]
SEOMD	Lk	ADF	0.621[b]
		NADF	0.680[b]
		ANDF	0.542[a]
SEOMD − CT	Ls	ADF	0.739[b]
		NADF	0.776[b]
		ANDF	0.784[b]
SEOMD − CT	Lk	ADF	0.638[b]
		NADF	0.696[b]
		ANDF	0.582[a]

[a] $p < 0.05$. [b] $p < 0.01$.

estimate of digestibility was obtained from the lignin permanganate content of NADF ($R^2 = 0.610$), while the Klason lignin content of NADF gave the lowest prediction ($R^2 = 0.320$).

Tannins, as components that may cause a decline in digestibility, can be introduced into the equation to predict digestibility. The correction could be done by subtracting the percentage of condensed tannins from the organic matter digestibility (Table 4). Permanganate lignin gave the lowest variation among samples in oak and the highest in olive and vine branches. In all plants, digestibility values calculated using the three different analytical routes were not significantly different. With the correction from tannins, the prediction of the *in vitro* value from sulfuric lignin content of fiber improved ($R^2 = 0.739, 0.776, 0.784$, Table 4), while change was not significant in the case of permanganate lignin ($R^2 = 0.638, 0.696, 0.582$). Plant tannins, as components with a negative effect upon digestibility of feeds, should be taken into account in the prediction of digestibility from chemical composition.

The effects of heating the plant material on tannin reactivity should not be overlooked, since oven-drying could causes a decrease in the solubility of the proanthocyanidins and an increase in the content of lignin and fiber content (Reed *et al.*, 1982). Additional research by comparing fresh material with air-dried, oven-dried, and lyophilized samples is needed. However, the problem of analyzing tannins in prepared dried feeds remains. The relationship between the assays for tannins and the biological effects need to be clarified, as well as the influence of the season on tannin content (Marquardt, 1989, 1993). A better knowledge of the chemistry of tannins is necessary to understand completely their nutritional significance (Hagerman and Butler, 1989).

ACKNOWLEDGMENT

This work was done at the Department of Animal Science of Cornell University. I thank Dr. Van Soest and Dr. Robertson for their guidance and advice and B. Giner, T. Hernandez, and T. Kinsman for their assistance. The Dirección General de Investigación Científica y Técnica of Ministerio de Educación y Ciencia of Spain, the Consejería de Educación of Comunidad de Madrid, and the Department of Animal Science of Cornell University supported this study.

LITERATURE CITED

AOAC. *Official Methods of Analysis*, 11th ed.; Association of Official Analytical Chemists: Washington, DC, 1980; p 125.

Austin, P. J.; Suchar, L. A.; Robbins, C. T.; Hagerman, A. E. Tannin-binding proteins in saliva of seer and their absence in saliva of sheep and cattle. *J. Chem. Ecol.* **1989**, *15*, 1335–1347.

Boza, J.; Guerrero, J. E. Valeur alimentaire de quelques sous-produits agricoles pour la chèvre. In *Nutrition et Systemes d'Alimentation de la Chèvre*; Morand Fehr, P., Bourbouze, A., Simiane, M., Eds.; ITOVIC-INRA: Tours, 1981; Vol. II, pp 635–642.

Conklin, N. L. The potential nutritional value to cattle of some tropical browse species from Guanacaste, Costa Rica, Ph.D. Thesis, Cornell University, Ithaca, NY, 1987.

Goering, H. K.; Van Soest, P. J. Forage fiber analysis. *Agriculture Handbook No. 379*; ARS, USDA: Washington, D.C., 1970.

Hagerman, A. E. Extraction of tannins from fresh and preserved leaves. *J. Chem. Ecol.* **1988**, *14* (2), 453–461.

Hagerman, A. E.; Butler, L. G. Choosing appropriate methods and standards for assaying tannin. *J. Chem. Ecol.* **1989**, *15* (6), 1795–1811.

Horvath, P. J. The nutritional and ecological significance of acertannins and related polyphenols. M.S. Thesis, Cornell University, Ithaca, NY, 1981.

Marquardt, R. R. Personal communication, 1993.

Marquardt, R. R. Dietary effects of tannins, vicine and convicine. Paper presented at *Recent Advances in Antinutritional Factors in Legume Seeds*; Proceedings of the First International Workshop on ANF in Legume Seeds; Wageningen: The Netherlands, Nov 23–25, 1989.

McCammon-Feldman, B. A critical analysis of tropical savanna forage consumption and utilization by goats. Ph.D. Thesis, University of Illinois, Urbana, IL, 1980.

Mueller-Harvey, I.; McAllan, A. B.; Theodoru, M. K.; Beever, D. E. Phenolics in fibrous crop residues and plant and their effects on the digestion and utilization of carbohydrates and protein in ruminant. 1988. Paper presented: *Plant Breeding and the Nutritive Value of Crop Residues*. Proc. ILCA Addis Ababa, Dec 7–10, 1987.

Rebolé, A.; Alvira, P.; González, G. Digestibility *in vivo* of ensiled grapevines (branches and leaves): influence of the system of analysis in the detergent fibre scheme on the prediction of digestibility. *Anim. Feed Sci. Technol.* **1988**, *22*, 173–177.

Rebolé, A.; Alvira, P.; González, G. Relationship among compositional data of fibrous by-products as determined by two different extractions in the detergent system of analysis. Influence on the prediction of digestibility. *J. Sci. Food Agric.* **1990**, *53*, 15–22.

Reed, J. D. Relationships among soluble phenolics, insoluble proanthocyanidins and fiber in East African browse species. *J. Range Manage.* **1986**, *39*, 5–7.

Reed, J. D. Phenolics, fiber, and fiber digestibility in bird resistant and non bird resistant sorghum grain. *J. Agric. Food Chem.* **1987**, *35*, 461–464.

Reed, J. D.; McDowell, R. E.; Van Soest, P. J.; Horvath, P. J. Condensed tannins: a factor limiting the use of cassava forage. *J. Sci. Food Agric.* **1982**, *33*, 213–220.

Reed, J. D.; Horvath, P. J.; Allen, M. S.; Van Soest, P. J. Gravimetric determination of soluble phenolics including tannins from leaves by precipitation with trivalent ytterbium. *J. Sci. Food Agric.* **1985**, *36*, 255–261.

Van Soest, P. J. *Nutritional Ecology of the Ruminant*; Comstock Publishing Associates, Cornell University Press: Ithaca, NY, 1987; pp 75–94.

Van Soest, P. J.; Robertson, J. B. *Analysis of Forages and Fibrous Feeds*, Laboratory Manual; Cornell University: Ithaca, NY, 1985.

Van Soest, P. J.; Conklin, N. L.; Horvath, P. J. Tannins in foods and feeds. *Proc. Cornell Nutr. Conf.* **1987**, 115–122.

Woodward, A. Chemical composition of browse in relation to relative consumption of species and nitrogen metabolism of livestock in Southern Ethiopia. Ph.D. Thesis, Cornell University, Ithaca, NY, 1988.

Received for review May 6, 1993. Revised manuscript received October 5, 1993. Accepted December 27, 1993.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, February 1, 1994.