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Inaccuracy of fecal analysis for estimating botanical composition of small ruminants' diets in Northeastern Brazil

by

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Abstract

Accuracy of fecal analysis to estimate botanical composition of a known diet was tested during the rainy season in Northeastern Brazil. Leaves of three highly-palatable plant species, an annual herbaceous vine, and a deciduous and an evergreen tree, were fed in known amounts to a sheep. Fecal analysis proved to be highly inaccurate. No epidermis of the annual vine was found in the fecal material, thus grossly underestimating this species. The technique also failed to accurately represent the browse contribution to the diet.

INTRODUCTION

Determining the botanical composition of diets consumed by grazing livestock has become a fundamental part of grazing research and is being used more frequently in management decisions. Because grazing animals demonstrate a high degree of selectivity, the proportion of plant species found in their diets is rarely similar to the forage available in the vegetation complex (Theurer et al. 1976).

The method of choice for determining diet composition is the esophageal fistula technique (Vavra et al. 1978; Theurer et al. 1976), in which animals themselves select representative samples. In addition to using fistula extrusa for botanical determinations, it can also be used for chemical analyses and to determine *in vitro* digestibility. Moreover, with an estimate of fecal output, *in vitro* digestibility values from extrusa samples can be used in calculating rates of intake. However, some researchers perceive the establishment and maintenance of a flock of esophageally fistulated animals as a major expense and technical limitation, therefore are hesitant to use the approach. Microscopic analysis of fecal materials is often viewed by these researchers as a simple alternative for determining botanical diet composition. Fecal sampling is much simpler than sampling with esophageally fistulated animals. Usually a small part of many defecations is collected, then these subsamples are pooled for final samples (Hansen and Lucich 1980).

These logistical advantages notwithstanding, there are currently many conflicting reports questioning the accuracy of fecal analysis.

Several serious problems with the technique appear to be responsible for the lack of agreement about the accuracy of the technique (Holechek et al. 1982). Differential digestion of plant species throughout the year has been cited as the most serious source of error in fecal analysis (Sanders et al. 1980; Slater and Jones 1971; Smith and Shandruk 1979). In controlled *in vitro* digestion studies of mixes of known species composition, Vavra and Holechek (1980) found grasses to be overestimated while forb and browse species were underestimated due to differential digestion.

Another source of error in fecal analysis is that digestion may cause plant cuticle to fragment, reducing reliability of quantification procedures (Slater and Jones 1971; Smith and Shandruk 1979). Also digestion may reduce the clarity and reliability of plant diagnostic features (Vavra et al. 1978; Johnson and Pearson 1981).

Few studies have compared results from fecal analyses to actual dietary composition in trials where animals were fed diets of known composition. Information for validating fecal analysis is non-existent for the rangelands of northeastern Brazil, where research is currently being conducted on improved methods for sheep and goat production on native *caatinga* rangelands.

Caatinga vegetation is a complex mix of low-growing deciduous trees and shrubs with an understory of annual forbs and grasses. Considering that many range researchers in this area had questioned the use of fecal analysis to examine livestock diets, the objective of this study was to assess the accuracy of the technique for use during the rainy season when plants are immature and growing rapidly.

Presumably, problems caused by differential digestibility would be the most serious at this season.

METHODS

This study was conducted at the Brazilian National Goat and Sheep Research Center located near Sobral, in Ceara' state. Leaves from jitirana peludo, an annual herbaceous vine (Ipomoea spp.), sabia, a deciduous tree (Mimosa caesalpiniaefolia), and jucazeiro, an evergreen tree (Caesalpinia ferrea) were hand-harvested twice daily in early February (1981), about 5 weeks after the onset of the annual rainy season. These three species were chosen because they are highly palatable to sheep and goats in the region; also we desired to compare dissimilar herbaceous and browse species.

Harvested leaves were fed fresh to an indigenous mature wether weighing 25 kg. The experiment was conducted in a shaded open-air 2-x 4-m stall. Daytime temperatures ranged from an average daily high of 33°C at 1500 hours to a low of 22°C at 0700 hours. The animal had free access to water and salt throughout the trial.

A 7-day preliminary feeding period was used to insure that only these three species remained in the animal's digestive tract. This preliminary period was immediately followed by a 3-day trial where intake of the three test species was closely monitored. Twice daily (at 0800 hours and 1430 hours) 700 g of jitirana peludo and 400 g each of sabia and jucazeiro were offered to the animal. Orts were collected and weighed at 0900 hr and 1530 hrs.

The dry matter (DM) content of each of the three species was determined on the third day of the trial by collecting 100 g of fresh

material and immediately transporting it to the laboratory where it was dried at 75°C for 48 hours in a forced-air oven. These DM values were used to calculate daily DM intake (DDMI), after subtracting orts weights. To insure that the high ambient temperature did not influence orts weights during the 3-day trial period, orts were transported to the laboratory, then dried in a forced-air oven before weighing.

Feces were collected fresh on the morning after the 3-day feeding period ended. Feces were immediately frozen at -17°C and stored for a 6-week period. Then the feces were thawed and dried before being ground in a Wiley mill through a 1-mm screen. The mean DDMI of each species over the last 2 days of the trial was used to compare with results of the fecal analysis, considering that sheep on roughage diets have a retention time for digestion of about 50 hours (Van Soest 1982).

