Estimating digestibility of oak browse diets for goats by in vitro techniques

ANASTASIOS S. NASTIS AND JOHN C. MALECHEK

Abstract

Predicting digestibility of shrubs is important to evaluating many of the world's rangelands. We examined laboratory procedures for predicting in vivo digestion of browse-alfalfa *(Medicago sativa)* mixed diets and how drying temperature and inoculum source affect digestibility. In addition, we considered the effect of oak tannin on pepsin activity and dry matter digestion. The commonly used Tilley and Terry (1963) two-stage in vitro digestion technique was a precise (r^2=0.97) but inaccurate predictor of in vivo apparent digestibility of mixed oak *(Quercus gambelii)* and alfalfa diets for goats. The Van Soest et al. (1966) neutral detergent method for predicting true digestibility was less precise (r^2=0.76). Estimates from the Goering and Van Soest (1970) summative equation were not correlated ($P \leq 0.05$) with in vivo digestion. Separate regression equations are necessary if in vitro methods are to predict accurately in vivo digestibility of browse diets. In vitro digestibility was inversely related to percentage of oak in the diets and the amount of oak in the inoculum donors' diets. High drying temperatures depressed digestibility of oak browse and this effect was greater for immature than for mature forage.

Key Words: in vitro digestibility, *Capra hircus, Quercus gambelii,* tannins

Digestion trials are important in determining the nutritional value of a forage. However, the time and expense involved in collecting sufficient forage to feed animals required for in vivo trials limits their application. Laboratory procedures requiring only small samples have frequently been used as alternatives (Pearson 1970). These include in vitro methods of estimating apparent (Tilley and Terry 1963, Barnes 1967) or true (Van Soest et al. 1966) digestibility. The summative equation of Goering and Van Soest (1970), which is based on chemically partitioned forage fractions, is another potential technique for indirectly estimating digestibility.

These techniques, when applied to conventional forages and within relatively narrow and well-defined boundaries of variation, have proven to be accurate and inexpensive. However, use of indirect methods to estimate in vivo digestibility of mature shrub species has been problematic (Short et al. 1974, Urness et al. 1977, Mould and Robbins 1981).

Secondary chemical compounds such as tannins may also complicate prediction of in vivo digestion (Burns et al. 1972, McLeod 1974). They can affect apparent digestibility of protein (Robbins et al. 1987) as well as microbial and enzyme activity (Tagari et al. 1965). The source of inoculum used in in vitro fermentations can potentially introduce yet another factor of variation (Knipfel and Troelsen 1966, Calder 1970, Milchunas and Baker 1982).

It is important that microdigestions be compared to standard in vivo digestion trials using the same or similar forage before results can be generally useful. We compare in vivo digestibility of oak-containing diets by goats *(Capra hircus)* with estimates derived from 3 microdigestion techniques. Effects of drying temperatures and composition of inoculum donors' diets on in vitro fermentation and the effect of oak tannin on pepsin activity and digestion are also reported.

Authors are with the Dept. of Range Wildlife Sci. 236, Univ. of Thessaloniki, Greece, and Dept. of Range Sci., Utah State University.

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Materials and Methods

Oak *(Quercus gambelii)* was collected in June, during the season of rapid growth, and in August, after twig elongation had ceased and stems had hardened. Oak browse included only terminal portions (up to 15 cm in length) of the current year's growth and was hand-plucked from various positions in the tree canopies. From the June collection a diet was formulated containing 80% oak and 20% alfalfa *(Medicago sativa).* From the later collection 4 diets were formulated containing 20, 40, 60, and 80% oak with alfalfa making up the complement. A pure alfalfa diet was used as the experimental control.

In vitro dry matter digestibility of these 6 diets was measured using techniques described by Tilley and Terry (1963) (hereafter called IVAP), Van Soest et al. (1966) (hereafter called IVND), and Goering and Van Soest (1970) (hereafter called (SUEQ). The Tilley and Terry technique was modified by terminating fermentation with HCl instead of HgCl.

Inoculum for in vitro digestion trials was obtained by vacuum aspiration of rumen fluid from 2 ruminally fistulated goats (donor animals) fed the test diets. The donor animals were fed the 6 diets in sequence beginning with pure alfalfa (control) and proceeding in order of increasing oak content. The 80% oak diet made from browse collected in June was fed last. Donor animals were allowed at least 5 days to adjust to each new diet before rumen fluid was collected for in vitro trials. Rumen inoculum corresponding to a specific test diet was termed a *source* of inoculum. For each source, all 6 diets were fermented in duplicate for each of the 2 in vitro techniques. The rumen fluid obtained from the 2 donors at any particular collection was aggregated and handled according to the procedures outlined by Tilley and Terry (1963).

