Comparison of Micro- and Macro- digestion Methods for Fiber Analysis

J.L. HOLECHEK AND M. VAVRA

Abstract

Micro-methods for analyses of neutral detergent fiber (NDF), acid detergent fiber (ADF), and permanganate lignin were compared to the macro-methods of Van Soest (1963) and Van Soest and Wine (1967, 1968). Differences between the two methods were small although the micro-methods gave better precision for ADF while the macro-method gave better precision NDF and lignin. Time and reagents needed for analysis were reduced over 60% with the micro-digestion methods.

In recent years the methods of Van Soest (1963) and Van Soest and Wine (1967, 1968) have become standards for analysis of fiber in forages and ruminant diets. Problems associated with these methods include time required for analysis and investment in equipment. The number of samples that can be digested is severely limited by the number of digestion and refluxing units. Waldern (1971) modified the macro-digestion procedures of Van Soest (1963) and Van Soest and Wine (1967, 1968) into micro-techniques that require less time, fewer chemicals and less equipment. However, he only evaluated the effectiveness of the micro-procedure with forage samples containing low neutral detergent fiber (<56%) and acid detergent lignin (<7%). The objectives of this study were to compare neutral detergent fiber, acid detergent fiber, and permanganate lignin values determined by a modification of the micro-digestion techniques of Waldern (1971) with the macro-digestion techniques of Van Soest (1963) and Van Soest and Wine (1967, 1968) using diet samples from esophageally fistulated cattle.

Methods and Materials

Diet samples were collected in 1976 on mountain range in northeastern Oregon. The botanical composition of these samples was determined using the procedures of Sparks and Malechek (1968). Five diet samples were chosen that represented different forage classes and seasons. In late spring (June 20–July 18) on northeastern Oregon mountain ranges forages are immature and highly digestible. Grasses and forbs are the major diet constituents (Holechek 1980). In late summer (August 15–September 14) diet shifts occur where availability or alternate forages allow. Cattle often consume shrubs in late summer and fall (September 14–October 12). Therefore, diets analyzed were high grass and high forb, late spring; high grass and high shrub, late summer; and high browse, fall. "High" refers to the diet constituents that comprised 40% or more of the total diet. Fifteen sub-samples from each of the 5 selected esophageal fistula samples were digested by the micro-digestion (Waldern 1971) and standard Van Soest (1963) and Van Soest and Wine (1967 1968) digestion procedures. Differences between the two methods were tested using a paired *t*-test according to Steel and Torrie (1960).

Procedure

The procedures of Van Soest (1963) were used as the standard for neutral detergent fiber (NDF) analysis. The procedures of Van Soest (1963) and Wine (1967, 1968) were used as the standards for acid detergent fiber (ADF) and permanganate lignin analyses. Reagents used were those outlined by Van Soest (1963) for NDF and Van Soest and Wine (1967, 1968) for ADF and lignin. The micro-digestion technique had some apparatus and procedural differences from that used by Waldern (1971), and is described a follows:

Apparatus

1) A cylindrical aluminum block with 28 holes.
   a) Block dimensions: 20.32 cm in diameter; 7.62 cm deep.
   b) Hole dimensions: 2.54 cm in diameter, 5.06 cm deep.
2) Chromalox 660 watt hevi-duty heater with rheostat.
3) Marbles, 25.4 mm in diameter to serve as condensers.
4) 25 × 225 mm test tubes.
5) Sintered glass crucibles as described by Van Soest (1963).
6) Vacuum pump for filtration.

NDF Procedure

1) Weight .35g air dried sample which has been ground through a #40 sieve into a test tube.
2) Add 35 ml of NDF solution.
3) Add 1 ml of decalin.
4) Place large marble on top of each test tube.
5) Place tubes in aluminum block and bring to boil gradually; boil at approximately 124°C for one hour. Feed particles collecting above digestion fluid level should be returned to the boiling mass by washing down the sides with a small amount of warm NDF solution.
6) Filter through a previously weighed sintered glass crucible using a light suction.

Authors, at the time of the research, were graduate assistant and professor of range nutrition, Oregon State University, Eastern Oregon Agricultural Research Center, Union 97883. J.L. Holechek is currently assistant professor of range science, Department of Animal and Range Sciences, New Mexico State University, Las Cruces 88003.
This paper is Oregon State Agricultural Experiment Station Technical Paper Number 5552.

Table 1. Comparison of neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin in esophageal fistula samples as obtained by 2 methods.

