Effects of Monoterpenoid Exposure on Ability of Rumen Inocula to Digest a Set of Forages

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Abstract

Rumen inoculum collected from wild mule deer on summer, fall, winter, and spring ranges in central Utah was equally effective in digesting alfalfa hay, orchardgrass hay, big sagebrush, curlleaf mahogany, antelope bitterbrush, and hips of sweetbrier rose. Alfalfa hay was the forage most easily digested. Inocula from deer that had not been exposed to big sagebrush and juniper monoterpenoids (essential oils) digested all test forages, including big sagebrush equally as well as inoculum from deer that had been exposed to big sagebrush monoterpenoids. We concluded that rumen microorganisms do not have to adjust to the presence of the monoterpenoids or other dietary changes.

Nagy et al. (1964) using in vitro techniques found that the monoterpenoids (essential or volatile oils) of big sagebrush *(Artemisia tridentata)* suppressed the growth of mule deer rumen microorganisms, decreased the rate of cellulose digestion and the production of gas and volatile fatty acids by the microorganisms. Oh et al. (1967, 1968) found that the monoterpenoid hydrocarbons (the monoterpenes) of Douglas-fir needles actually enhanced in vitro microbial fermentation in sheep and deer inoculum. Sesquiterpenes were also found to be stimulatory. Microbial fermentation, however, was suppressed in the presence of the oxygenated monoterpenoids (alcohols, esters, aldehydes) of Douglas-fir (Oh et al. 1967, 1968; Longhurst et al. 1969). After a review of the literature, Welch and McArthur (1979) concluded that the results of in vitro and in vivo digestibility trials of big sagebrush did not support the contention that monoterpenoids suppressed digestion in mule deer. It was suggested by Nagy et al. (1964), Welch and McArthur (1979), and Van Soest (1981) that microorganisms in the rumen may be able to adapt to big sagebrush monoterpenoids. Observations made by Oh et al. (1967) support the adaptation idea. They found that citonellal (aldehyde), an oxygenated monoterpenoid, inhibited rumen microorganisms from sheep and deer that had no access to Douglas-fir needles (a monoterpenoid source), but produced no effect upon rumen microorganisms from deer that had access to Douglas-fir.

Welch and Pederson (1982) through in vitro means, found big sagebrush to be a highly digestible browse for wintering mule deer. They collected rumen inoculum from wild mule deer that had been feeding on big sagebrush for about 3 weeks. This was to ensure that the rumen microorganisms had fully adapted to the presence of big sagebrush monoterpenoids. Although that study (Welch and Pederson 1982) demonstrated that rumen inoculum readily digested big sagebrush, it did not furnish evidence concerning the ability of rumen microorganisms to adapt to the presence of monoterpenoids.

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If adaptation is important, the inoculum with unadapted rumen microorganisms should digest big sagebrush forage less readily than inoculum with adapted microorganisms. Therefore, we undertook this study to test the hypothesis that rumen inoculum collected from mule deer not consuming big sagebrush (summer range) would digest significantly less dry matter of winter samples of big sagebrush forage than inoculum collected from mule deer consuming big sagebrush (winter range).

Materials and Methods

During 1979-1980, we collected rumen inoculum from 13 wild mule deer at seven points in time: July 18, September 7, October 29, December 3, January 14, February 25, and April 17. Two mule deer were sacrificed for each time period, with the exception of October 29 when one deer was taken. Deer were obtained from native ranges in central Utah. The method we used to collect rumen inoculum was described by Nagy et al. (1964).

The collections of rumen inocula were tested against four native forages and two dry roughages collected from one site in central Utah and at one point in time. Native forages included the current year's growth of big sagebrush, curlleaf mahogany *(Cercocarpus ledifolius),* antelope bitterbrush *(Purshia tridentata),* and the hips from sweetbrier rose *(Rosa eglanteria).* These forages were collected during midwinter and ground with a motorized steel mortar and pestle to a fine powder in liquid nitrogen (Welch and Pederson 1982). During the early spring of 1979, samples of alfalfa *(Medicago sativa)* and orchardgrass *(Dactylis glomerata)* were collected, dried, and ground in a Wiley mill (1 mm). All forages were stored in a freezer (-35° C).

We used the in vitro digestion procedure outlined by Pearson (1970), except that 1.0 g of fresh tissue was used for the four native forages. The dry matter content was determined for all forage samples digested. Rumen inoculum for each deer was tested separately. Burbank et al. (1979) reported that the pH of the buffer-rumen inoculum can significantly affect the digestive ability of the inoculum. We found that the pH of the rumen fluid collected from the 13 deer varied from 6.5 to 6.8, therefore, the pH of the buffer-rumen inoculum was adjusted to a pH of 6.8.

