Constituents of In Vitro Solution Contribute Differently to Dry Matter Digestibility of Deer Food Species

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Highlight: *This study assessed the contribution of chemical constituents used in the in vitro technique by Tilley and Terry on digestibilities of five species of plants. Apparent digestibility was lowest, 28–29%, for water alone, buffer alone, and buffer plus pepsin. Dry matter loss increased to 32–33% with either buffer + alcohol + HCl or buffer + alcohol + HCl + pepsin. Highest apparent digestibility, 44%, was reached with the addition of white-tailed deer inoculum. HCl contributed significantly to digestion while pepsin did not. Degree of digestion varied among the five species of plants tested.*

Dry matter digestibility of deer food is commonly used to estimate deer range carrying capacity. The in vitro technique of Tilley and Terry (1963) is gaining popularity over the in vivo method; however, little is known about the response of food plants to the individual components of the in vitro technique. Basically, this technique involves digesting plant material in an artificial rumen with a solution comprised of water, buffer, pepsin, alcohol, hydrochloric acid, and rumen fluid. Barnes (1966) evaluated these components by testing dry matter disappearance of grasses and legumes with the following solution combinations: buffer, buffer with pepsin; buffer with rumen fluid; and buffer, rumen fluid, and pepsin. He used a 48-hour digestion period for incubation of the sample with buffer and buffer with rumen fluid, followed by a 24-hour incubation period with pepsin. Dry matter disappearance was 33.2% with buffer alone, 41.8% with buffer and pepsin, 47.9% for buffer and rumen fluid, and 59.7% with the complete solution mixture of buffer, rumen fluid, and pepsin.

Pearson (1970), in a study similar to Barnes' (1966), evaluated three combinations of in vitro solution components for their ability to digest dry matter of grasses and shrubs from the Arizona chaparral. His data showed an average dry matter loss for the buffer alone to be 20.9%, microorganisms digested 21.9%, and the acid-pepsin accounted for 7%.

Neither Barnes (1966) nor Pearson (1970) tested dry matter disappearance of forbs, evergreen grasses, or evergreen shrubs. They did not test with water alone, nor did they separate the acid-pepsin fractions. The

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Table 1. Mean dry-matter digestibilities (%) for five species of plants determined with six treatment solutions.

Solution	Chokecherry	Western yarrow	Roughleaf ricegrass	Cream peavine	Kinnikinnick	Solution mean[1]
Distilled water	22.6	21.2	25.0	26.5	43.1	27.7 a
Buffer	22.7	25.8	22.9	30.4	43.2	29.0 a
Buffer + pepsin	23.4	26.5	24.5	30.0	42.5	29.3 a
Buffer + alcohol + HCl	25.8	26.5	27.0	35.0	47.2	32.3 b
Buffer + alcohol + HCl + pepsin	24.9	29.4	28.9	36.3	47.4	33.4 b
Buffer + alcohol + HCl + pepsin + rumen fluid	38.2	40.2	44.4	47.4	50.2	44.1 c
Species mean[1]	26.3 k	28.3 k	28.8 k	34.2 l	45.6 m	

[1] Means were grouped by Duncan's Multiple Range Test. Means of solutions (columns) and species (rows) followed by common letter designation were not significantly different ($\alpha = 0.05$).

purpose of this study was to determine how digestibility ratings of selected Black Hills plants are affected by various components of the Tilley and Terry (1963) technique.

Study Area and Methods

Plant materials consisting of leaves and stems of five species of plants were collected during November 1970 from the Black Hills Experimental Forest, 50 kilometers west of Rapid City, S. Dak., at an elevation of 1,768 meters. The plant material tested for digestibility consisted of dead herbage from cream peavine (*Lathyrus ochroleucus* Hook) and western yarrow (*Achillea millefolium* L.), and live herbage from roughleaf ricegrass (*Oryzopsis asperfolia* Michx.) and kinnikinnick (*Arctostaphylos uva-ursi* (L.) Spreng.). Stems from dormant chokecherry (*Prunus virginiana* L.) were also tested.