We determined the effect of drying temperature upon in vitro digestion in a completely random experiment. Samples of oak browse were hand harvested at monthly intervals throughout the growing season and subsamples of these materials were freeze-dried at -2° C, air-dried at 0° C and 25° C, and oven-dried at 55° C, 65° C, and 100° C. Subsamples of the material were then subjected to in vitro digestion using the IVAP procedure.

In addition, tannin content of the diets (expressed as tannic acid equivalent) was determined by the spectrophotometric method of Burns (1963); Folin Denis reagent was used. To determine whether tannins contained in oak had any effect on activity of pepsin used in the second stage of the IVAP procedure, an enzyme activity test was conducted according to the methods of Tang (1970). Each of the 6 diets was prepared for the test by refluxing a 0.5-g sample in 100 ml of distilled hot water for 6 hrs. After cooling, the mixture was centrifuged for 10 min at 500 G to remove suspended solids. Then, 50 ml of the supernatant was transferred to a 250 ml Erlenmeyer flask and 2 ml of pepsin solution (concentrations 5%, W/V) was added. From this point, the steps outlined by Tang (1970) were followed. Results are reported as the quantity (micromols) of diliodotyrosine liberated per minute (Ryle 1970) from N-Acetylphenylalanyl-l-diliodotyrosine (APD) by pepsin under the conditions of the assay.

Results from experiments on the 3 microdigestion techniques were analyzed by regression procedures. In vitro values were regressed on corresponding in vivo digestibility values determined

in a related digestion balance experiment (Nastis and Malechek 1981). Regression procedures were also used to relate oak content of diets to their in vitro and in vivo digestibilities. Results of the enzyme test, the inoculum source effect, and the drying-temperature tests were evaluated statistically by analysis of variance procedures. Where significant effects were found by analysis of variance, an LSD test (Steel and Torrie 1980) was applied to isolate specific differences between means at the 0.05 probability level.

Results and Discussion

Diet Composition and Digestibility

Digestibility of diets that contain mature browse is typically low (Short et al. 1972, Urness et al. 1975). Nastis and Malechek (1981) reported 46.7% apparent digestibility for the mature browse used in this study. When such material is added to an alfalfa diet in a progressive fashion as in this study, the orderly decline of digestibility that we observed (Fig. 1) might be expected. However, the

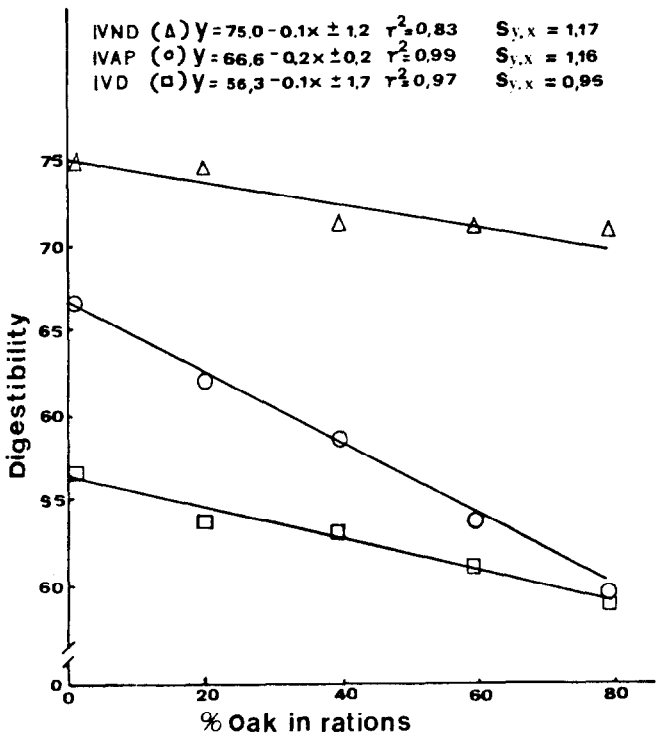

Fig. 1. *Relations of IVAP digestibility (Y), IVND digestibility (Y) and in vivo digestibility (Y) by goats (IVD) to oak foliage content of experimental diets (X).*

explanation for the decline, especially in an in vitro system and when oak is the browse component, is not obvious. Some of the decline is undoubtedly due to the effect of poorly digestible components (Nastis and Malechek 1981). Another factor is the influence of tannins. Tannins have the property of precipiting proteins from aqueous media (Swain 1979, Robbins et al. 1987). They form complexes with both plant and animal proteins which are insoluble or variably soluble (McLeod 1974, Rosenthal and Janzen 1979, Robbins et al. 1987). Tannin precipitated proteins, though, are digested in vivo up to 88% as reported by Robbins et al. (1987). This shows that the over-all digestion is only slightly affected by the reduced protein digestion.

