In Vitro Digestibility among Accessions of Big Sagebrush by Wild Mule Deer and Its Relationship to Monoterpenoid Content

BRUCE L. WELCH AND JORDAN C. PEDERSON

Abstract

Results of in vitro digestibility trials indicate that big sagebrush (*Artemisia tridentata*) is a highly digestible browse for wintering mule deer. Subspecies *tridentata* (62.1% digested dry matter) was more highly digested than subspecies *vaseyana* (53.2% digested dry matter) and subspecies *wyomingensis* (51.4% digested dry matter). On an accession level, some accessions of big sagebrush were more highly digested than others. The accessional range was from 44.6% digested dry matter to 64.8%. No relationship was found between total monoterpenoids (essential or volatile oils) content and digestibility.

Some accessions of big sagebrush (*Artemisia tridentata*) have been found to contain significantly higher levels of monoterpenoids (essential or volatile oils) than others (Welch and McArthur 1979, Welch and McArthur 1981). Our interest in the monoterpenoid content stems from the reports of Nagy et al. (1964), Oh et al. (1968), and Longhurst et al. (1969) that in high enough concentrations, these compounds may adversely affect deer digestion. If

monoterpenoids of big sagebrush do suppress digestion, we could design breeding and selection schemes aimed at developing strains of big sagebrush low in monoterpenoids. Welch and McArthur (1979) questioned the hypothesis that monoterpenoids cause digestive problems. Our main concern is that it requires a lot of time and effort to select for or against a given characteristic such as low monoterpenoids when in fact they may not be adversely affecting digestion. Therefore, we undertook this study to determine the relation between accessions of big sagebrush with varying amounts of total monoterpenoids and their in vitro digestibility. Other non-monoterpenoid producing browse species were included in the study for comparison.

Materials and Methods

From a uniform shrub garden at the Snow Field Station¹ at Ephraim, Utah, nine accessions of *Artemisia tridentata* (big sagebrush) were selected to study in vitro digestibility using wild mule deer rumen inoculum. These nine accessions contained different levels of total monoterpenoids (Welch and McArthur 1981). All three subspecies of *A. tridentata* were equally represented (three accessions each). Subspecies were identified by morphological (Beetle and Young 1965, Winward and Tisdale 1977, McArthur et

Authors are research plant physiologist, Intermountain Forest and Range Experiment Station, Shrub Sciences Laboratory, Provo, Utah 84601; and regional game manager, Utah State Division of Wildlife Resources, Springville, Utah 84663.

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al. 1979), and chemical criteria (Stevens and McArthur 1974). Within each accession, five plants were selected at random. Vegetative samples of current-year growth were collected at random over the entire crown of each plant. The tissue collected would represent the portion most commonly consumed by wintering mule deer, that is the first-two terminal inches of the stems with leaves. Collections were made on January 18, 1978. Samples collected from a given plant were placed in a paper bag and frozen on-site with dry ice. All samples were collected within a 90-minute period (1000-1130) to avoid diurnal variation in monoterpenoid concentration (Nicholas 1973). Samples were stored in an ultra-low freezer (-35° C) until needed for grinding. Before grinding, the samples—stems with leaves—from the five plants of a given accession were pooled, ground together, and thoroughly mixed.

Samples were ground with a motorized steel mortar and pestle. Liquid nitrogen was poured over the tissue to aid in grinding the tissue to a fine powder.

After grinding, the samples were placed in plastic bottles fitted with airtight caps and stored in an ultra-low freezer (-35° C).

Current-year growth was collected from five plants of each of the following: *Cercocarpus ledifolius* (curlleaf mahogany), *Cercocarpus montanus* (true mahogany), and *Purshia tridentata* (antelope bitterbrush). Also, hips were collected from five plants of *Rosa eglanteria* (sweetbrier rose). Samples were pooled on a species basis and ground as described for the nine accessions of big sagebrush.

We used the in vitro digestion procedure as outlined by Pearson (1970), except 1.0 g of fresh tissue was placed in digestion tubes. The dry matter content was determined for all plant samples digested. Inoculum was collected from four deer killed by personnel from the Utah State Division of Wildlife Resources. The four deer harvested for this study were killed on January 15, 22, 29, and February 5, 1979. Deer were taken from winter ranges dominated by big sagebrush. This was done to enhance the probability that the deer harvested had been eating big sagebrush long enough to have allowed the rumen microorganisms to adapt fully to the monoterpenoids (Nagy et al. 1964, and Oh et al. 1967).

