Effect of drying method on the nutritive composition of esophageal fistula forage samples: influence of maturity

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Abstract

Researchers continue to oven- and air-dry extrusa samples from esophageally fistulated animals, despite evidence that this practice leads to erroneous nutritional evaluations. Two studies were conducted to determine how method of drying affects the nutritional composition of esophageal extrusa collected from sheep and goats browsing forage at different stages of maturity. In trial 1, extrusa collected from free-ranging sheep and goats in Northeast Brazil from July to April was freeze- or oven-dried (40° C) and analyzed for neutral detergent fiber (NDF) and lignin (L). NDF and L were artificially elevated (31–93%) in oven-dried extrusa collected from January to April when forage was immature. No differences were observed in the fiber content of oven- and freeze-dried extrusa collected during the dry season (July–December). In trial 2, *Dactylis glomerata, Medicago sativa, Acer grandidentatum* and *Purshia tridentata* were hand-harvested and fed to esophageally fistulated sheep. Extrusa was either freeze-, air- or oven dried (40° C) and analyzed for hemicellulose, cellulose, L and in vitro organic matter digestibility (IVOMD). Hemicellulose and L concentrations were significantly increased in air- and oven-dried forage for all plant species. Cellulose was least affected by method of drying. IVOMD was depressed most by oven- and air-drying in species containing phenolic compounds. When there were significant treatment by period interactions, method of drying was most critical early in the growing season. Results reemphasize that freeze-drying extrusa is an important preliminary step to obtain accurate nutritional information.

Key Words: esophageal extrusa, drying method, maturity, fiber, in vitro digestibility

Esophageally fistulated animals are commonly used to monitor the nutrient composition of diets from free-ranging livestock. Extrusa (i.e., forage collected via an esophageal fistula) is frequently oven- or air-dried prior to nutritional analyses (McCollum et al. 1985, Judkins et al. 1985, Rosiere and Vaughn 1986, Long et al. 1986, Holechek et al. 1987, Adams et al. 1987, Hakkila et al. 1987) despite reports that oven- or air-drying elevates fiber content and depresses in vitro digestibility of extrusa (Acosta and Kothmann 1978, Engels et al. 1981, Smith et al. 1967, Lesperance et al. 1974). Drying extrusa even at low temperatures (25–40° C) may cause losses in organic matter (Acosta and Kothmann 1978) and/or result in nonenzymatic browning, a chemical reaction involving the condensation of sugar residues and amino acids followed by polymerization to form a brown complex containing 11% nitrogen with physical properties similar to lignin. Unsaturated oils and phenolic compounds, such as tannins, may copolymerize in the reaction. The resulting substance has been called artifact lignin (Van Soest 1965). The alternative to oven- or air-drying is freeze-drying, which reportedly causes the smallest degree of change in the fiber fractions and digestibility of extrusa (Lesperance and Bohman 1964).

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Studies of the effects of drying method on the nutritional quality of extrusa have been limited to forages consumed at a single stage of maturity and to non-woody species containing few or no phenolic compounds. Method of drying may be more important at certain times of the year and when extrusa contains forages high in phenolic compounds. This study determined how drying method affected the chemical composition of extrusa collected throughout the growing season, and extrusa high in phenolic compounds.

Materials and Methods

Trial 1

The study site was located at the Brazilian National Goat Research Center 10 km from Sobral, Ceará state, Brazil. The climate in this portion of Brazil is characterized by distinct wet and dry seasons. The dry season typically extends from June to December, and the wet season from January to May. The vegetation of this area, called *caatinga,* is a heterogeneous mix of deciduous trees and shrubs with an herbaceous understory. Samples of the native vegetation were collected at approximately monthly intervals from July to April using 16 esophageally fistulated sheep and goats. Each sample collected was mixed thoroughly and divided into 2 portions: half was oven-dried at 40° C immediately at the study site and the other half was frozen at –20° C and stored for 12 months prior to freeze-drying. The oven-dried portion was analyzed at the National Goat Center for neutral detergent fiber (NDF) and lignin (Goering and Van Soest 1970). The frozen portion was packed in dry ice and flown to Utah State University, freeze-dried, and analyzed for NDF and lignin by the same procedures.

Trial 2

Orchardgrass *(Dactylis glomerata),* alfalfa *(Medicago sativa),* maple *(Acer grandidentatum),* and bitterbrush *(Purshia tridentata)* were hand-harvested at various stages of maturity (Table 1) at

Table 1. Date of collection and phenological description of forages fed to esophageally fistulated sheep.