Oak tannin reduced pepsin activity (Table 1) as oak content of the diet increased. However, reduction in enzyme activity was no different in the two 80% oak diets, although the diets differed in tannin content. This may indicate that tannins from mature oak foliage are more effective in reducing enzyme activity than tannins from immature oak. In the present experiment, mature oak browse

Table 1. Pepsin activity as influenced by oak tannin content of diets. Enzyme concentrations and amounts of forage were similar to those in the Tilley and Terry (1963) in vitro procedure.

Diets		Tannin	Quantity of substrate
Oak	Alfalfa	content%	liberated (μmol/min)
00	100	1.0	13.0 ± 0.2a [1]
20	80	2.4	12.9 ± 1.0ab
40	60	3.2	10.4 ± 1.5bc
60	40	4.8	7.9 ± 0.9cd
80	20	6.9	5.6 ± 1.8d
80[2]	20	8.9	8.8 ± 1.4d

[1]Quantity of diiodotyrosine liberated from the substrate (±95% confidence intervals) followed by common letters are not significant by $P \leq 0.05$ different.
[2]Oak collected in June during the season of rapid growth. Oak of the other diets was collected in August after elongation had ceased and stems had hardened.

contained less fiber than did alfalfa (31.7% vs. 39.6% ADF) but considerably more tannin (8.7% vs. 1.0%) (Table 1) and its digestibility was significantly lower (Fig. 2). Similarly, Burns et al. (1972) reported results for Serica lespedeza *(Lespedeza cuneata),* which is

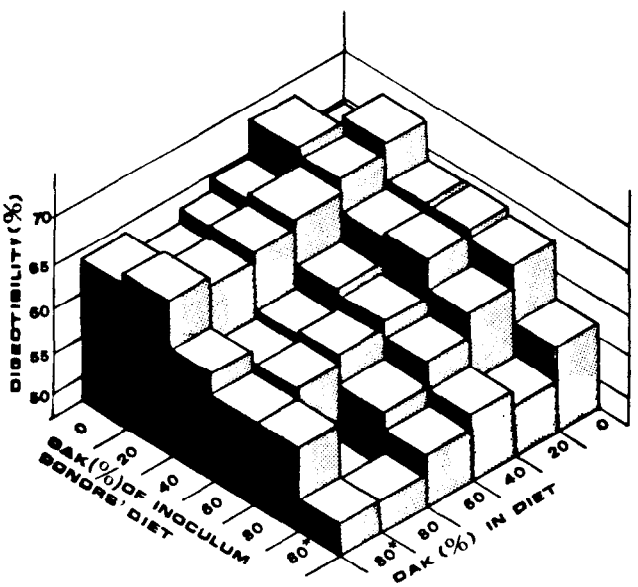

Fig. 2. *In vitro digestibility (%) of an alfalfa diet and 5 oak-containing diets in relation to composition of the inoculum donors' diets. Values are means of estimates from the Tilley and Terry (1963) method and the Van Soest et al. (1966) method. (*) Oak collected in June during the season of rapid growth. Oak in the other diets was collected in August after elongation had ceased and stems had hardened.*

similar to alfalfa in fiber content but contains almost 3 times as much tannin and was 14.2 digestibility units less digestible. The tannins' inhibitory properties might be involved in our estimates of digestibility. Tagari et al. (1965) also reported that tannins act as inhibitors affecting microbial fermentation and/or enzymatic proteolysis.