The plant species selected are representative of several major groups of fall foods eaten by deer in the ponderosa pine (*Pinus ponderosa* Laws.) habitat type. Cream peavine is a highly palatable legume while western yarrow is an important forb in the deer diet. Roughleaf ricegrass remains green throughout the fall and winter as does the evergreen prostrate shrub–kinnikinnick–both staple dietary items. Chokecherry twigs are a favorite food of white-tailed deer on the area.

The plant material was air dried, then ground in a Wiley mill to pass through a 1-mm screen mesh size. A 0.5-g sample was placed into a 100-ml centrifuge tube. Each plant species was tested in duplicate, and a minimum of five controls (tubes with no plant material added, but subjected to the complete in vitro procedure) were included in each digestion run.

The six test solutions were as follows:

1. Distilled water only;
2. Buffer solution kept at pH 6.7–6.9 (Tilley and Terry, 1963);
3. Buffer solution with the addition (after 48 hours) of isoamyl alcohol and 2.2 N hydrochloric acid (HCl);
4. Buffer plus the addition of pepsin after 48 hours;
5. Buffer with the addition (after 48 hours) of isoamyl alcohol, 2.2 N HCl, and pepsin; and
6. The complete solution, including the addition of rumen fluid collected from white-tailed deer (*Odocoileus virginianus dacotensis* Goldman and Kellogg).

All samples were maintained at 39°C for the two 48-hour periods. Following incubation, all samples were filtered under vacuum. The filter containers were oven dried and weighed.

The results were subjected to a two-way analysis of variance with repeated measurements (Dixon and Massey, 1969) and Duncan's Multiple-Range Test (Woolf, 1968) with all tests at $\alpha = 0.05$ level.

The weight of residue in the controls was subtracted from residue in containers, and loss of weight during the digestion process reported as dry matter disappearance, or apparent dry matter digestibility.

Results and Discussion

Duncan's Multiple-Range Test separated the results from the six solutions into three groups (Table 1). Dry-matter disappearance for the lowest group (water, buffer, and buffer + pepsin) was 27.7–29.3%. The addition of isoamyl alcohol and hydrochloric acid to the buffer solution alone and to the buffer plus pepsin solution significantly increased ($\alpha = 0.05$) dry matter disappearance to 32.3 and 33.4%, respectively. There was no significant difference between the buffer + HCl and buffer + HCl + pepsin solutions, however, indicating that HCl contributes a significant portion to the total digestibility of forage, while pepsin contributes insignificantly.

Pepsin, which breaks down protein, may have contributed insignificantly because of the low protein values generally found in dormant or dead plants. However, neither kinnikinnick nor roughleaf ricegrass (both green, living, and presumably higher in protein) showed a response with the addition of pepsin.

When the forage samples were inoculated with rumen fluid, following the complete procedure of the in vitro technique, dry-matter loss increased to an average of 44.1%, an increase of 14.8–16.4 percentage points over the first group and 10.7–11.8 percentage points above the second group. These results follow a trend similar to that found by Pearson (1970).

Chokecherry, western yarrow, and roughleaf ricegrass were similar in dry-matter loss among treatments (Table 1). Cream peavine, which had a mean of 34.2% dry-matter loss, was significantly more digestible. Kinnikinnick, however, had the highest dry-matter loss, 45.6%, which may be partially accounted for by its evergreen growth habits. Kinnikinnick was significantly more digestible than all other species of plants. There were no significant treatment-species interactions.

Of major importance is the relatively high dry-matter disappearance of all the plant groups in distilled water, ranging from a low of 21.2% for western yarrow to a high of 43.1% for kinnikinnick. The high solubility of kinnikinnick in water indicates a high intra-cellular carbohydrate and mineral content. This may be a reason kinnikinnick is one of the most important browse species during winter on pine sites in the Black Hills (Hill, 1946). Although kinnikinnick was the most digestible of the plants tested, microbial action only increased in vitro digestibility from 47.4 to 50.2 percentage points. In other words, the solution mixture of buffer + alcohol + HCl + pepsin accounted for 94% of the dry-matter

disappearance. The waxy leaf surface of kinnikinnick may have inhibited cell wall digestibility by the rumen microorganisms. Dry-matter disappearance was increased most from the addition of rumen fluid for roughleaf ricegrass and chokecherry. The surprising and most significant discovery was the negligible effect of pepsin on the dry-matter disappearance of these plant species.

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