The data were expressed as percent of digestible dry matter. Percentages were transformed (arcsin) for performing analysis of variance and Hardley's range test. Completely random analysis of variance was used to detect significant differences in digestibility among subspecies of big sagebrush, among nine accessions of big sagebrush, and for all species of browse studied (sagebrush, bitter-

Table 1. In vitro digestibility of nine accessions of *Artemisia tridentata* (big sagebrush) and four other species of browse (hips of sweetbrier rose¹, curlleaf mahogany², true mahogany³, and bitterbrush⁴). Data expressed as percent digestible dry matter.

Big sagebrush accession or other browse species	Date of digestion trials				Average
	1/15/79	1/22/79	1/29/79	2/5/79	
Clear Creek (t) ⁵	69.9	59.8	59.5	70.0	64.8
Dove Creek (t)	67.9	62.0	61.2	67.1	64.6
Loa (t)	61.1	55.5	55.0	56.3	57.0
Indian Peaks (v)	63.3	51.8	55.2	52.7	55.8
Benmore (v)	64.7	53.0	53.6	49.4	55.2
Kaibab (w)	56.3	53.7	55.5	54.1	54.9
Milford (w)	58.9	50.5	58.6	50.4	54.6
Sardine (v)	53.5	48.6	49.0	43.8	48.7
Trough Springs (w)	47.1	40.5	45.0	45.6	44.6
Rose hips ¹	56.3	52.2	40.1	47.6	49.1
Curlleaf ²	49.5	41.3	45.8	42.0	44.7
Mahogany ³	26.2	18.2	16.2	18.8	20.0
Bitterbrush ⁴	24.7	14.3	21.5	18.7	19.8

¹*Rosa eglanteria*.
²*Cercocarpus ledifolius*.
³*Cercocarpus montanus*.
⁴*Purshia tridentata*.
⁵t = *Artemisia tridentata* ssp *tridentata*
v = *A. tridentata* ssp *vaseyana*
w = *A. tridentata* ssp *wyomingensis*

brush, rose hips, curlleaf, and true mahogany). For significant *F* ratios, Hardley's range test was used to compare treatment means (Snedecor and Cochran 1967).

The monoterpenoid content for each accession of big sagebrush was determined by using the methods outlined by Welch and McArthur (1981). Correlation analysis and stepwise multiple regression analysis were used to relate monoterpenoid content of nine accessions of big sagebrush to in vitro digestibility (Steel and Torrie 1960). Also, the crude protein content for each accession of big sagebrush was determined by the Kjeldahl method (Association of Official Agricultural Chemists 1965). As with monoterpenoid content, crude protein content was related to in vitro digestibility by correlation analysis and stepwise multiple regression analysis (Steel and Torrie 1960).

We wanted to know what was happening to the monoterpenoids during the digestibility trials. Therefore, we modified the digestibil-

Table 2. Comparisons (Hardley's range test) of the in vitro dry matter digestibility among three plant groupings.

Plant grouping					
Subspecies of big sagebrush ¹		big sagebrush		9 accessions of big sagebrush and 4 other browse species ²	
Subspecies	% digested dry matter	Accessions	% digested dry matter	Accession or species	% digested dry matter
tridentata	62.1a ³	Clear Creek	64.8a ³	Clear Creek	64.8a ³
vaseyana	53.2b	Dove Creek	64.6a	Dove Creek	64.6a
wyomingensis	51.4b	Loa	57.0b	Loa	57.0b
		Indian Peaks	55.8b	Indian Peaks	55.8b
		Benmore	55.2b	Benmore	55.2b
		Kaibab	54.9b	Kaibab	54.9b
		Milford	54.6b	Milford	54.6b
		Sardine	48.7bc	Rose hips	49.1bc
		Trough Springs	44.6c	Sardine	48.7bc
				Curlleaf	44.7c
				Trough Springs	44.6c
				Mahogany	20.0d
				Bitterbrush	19.8d

¹*Artemisia tridentata*.
²Rose hips-sweetbrier-*Rosa eglanteria*
Curlleaf-curlleaf mahogany-*Cercocarpus ledifolius*
Mahogany-true mahogany-*Cercocarpus montanus*
Bitterbrush-antelope bitterbrush-*Purshia tridentata*.
³Values sharing the same letter superscript are not significantly different at the 5% level.

ity trials by adding three digestive tubes that contained only buffer. To these tubes, we added specific amounts of α -pinene and d-camphor—2.5 ug/ul. These tubes were incubated and treated like other tubes except they did not contain plant tissue or rumen inoculum. They also did not receive the acid-pepsin, and sodium carbonate treatments. After the first incubation period, we extracted the solution with absolute ether and used gas chromatography to detect any changes in the monoterpenoid content.