In vitro techniques generally either underestimate in vivo digestibility of browse forage (Urness et al. 1977, Sidahmed et al. 1981) or provide close estimates (Newman and McLeod 1973). But when browse high in tannins and related phenolic compounds is tested, in vivo digestion is likely to be overestimated by in vitro methods (Fig. 3) because of increased fecal excretion of protein. In a related study (Nastis and Malechek 1981), we observed that increasing levels of oak in goats' diets led to elevated levels of fecal nitrogen excretion and progressively lower levels of apparent digestion of cellular constituents. The overestimation of in in vivo digestibility

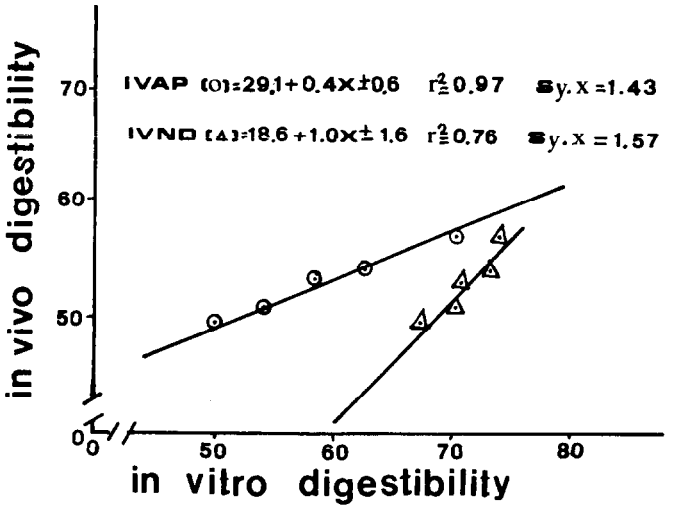

Fig. 3. *Relation of digestibility determined by the in vitro acid pepsin method (IVAP) and the in vitro neutral detergent method (IVND) to in vivo digestibility.*

can be attributed mainly to the low in vivo digestion of cell contents. Maximum in vivo digestibility of oak browse cell contents (Nastis 1977) was 74.8±2.0% as compared to 98±2.5% assumed for conventional forages by Van Soest (1967).

Comparison of In vitro Techniques

Regression analysis relating the 2 in vitro techniques to in vivo digestion (Fig. 3) indicated that the IVAP method was more precise, (r^2=0.97, Sy.x=1.43) than the IVND procedure (r^2=0.76, Sy.x=1.57). Results using the SUEQ were not significantly correlated with in vivo digestion ($P \leq 0.05$). Averaged over all inoculum sources and diets, the mean estimate provided by IVND was 71.0% and 56.5% for IVAP, compared to a mean of 52.9% for in vivo trials.

The discrepancy between the 2 in vitro techniques can be explained partly in terms of differences in their principles. The IVAP approach provides estimates of apparent digestibility (Tilley and Terry 1963) whereas the IVND procedure estimates true digestibility (Van Soest et al. 1966).

Our estimates of digestibility derived from the summative equation (Goering and Van Soest 1970) were not significantly ($P \leq 0.05$) correlated with in vivo digestion.

Two points should be kept in mind when rationalizing the utility of in vitro techniques for estimating in vivo digestibility of oak-containing diets and possibly other diets high in tannin-containing browse. First, the Tilley and Terry (1963) procedure seems to provide precise estimates of digestibility which may not be accurate unless corrected (e.g., Fig. 3). This, of course, means that a limited number of in vivo digestion trials must be conducted.

Secondly, estimates of true digestibility (Van Soest et al. 1966) are valuable in certain contexts, but in the case of diets such as those used in this study, the factors contributing to high excretion of metabolic fecal components and affecting the extent of cell constituents digestion cannot be overlooked. Until these relationships are better understood, in vitro procedures that estimate apparent digestibility would seem to have the greatest utility in determinations of dietary quality.

Inoculum Donors' Diet

Inoculum source had a significant effect on in vivo digestibility with a tendency towards lower values as the composition of donors' diets increased in oak content (Fig. 2). When donors were fed a diet composed of oak collected in June, even lower in vitro digestibility resulted. Moreover, composition of donors' diets and composition of test diets interacted significantly ($P \leq 0.05$). Test diets containing high percentages of oak had lower in vitro digestibility estimates when the inoculum donor's diet contained higher levels of oak than when it contained only alfalfa or small amounts of oak. Figure 2 indicates that digestibility of diets containing less than 60% oak was slightly affected by the donors' diet, while digestibility of diets containing 80% oak was influenced by the inoculum donors' diet.

The interaction of in vitro methods with inoculum sources was also significant. Digestion by IVAP was more affected by inoculum source than was IVND digestion (Table 2). The IVAP seems to

Table 2. In vitro digestibility (%) of dry matter, with inoculum from donors consuming oak-alfalfa diets in varying proportions, by Tilley and Terry (1963) and Van Soest et al. (1966) methods averaged over all diets.