Diet sample material	NDF		ADF		Lignin	
	Micro	Macro	Micro	Macro	Micro	Macro
High grass, late spring						
Mean	56.72	58.91	39.41	39.26	7.10	6.73
Std. Dev.	2.10	1.68	.61	.92	.31	.42
Coef. Var.	3.70	2.85	1.55	2.34	4.37	6.24
High forb, late spring						
Mean	53.67	54.15	43.52[a]	42.90[b]	9.01	9.10
Std. Dev.	2.29	2.99	.71	.64	.58	.43
Coef. Var.	42.7	5.52	1.53	1.49	6.44	4.73
High grass, late summer						
Mean	67.81	66.10	52.76	53.18	10.93	11.25
Std. Dev.	2.14	3.16	.35	1.25	.51	.38
Coef. Var.	3.16	4.78	.66	2.35	4.67	3.38
High browse, late summer						
Mean	61.91	63.21	53.17	53.17	13.50[a]	14.18[b]
Std. Dev.	3.56	2.10	.87	1.00	.54	.46
Coef. Var.	5.75	3.32	1.64	1.86	4.00	3.24
High browse, fall						
Mean	68.26	67.19	56.89	56.78	10.60[a]	19.33[b]
Std. Dev.	2.12	1.11	.33	.67	.46	.68
Coef. Var.	3.11	1.65	.58	1.18	2.47	3.52

[a,b] Means with different letters are significantly different ($P<.05$).

7) Wash with hot water.
8) Repeat the washing with acetone until no more color is removed.
9) Dry at 100° C and weigh.

ADF Procedure

1) Weigh .35 g air dried sample, which has been ground through a #40 sieve, into a test tube.
2) Add 35 ml of ADF solution.
3) Add 1 ml of decalin.
4) Use procedures 4-8 of NDF procedure.

Lignin Procedure

1) Place crucibles containing dried and weighed ADF in a shallow enamel or glass plan containing 1 cm cold water. ADF in crucibles should not get wet.
2) Fill the crucibles half full with saturated potassium permanganate and buffer solution. Use a short glass rod to break lumps and draw permanganate solution up on sides of crucibles.
3) Allow crucibles to stand at room temperature for 90 minutes. Add more mixed permanganate solution if necessary. Purple color must be present at all times.
4) Remove crucibles to filtering apparatus and one by one, suck them dry.
5) Fill the crucibles again half full with demineralizing solution. With a glass rod move the contents taking care that all feed particles are under the solution and the sides of the crucibles are rid of all color.
6) After 5 minutes suck the crucibles dry and refill halfway with demineralizing solution. Rerinse the sides of the crucibles. Continue the treatment until the filter is white.
7) Fill and thoroughly wash crucibles and contents with 80% ethanol. Suck dry and repeat wash.
8) Wash twice with acetone as with ethanol.
9) Dry at 100° C and weigh.

Results and Discussion

The comparison of means for neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin in Table 1 shows significant differences existed between the two methods in 2, 1 and 2 of the 5 samples used for NDF, ADF and lignin analyses. The largest difference between means was obtained for NDF in a high grass sample. Coefficients of variation were highest for lignin followed by NDF and ADF analyses with both methods. The absolute differences between the two methods for ADF and lignin for a given diet sample were relatively small varying from .48 to 2.19 for NDF, .11 to .62 for ADF, and .10 to .73 for lignin. From a biological standpoint these differences appear relatively unimportant. In 4 of the 5 diet samples, variation in ADF values was higher for the macro-method than for the micro-method. However for NDF and lignin, less variation was associated with the macro-method in 3 of the 5 samples. When the macro-method was used, several NDF and ADF sub-samples presented filtering problems which at times resulted in erratic values and the necessity to rerun the sample. Waldern (1971) worked with samples that ranges from .31 to 7.38 percent acid detergent lignin. Data presented herein covered higher lignin levels that could typically be found in range forage and show the amount of variation associated with permanganate lignin.

In our study we did not run sequential analyses for NDF and ADF. Recent research by Mould and Robbins (1981) indicates that sequential analyses should be used for NDF and ADF if a highly precise determination of these fiber components is desired for browse. They also found that fiber partitioning for browse species was more accurate when sodium sulfite was left out of the NDF solution. We used sodium sulfite for NDF analyses in our study.

Compared to the macro-method the amount of time required to run 100 samples was reduced 75% with the micro-method because over four times as many samples were run at once, and filtration was faster and presented fewer problems. Another advantage of the micro-method was that a lower volume of solution was required per sample thereby reducing reagent use 65%. Our results may have been altered slightly if NDF and ADF analyses had been conducted sequentially and sodium sulfite had not been used in the NDF solution.

Literature Cited

Holechek, J.L. 1980. The effects of vegetation type and grazing system on performance, diet and intake of yearling cattle. Ph.D. Thesis. Oregon State Univ., Corvallis. 247 p.

Mould, E.D., and C.T. Robbins. 1981. Evaluation of detergent analysis in estimating the nutritional value of browse. J. Wildl. Manage. 45:937:947.

Sparks, D.R., and J.C. Malechek. 1968. Estimating percentage dry weight in diets using a microscopic technique. J. Range Manage. 2:264-265.

Steel, R.G., and J.H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc. New York. 532 p.

Van Soest, P.J. 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J. Ass. Offic. Anal. Chem. 46:829-835.

Van Soest, P.J., and R.H. Wine. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. J. Ass. Offic. Anal. Chem. 50:50-56.

Van Soest, P.J., and R.H. Wine. 1968. The determination of lignin and cellulose in acid detergent fiber with permanganate. J. Ass. Offic. Anal. Chem. 51:780-787.

Waldern, D.E. 1971. A rapid micro-digestion procedure for neutral and acid detergent fiber: Can. J. Anim. Sci. 51:67-69.