Species	Collection Date	Phenological Description
Orchardgrass	May 13	Early boot
	June 6	Dough
	July 18	Vegetative regrowth
	August 27	Mature and dry
Alfalfa	May 14	Vegetative
	June 19	Full-bloom
	July 17	Dough
	October 4	Mature
Bitterbrush	June 4	Stems growing
	July 4	Stems growing
	August 28	Stems mature
	October 5	Leaves senescent
Maple	June 1	Young leaves, fully developed
	July 2	Mature leaves
	August 27	Mature leaves
	September 25	Leaves senescent
	October 3	Fallen leaves

Utah State University's Green Canyon research facility, Logan, Utah. Bitterbrush and maple were selected because they are known to contain phenolic compounds (Burritt et al. 1987). Orchardgrass and alfalfa leaves and stems were clipped, divided into thirds, and fed individually to 3 esophageally fistulated sheep. Bitterbrush and maple branches were harvested, suspended from a fence, and fistulated sheep were allowed to selectively browse the foliage. Upon collection of extrusa, a sample from a particular animal was divided into thirds and randomly assigned to 1 of 3 drying treatments: air-dry, oven-dry (40° C), and freeze-dry. Subsamples to be freeze-dried were frozen at –4° C and stored for 1 to 3 months prior to freeze-drying. Oven- and air-dried samples were dried immediately. Samples were normally dry within 24 hours for all drying treatments. After drying, samples were ground to pass a 1-mm screen and analyzed sequentially for hemicellulose, cellulose, and lignin (Goering and Van Soest 1970) and in vitro organic matter digestibility (IVOMD) (Goto and Minson 1977). Detergent fiber hemicellulose and cellulose were used rather than NDF or acid detergent fiber in order to observe which cell wall constituents were altered as a result of drying method.

Data from trial 1 were analyzed using a randomized block design; numbers of blocks (sheep and goats) varied from 9 to 16 per period. For trial 2, data were analyzed using a randomized block design with 3 blocks (animals). Drying method was the main effect and stage of maturity was the subplot in both analyses. Levels of significance between treatment means was 0.05, using least significant differences (LSD) (Neter and Wasserman 1974).

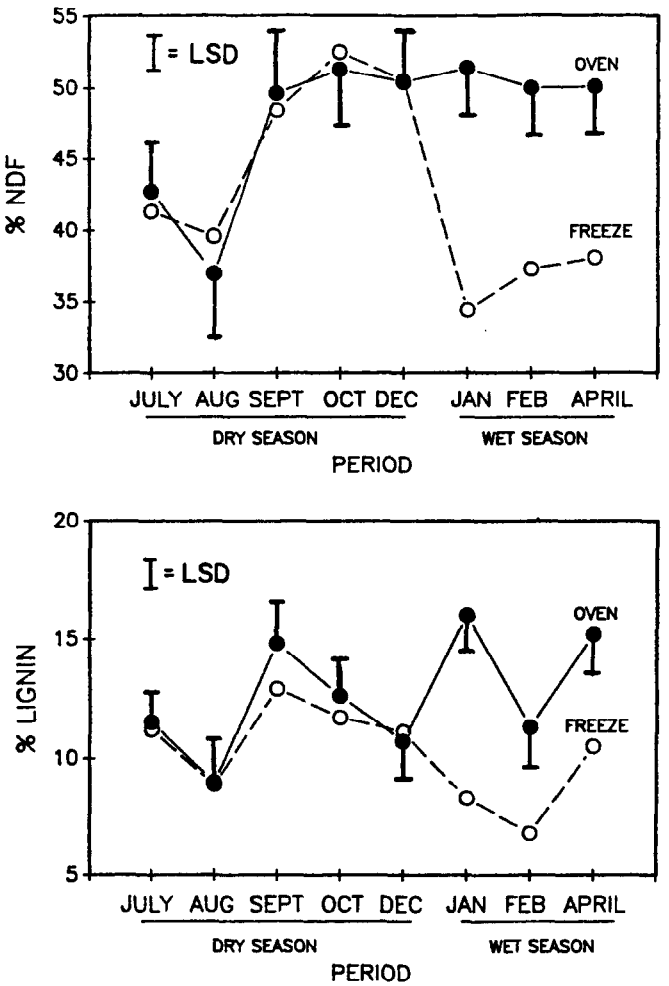

Fig. 1. *Effect of oven (40° C) and freeze- drying on the neutral detergent fiber (NDF) and lignin (dry matter basis) content of extrusa from esophageally fistulated sheep and goats browsing* caatinga *vegetation in Northeast Brazil.*

Results

Trial 1

Method of drying had no effect on the NDF or lignin composition of extrusa collected during the dry season (July to December) (Fig. 1). However, both NDF and lignin were elevated ($P<.05$) in the extrusa collected during the wet season (January to April). This increase due to oven-drying was so extreme that extrusa collected during the wet season appeared to contain more NDF than extrusa collected during the early dry season. Oven-dried extrusa collected in January had the highest lignin value, even though forage collected at this time of the year was actively growing and should have contained the lowest levels of structural components.