Results

The raw data of the four in vitro digestibility trials are given in Table 1. Significant *F* ratios were detected (Table 2) for the following effects: subspecies of big sagebrush, accessions of big sagebrush, and for all browse species studied. Subspecies *tridentata* (62.1% digested dry matter) was more highly digested by in vitro means than subspecies *vaseyana* (53.2% digested dry matter) and subspecies *wyomingensis* (51.4% digested dry matter). Clear Creek Canyon (64.8% digested dry matter) and Dove Creek (64.6% digested dry matter) accessions of big sagebrush (*Artemisia tridentata*) were more highly digested than the other seven accessions. Rose hips (*Rosa eglanteria*) and curleaf mahogany (*Cercocarpus ledifolius*) were the only other browse species that were as digestible as some of the accessions of big sagebrush, but were not as digestible as the Clear Creek Canyon and Dove Creek accessions (Table 2). Mahogany (*Cercocarpus montanus*) and bitterbrush (*Purshia tridentata*) were significantly the least digestible of all plants tested (Table 2).

Our findings in relating monoterpenoid content and crude protein content of the nine accessions of big sagebrush to percent digested dry matter are given in Table 3. Total monoterpenoids were not significantly related to digestibility ($r = -0.09$). The concentrations of α -pinene ($r = -0.56$), 1,8 cineol ($r = -0.40$), α -thujone ($r = -0.06$), β -thujone ($r = -0.39$), and terpineol ($r = -0.10$) were not significantly related to percent digested dry matter. Camphene ($r = -0.75$) and camphor ($r = -0.75$) were significantly related to percent digested dry matter, but not at the 1% level. The crude protein level ($r = 0.80$) was significantly related to percent digested dry matter at the 1% level.

Stepwise multiple regression analysis revealed that the content of crude protein accounted for 64% of the variation of digestible dry matter among the nine accessions of big sagebrush. An additional 25% of the variation was accounted for by adding camphor content to the regression equation. Other variables, total monoterpenoids, and individual monoterpenoids contributed only 1% or less to the regression.

We found that 100% of the α -pinene added to control flasks (buffer only) was lost. Loss of camphor was 17.3%. All three control flasks, however, contained a white condensate ring around

the neck of the flask. This condensate ring was located about 70 mm above the surface of the digestion solution and was identified to be camphor.

Discussion

Our results indicate that big sagebrush is a highly digestible food for wintering mule deer. Other workers agree with the results we have obtained (Sheehy 1975, Urness et al. 1977, and Wallmo et al. 1977). Unfortunately, as Wallmo et al. (1977) pointed out, preparatory techniques such as oven drying may result in large losses of monoterpenoids, thus introducing bias. Also, Welch and McArthur (1979) pointed out possible large losses of monoterpenoids due to freeze drying. We ran a test to determine the loss of monoterpenoids due to oven drying and freeze drying. We found that oven drying at 100° C caused a total loss of monoterpenoids. Freeze drying caused a 78% loss. We were able to overcome these preparatory techniques by grinding the big sagebrush tissue (fresh), submerged in liquid nitrogen, with a motorized steel mortar and pestle.

We found very little relationship between digestibility and total monoterpenoid content. Connolly et al. (1980) found similar results using Douglas-fir and deer rumen inocula. On an individual monoterpenoid basis, camphene and camphor had a significant negative effect on digestion. We are unable to explain the significant negative effect camphene and camphor appear to have on digestibility among the nine accessions of sagebrush. Milford, Loa, and Clear Creek accessions contained about the same amount of camphor, but Clear Creek was significantly more digestible than the Milford and Loa accessions. This is also true for camphene. Trough Springs, Sardine Canyon, and Kaibab accessions contained the same amount of camphene but Kaibab was significantly more digestible than the Trough Springs. Our reluctance to accept the statistically significant effects of camphene and camphor on digestions stems from two points: First, we selected the accessions purposely for varying amounts of total monoterpenoids not for individual monoterpenoids—our test for individual monoterpenoids may not be valid; and secondly, in vitro digestibility values for big sagebrush are among the highest recorded for shrubs (Welch and McArthur 1979). In the multiple regression equation, camphor content accounted for about 25% of the digestibility variation among the accessions, whereas, camphene accounted for less than 1%. From a biological point, the impact of these two monoterpenoids must be small. If not, the high percentage of digested matter would not have been obtained. Crude protein levels had a significant positive relation to digestibility.