Inoculum donors' diet			
% Oak	% Alfalfa	Tilley and Terry apparent	Van Soest true
00	100	58.8 ± 3.0a[1]	72.1 ± 4.2ab
20	80	57.5 ± 3.0b	73.3 ± 1.6a
40	60	57.8 ± 3.0b	71.8 ± 3.6b
60	40	57.3 ± 2.6b	71.8 ± 1.3b
80	20	55.8 ± 3.1c	70.9 ± 0.9c
80[2]	20	51.6 ± 3.3d	67.0 ± 1.5d
Average		56.5 ± 0.3	71.1 ± 0.3

[1]Digestibility averages (±95% confidence intervals) in a particular column followed by common letters are not significantly different ($P \leq 0.05$).
[2]Oak collected in June during the season of rapid growth. Oak in the other diets was collected in August after elongation had ceased and stems had hardened.

be sensitive to factors affecting the microbial population, such as tannins which most probably covary with nitrogen and fiber content of forage. Results of the enzyme activity test suggested some reduction in pepsin activity owing to compounds extracted from oak-containing diets (Table 1). The IVND, which differs in the second stage from IVAP by including digestion with neutral detergent, seems to dissolve fractions undigested by the IVAP procedure, thus yielding higher digestibility values.

Phenological Stage and Drying Temperature

In vitro digestibility of oak foliage was significantly affected by drying temperatures (Fig. 4). Lower digestibilities were associated with higher drying temperatures. Van Soest (1965) demonstrated that heating in the presence of moisture alters carbohydrate structure and diminishes nitrogen solubility by forming insoluble polymers that are measured in the lignin fraction.

Phenological stage of plant material also caused significant variation in in vitro digestibility. Averages of in vitro digestion across all drying temperatures decreased through the growing season (Fig. 4). Oak collected in June had a higher digestibility ($P \leq 0.05$) than oak collected in July and they were both higher than from oak collected in August and September. However, there were no significant differences between the last 2 sampling periods. Chemical composition of oak through the growing season showed a gradual decrease in crude protein from 20% to 13%, an increase in acid detergent fiber from 29% to 36%, and a slight increase in lignin content from 10% to 11%. These chemical changes probably contributed to the decrease in digestibility of oak over the range of phenological stages.

Interactions between phenological stage and drying temperature (Fig. 4) were also significant ($P \leq 0.05$). High temperature depressed IVAP digestibility to a greater extent in early-harvested material than in late-harvested material. This is probably related to the higher concentrations of moisture, soluble carbohydrates, and nitrogen-containing compounds in young foliage, which upon heating, provide substrates for formation of the insoluble, dark-colored polymers discussed by Van Soest (1965) and Raguse and Smith (1965). Findings from this experiment suggest that results of in vitro digestibility assays on oak browse diets may be comprom-

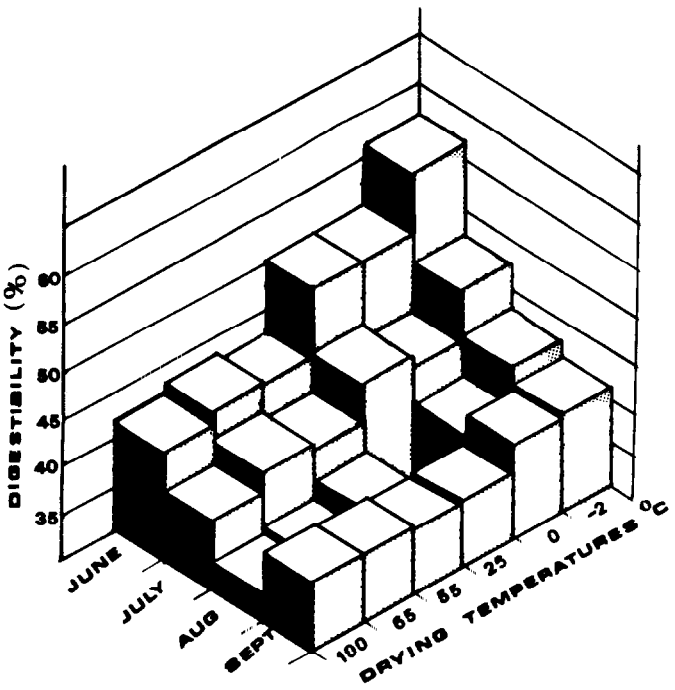

Fig. 4. *Effects of drying temperature and stage of maturity on oak foliage digestion determined by the Tilley and Terry (1963) in vitro method.*

ised by any drying procedure other than freeze-drying.

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