While biases may exist in these data due to between-laboratory variation, we feel the data are accurate because of the similarities between the dry season values. At the time of this study, our laboratory in Brazil had no freeze-drier. Obtaining permission to transport samples between Brazil and the United States is extremely difficult because Brazil is a foot-and-mouth area. This factor impeded analyzing samples from different drying methods in the same laboratory.

Trial 2

Extrusa hemicellulose content increased ($P<.05$) in all species due to method of drying (Table 2). In addition, there was a significant treatment by period interaction for maple and orchardgrass.

Table 2. Effect of freeze-, air- and oven-drying (40° C) on the hemicellulose (dry matter basis) content of extrusa collected from sheep.

	Drying Method		
Species	Freeze	Air	Oven
Orchardgrass			
May	20.1a[1]	24.0b	24.8b
June	26.6a	27.1ab	30.0b
July	22.7a	29.4b	30.8b
August	26.3a	27.1a	27.0a
Mean	23.9	26.9	28.2
Alfalfa[2]			
Mean	10.1a	11.3b	12.3c
Bitterbrush[2]			
Mean	10.1a	11.1b	11.5b
Maple			
June	11.1a	13.6b	14.4c
July	10.6a	12.1b	13.0c
August	8.9a	9.8b	10.1b
September	9.3a	9.6a	9.7a
October	9.1a	9.4a	9.4a
Mean	9.8	10.8	11.3

[1]Row means with different letters differ ($P<0.05$).
[2]No treatment by period interaction. Mean value is the average over all collection periods.

Early in the season, oven-dried maple and orchardgrass extrusa contained more hemicellulose than freeze-dried extrusa, but these differences diminished over time.

Freeze-dried extrusa contained less cellulose than oven- or air-dried samples for orchardgrass, alfalfa, and bitterbrush (Table 3). There were no differences in the cellulose content of maple. There were no treatment by period interactions for cellulose.

Percentage lignin was higher in oven- and air-dried samples than in freeze-dried samples (Table 4). The average lignin content of oven-dried bitterbrush extrusa was 39% higher than freeze-dried bitterbrush extrusa. There was a significant treatment by period interaction for grass extrusa. Air- and oven-drying elevated the lignin content of orchardgrass vegetative regrowth 100% and 147%, respectively.

Air- and oven-drying depressed IVOMD ($P<.05$) in all species

Table 3. Effect of freeze-, air- and oven-drying (40° C) on the cellulose (dry matter basis) content of extrusa collected from sheep.

Species[1]	Drying Method		
	Freeze	Air	Oven
Orchardgrass	26.3a[2]	26.8b	27.4c
Alfalfa	20.8a	21.4ab	21.6b
Bitterbrush	19.1a	19.6b	20.0b
Maple	15.1a	15.1a	15.2a

[1]No treatment by period interactions for any species. Mean value is the average over all collection periods.
[2]Row means with different letters differ ($P<0.05$).

Table 4. Effect of freeze-, air- and oven-drying (40° C) on the lignin (dry matter basis) content of extrusa collected from sheep.

Species	Drying Method		
	Freeze	Air	Oven
Orchardgrass			
May	3.8a[1]	4.5a	4.7a
June	5.7a	6.3ab	7.2b
July	3.4a	6.9b	8.4c
August	8.0a	8.3a	8.6a
Mean	5.2	6.5	7.2
Alfalfa[2]			
Mean	7.6a	8.0b	8.2b
Bitterbrush[2]			
Mean	7.4a	9.8b	10.3b
Maple[2]			
Mean	7.6a	7.8ab	7.9b

[1]Row means with different letters differ ($P<0.05$).
[2]No treatment by period interaction. Mean value is the average over all collection periods.

Table 5. Effect of freeze-, air- and oven-drying (40° C) on the IVOMD of extrusa collected from sheep.