This lack of a relationship between digestibility and total monoterpenoid content and the high percentage of digested dry matter contrasted with the findings or statements of Nagy et al. (1964),

Table 3. The relationship between percent digestible dry matter (in vitro) and total monoterpenoids (essential oils), individual monoterpenoids, and crude protein of nine accessions of big sagebrush.¹

Accession	% digested dry matter	Total monoterpenoids	α -Pinene	Camphene	1,8 Cineol	α -Thujone	β -Thujone	Camphor	Terpineol	Crude protein
Trough Springs	44.6	1.41 ²	.09	.09	.08	.00	.00	.89	.02	11.0
Sardine	48.7	1.74	.02	.11	.06	.25	.58	.62	.05	10.5
Milford	54.6	.99	.05	.03	.01	.13	.04	.22	0	11.2
Kaibab	54.9	.93	.11	.11	.02	.00	.00	.63	.01	11.9
Benmore	55.2	2.89	.00	.00	.09	.96	.49	.05	.32	10.0
Indian Peaks	55.8	1.72	.00	.04	.12	.03	.02	.31	.01	11.2
Loa	57.0	1.91	.01	.03	.09	.13	.55	.28	.05	14.5
Dove Creek	64.6	1.70	.00	.00	.02	.07	.71	.13	0	16.0
Clear Creek	64.8	.95	.00	.00	.02	.05	.31	.24	.01	15.3
<i>r</i>	—	-.09	-.56	-.75	-.40	-.06	-.39	-.75	-.10	.80
<i>r</i> ²	—	.008	.31	.56	.16	.003	.15	.56	.01	.64

¹*Artemisia tridentata*.

²Data for monoterpenoids expressed on a percent dry matter basis.

Nagy and Tengerdy (1968), Oh et al. (1968), Dietz and Nagy (1976), Wallmo et al. (1977), Nagy and Regelin (1977), Carpenter et al. (1979). In general, these workers have shown that at certain levels, monoterpenoids suppressed the in vitro growth of rumen microorganisms, rate of cellulose digestion, and the rate of gas and volatile fatty acids production of deer rumen microorganisms. Estimates of the amount of big sagebrush that deer can consume before the monoterpenoids start to interfere with digestion varies from 15 to 50% of the diet (Nagy et al. 1964, Nagy and Tengerdy 1968, Dietz and Nagy 1976, Wallmo et al. 1977).

Although we do not question the results of these in vitro studies or estimates of the amount of big sagebrush that can be safely eaten, we do question whether the suggested suppression by monoterpenoids occurs under range conditions. First, in vivo digestion trials conducted by Smith (1950), Bissell et al. (1955), and Dietz et al. (1962) determined the total digestible nutrients (TDN) content of big sagebrush to be 70.2%, 55.9%, and 58.9%, respectively. Nagy 1979 citing the protein digestion coefficients from these studies concluded that big sagebrush digestibility is poor to fairly poor. We cannot understand how a roughage with a reported mean TDN content of about 59% can be considered poor to fairly poor in digestibility (see review by Welch and McArthur 1979). High quality alfalfa has a TDN content of about 53% (Morrison 1961, National Academy of Sciences 1964). Secondly, we propose that the monoterpenoids are lost from the rumen and therefore do not interact significantly with the rumen microorganisms. This hypothesis is based on our observations that α -pinene was lost from the in vitro digestion tubes and that camphor formed a condensed ring about 70 mm above the surface of the in vitro digestion solution. The force that drove these compounds (pinene and camphor) out of the digestion solution was heat. Apparently 38.5° C, which is close to the normal body temperature of mule deer, is sufficient to volatilize the monoterpenoids (Moen 1973). We can visualize three ways in which monoterpenoid levels in the deer rumen can be greatly reduced: (1) loss through mastication and rumination, (2) heat from the body volatilizing the monoterpenoids resulting in the monoterpenoid being expelled from the rumen by eructation, and (3) possible adsorption in the rumen and excretion through the kidneys (Cook et al. 1952, Annison 1965). We will be testing this hypothesis in the near future. If the hypothesis is true, we will be able to explain the apparent conflict between the in vitro studies showing the monoterpenoid suppress deer rumen microorganisms and the results of our study showing that big sagebrush is a highly digestible forage for deer.

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