Fecal analysis was done using the microhistological technique (Sparks and Malechek 1968). Reference microslides of each species and microslides of fecal material were prepared by modifying the technique of Smith and Shandruk (1979). Approximately 1 gram of ground material was placed in a 100 ml beaker and covered with a 10% (v:v) solution of nitric acid. After allowing this mixture to stand for 12 hours at 20°C, it was brought to a boil over an alcohol burner and allowed to boil for about 30 seconds. After cooling, the mixture was deacidified by adding an equal volume of 10% (w:v) sodium hydroxide. The sample was then rinsed briefly with distilled water over a 200-mesh screen. The material was returned to the beaker, covered with ethanol, and

gently stirred for about 1 minute. It was again transferred to the 200-mesh screen to remove the alcohol, then replaced in the beaker and stained for 2 minutes with crystal violet. Excess stain was removed by repetition of the screening procedure. A small portion of the material was placed on a microslide and a coverslip affixed using Karo syrup as a mounting medium. The slides were dried for 3 days in a forced-air oven at 60°C.

Quantification of the botanical composition of fecal samples followed Hansen (1971). Two sets of five microslides were made from each sample. At 100x magnification, twenty fields were systematically located on each slide. Slides were read by an independent researcher who did not know the composition of the ingested diet, beyond knowing the three plant species included. Plant fragments were recorded as present or absent in each field (i.e. frequency); the total number of fields per set of slides equaled 100.

Dry weight percentages were calculated for each species using the procedures of Sparks and Malechek (1968). After percent frequency was calculated, particle density was determined and converted to relative density. Relative density expressed as a percent was used as a direct estimate of the percent dry weight of each species in the known diet.

RESULTS AND DISCUSSION

Fecal analysis results are compared with the actual diet composition in Table 1. No epidermis of jitirana peludo was found in the fecal material. Numerous solitary trichomes survived the digestive process, however presence of jitirana based on trichome fragments was not recorded, for several reasons.

First, the trichomes of jitirana were never attached to recognizable epidermal tissue. Occasionally trichomes would be attached to an undistinguishable clump of cells at the base. Secondly, the trichomes of jitirana are a very common unicellular type, thereby greatly reducing their reliability as a diagnostic feature when detached from the epidermis. One could conceivably count the presence of very unusual or unique solitary trichomes of certain plant species, but this was not the case here. Of the three species used, jitirana was the only species with trichomes; however, in a diet study involving many caatinga plant species, numerous other plant species would have similar unicellular trichomes which are easily detached. Thirdly, easily detached trichomes of pubescent plant species, if recorded as definitive evidence for the presence of a species, can lead to overestimation of that species and subsequent underestimation of other species (Sanders et al. 1980). Detached trichomes, when recorded in the absence of diagnostic epidermal tissue, may result in frequencies of 100% for pubescent plants like jitirana.

Jitirana was very succulent during this period of active growth (12% dry matter) and apparently the epidermis was destroyed in the digestive tract. Slater and Jones (1971) reported similar results with ladino clover. When the preparatory treatment with nitric acid was applied to fresh jitirana leaves (to prepare reference slides), no epidermal destruction was noted, thus we discounted the possibility that slide preparation affected results.

Cutinized epidermis, which is characteristic of perennial plant species, reportedly passes virtually intact through the digestive tract (Regal 1960; Hanna et al. 1973; Chatterton and Powell 1974). However, annual plant species with relatively low levels of cutin and lignin in the epidermis are more likely to disappear (Smith and Shandruk 1979; Vavra et al. 1978; Vavra and Holechek 1980; Slater and Jones 1971; Storr 1961; Regal 1960). Chemical analysis revealed relatively lower cutin levels (4.0% of dry matter) for jitirana as compared to the other two species (6-8%) (Pfister, unpublished data).

Sabia was grossly overestimated while jucazeiro was underestimated by about 25% (Table 1). While the epidermis of jitirana was easily distinguishable from sabia and jucazeiro, considerable difficulty was encountered at times in separating sabia and jucazeiro. Each species has several characteristic cell patterns, only one of which in each species was dissimilar enough to be reliable in separating the two plants.

Sabiá may have been overestimated because of its relatively higher degree of lignification (17%) and cutinization (8.0%) (Pfister, unpublished data). Much larger amounts of recognizable sabia probably survived the digestive process and were identifiable in the feces. Also the epidermis of sabia may have fragmented, leading to overestimation. Slater and Jones (1971) reported excessive fragmentation of tropical legumes after digestion.

CONCLUSIONS

Our results indicate potentially serious problems in using fecal analysis with wet season caatinga forages. Although fecal analysis

has proven accurate with perennial species, especially grasses (Smith and Shandruk 1979; Scotcher 1979), the method must be used with caution in the caatinga where grasses are a prominent dietary component for only one or two months of the year. In Africa, investigators do not attempt to use fecal analysis to identify dicots because of limitations of the technique (Scotcher 1979).

Presently the fecal analysis technique appeals to many range researchers in northeastern Brazil and elsewhere because of the ease of collecting samples and the desire to avoid caring for esophageally fistulated animals. However, fecal analysis provides no shortcuts. Problems with the technique must be recognized and dealt with, and competent technicians trained in the very tedious procedure before fecal analysis can become an accurate range research tool.

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Table 1. Results of fecal analysis compared to actual dry matter diet composition.

Plant Species	Fecal estimation (%) Set A	Set B	Actual Diet Composition (%)
Jitirana peludo	0.0	0.0	22.8
Sabia	69.8	72.4	38.3
Jucazeiro	30.2	27.6	38.9