Species	Drying Method		
	Freeze	Air	Oven
Orchardgrass[1]			
Mean	58.9a[2]	56.2b	55.6c
Alfalfa[1]			
Mean	65.8a	65.6a	65.0a
Bitterbrush[1]			
Mean	58.7a	51.3b	49.7c
Maple			
June	71.5a	60.0b	59.9c
July	61.4a	55.2b	51.2c
August	63.1a	57.0b	56.7b
September	61.3a	57.0b	54.6c
October	59.0a	57.2a	56.0b
Mean	63.3	57.3	55.7

[1]No treatment by period interaction. Mean value is the average over all collection periods.
[2]Row means with different letters differ ($P<0.05$).

but alfalfa (Table 5). There was a significant treatment by period interaction for maple. Trends in the IVOMD of maple were similar to the other constituents, immature samples were more affected by method of drying than mature samples. It also appears that extrusa containing phenolic compounds (maple and bitterbrush) are more susceptible to changes in IVOMD due to method of drying than either grass or alfalfa extrusa. IVOMD values for maple and bitterbrush were depressed 12 to 15% by oven-drying while orchardgrass IVOMD was depressed 6%.

Air-drying was not superior to oven-drying. Although air-drying frequently resulted in lower fiber and higher digestibility values than oven-drying, data from air-dried samples still differed significantly from data obtained from freeze-dried samples.

Discussion

Differences between drying methods are probably a result of nonenzymatic browning of extrusa during the oven- and air-drying processes. Van Soest (1982) reported that oven-drying below 60° C is relatively safe although small amounts of artifact lignin may still be produced. Grant and Campbell (1978), however, reported that oven-drying at 45° C increased lignin and acid detergent fiber and decreased digestibility in the species they studied. Changes due to drying method may be even more extreme when extrusa is dried. Other researchers (Acosta and Kothmann 1978, Lesperance and Bohman 1964) compared the effects of oven- and freeze-drying on the chemical composition of extrusa and hand-clipped forage and found that changes were greatest in extrusa samples. Our study indicates that large differences in nutritive composition may occur even when extrusa is dried at low temperatures (40° C or air-dried). Extrusa samples are typically saturated with saliva, resulting in increased moisture and pH and therefore increasing nonenzymatic browning of extrusa during oven- or air-drying (Van Soest 1965, Goering et al. 1973, Hodge 1953). Acosta and Kothmann (1978) speculated that changes due to oven- or air-drying are due to losses in organic matter. However, organic matter losses were small (2–4%) or nonexistent in our oven- and air-dried samples and could not account for the changes observed in our study.

Van Soest (1965) reported that nonenzymatic browning increased the lignin content of forage. However, in our study, changes occurred in all fractions, depending upon species and stage of maturity. Hemicellulose content increased with drying temperature. Jones and Bailey (1972) reported that oven-drying could denature protein in plant material and render it insoluble in hot neutral detergent. The digestibility of extrusa was also depressed. Forages containing phenolic compounds (bitterbrush and maple) were the most susceptible. This could be due to proteins complexing with tannin as well as carbohydrates. Oven- or air-drying may have decreased the solubility of these tannin complexes in vitro. Cellulose was least affected by oven- or air-drying, as noted by Acosta and Kothmann (1978).

Generally, extrusa containing immature forage was the most susceptible to oven or air-drying, probably due to higher concentrations of protein and soluble carbohydrates contained in immature forages. Air- and oven-drying, however, had deleterious effects on the lignin content and IVOMD of bitterbrush throughout the growing season.

Changes due to method of drying in this study were large enough to lead to erroneous conclusions about forage quality. For instance, data from oven-dried extrusa in trial 1 indicated that the fiber content of diets from animals grazing *caatinga* vegetation was highest in January even though forage was immature and actually contained relatively little fiber. The lignin content of oven-dried extrusa containing regrowth or mature orchardgrass was similar, when in reality mature orchardgrass contained 2 1/2 times more lignin than did regrowth. This finding is particularly relevant considering that much of the forage harvested by esophageally fistulated livestock during grazing trials is vegetative regrowth. Finally, the IVOMD of freeze-dried maple extrusa decreased over the growing season but decreased only slightly in oven- or air-dried extrusa. Therefore data from oven-dried material erroneously indicated that the digestibility of maple forage changed very little

during the growing season.

The results of this study indicate that freeze-drying appears to be the only reliable means of sample drying. Freeze-drying is expensive but the equipment is essential for laboratories engaged in grazing research. Results on the nutritive value of forages harvested by esophageally fistulated animals that have not been freeze-dried, are suspect until shown to be accurate.

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