Data were expressed as percent of digestible dry matter. Percentages were transformed (arcsin) for statistical analysis. Analysis of variance for a completely randomized design was used to evaluate differences in treatment effects. Treatment effects were measured by the ability of the various inoculum sources to digest each forage. Hartley's range test was used to compare inoculum collection dates—the treatment means (Snedecor and Cochran 1967).

Rumen contents of all 13 deer were saved to determine the amount of big sagebrush and Utah juniper *(Juniperus osteosperma)* in the diet (Cluff et al. 1982).

Results

Amount of big sagebrush and juniper in the diet of the mule deer

Table 1. Percent of big sagebrush and juniper in the diets of wild mule deer collected during 1979-1980. Two deer per date, except for October.

Forage	Date						
	July 18	Sept. 7	Oct. 29	Dec. 3	Jan. 14	Feb. 25	Apr. 17
Big sagebrush	0	0	<0.1	<0.1	26.0	1.0	<0.1
	0	0	—	23.8	38.5	7.0	2.6
Juniper	0	0	0	0	7.0	89.4	70.5
	0	0	—	4.1	27.7	50.9	44.3

is presented in Table 1. Consumption of big sagebrush and juniper increased from zero during the summer months to an upper range of about 90% of the diet during the winter months.

The results of our seven in vitro digestibility trials are summarized in Table 2. There were no significant differences (P=0.01) in the digestive effectiveness of the mule deer rumen inocula collected at seven points in time.

Rumen inocula collected in July and September, that is, inocula not exposed to big sagebrush or juniper monoterpenoids, digested big sagebrush[1] (64 to 70% digested dry matter) as well as inoculum collected in December and January that had been exposed to monoterpenoids (67 to 69% digested dry matter.)

Alfalfa was the most digestible, 72%, of the six forages tested, followed by big sagebrush and orchardgrass at 67% and 66%, respectively. Our results and those of Smith (1952) showed alfalfa hay to be a highly digestible deer food. This would suggest that alfalfa hay could be used as a winter feeding supplement for short periods during severe winters (Urness 1980). Rose hips, curlleaf, and bitterbrush were the least digestible at 53%, 42%, and 18% respectively. These results agree closely with a study by Welch and Pederson (1982).

Discussion

Our results indicate that rumen inocula not exposed to big sagebrush or juniper monoterpenoids can digest big sagebrush as well as rumen inocula exposed to the monoterpenoids. Rumen inocula obtained from mule deer (Jan. and Feb.) consuming high levels (62%) of monoterpenoid-containing plants digested all six forages as well as rumen inocula obtained from mule deer consuming plants devoid of monoterpenoids. Pearson (1970) also reported a lack of significant difference in the digestive ability of rumen inocula collected from members of the same ruminant species consuming different diets.

There are two schools of thought concerning big sagebrush and digestibility. One school believes that the monoterpenoids of big sagebrush cause digestive problems in wintering mule deer when big sagebrush exceeds 20 to 30% of the diet (Nagy et al. 1964, Nagy and Tengerdy 1968, Dietz and Nagy 1976, Nagy and Regelin 1977, Wallmo et al. 1977). The other school believes that wintering mule deer will, through two or three mechanisms, eliminate significant interaction among the monoterpenoids and the rumen microorganisms (Welch and McArthur 1979, Van Soest 1981, Welch and Pederson 1982, Cluff et al. 1982, White et al. 1982). Also, evidence that rumen microorganisms might be able to adapt to the presence of oxygenated monoterpenoids has been reported by Oh et al. (1967). Tabular data presented by Nagy and Tengerdy (1968) suggest some degree of adaptation (see Welch and McArthur 1979, Van Soest 1981).

As pointed out by Welch and McArthur (1979), neither in vitro nor in vivo digestibility trials of big sagebrush support the contention that monoterpenoids suppress the digestion of forage by mule deer. Wallmo et al. (1977) states that caution must be used in interpreting some of the in vitro trials because some of the preparatory methods used could have resulted in large losses of

[1]It should be noted that the total monoterpenoid content of the big sagebrush sample was 2.1% of the dry matter.

Table 2. The ability of wild mule deer rumen inocula collected at seven points in time to digest a set of forages. Data expressed as % of dry matter digested.

Forage	July 18	Sept. 7	Oct. 29	Dec. 3	Jan. 14	Feb. 25	Apr. 7	Mean
Alfalfa	74[1]	74	71	72	71	70	70	72
	74	73	—	72	73	73	70	
Big sagebrush	70	65	65	68	69	62	68	67
	64	66	—	68	67	69	68	
Orchardgrass	74	73	66	67	66	62	64	66
	66	68	—	67	62	61	72	
Rose hips	56	53	53	56	50	53	45	53
	62	54	—	51	50	50	50	
Curllcaf mahogany	46	39	41	43	42	42	40	42
	39	39	—	42	40	45	53	
Bitterbrush	17	18	19	20	18	20	16	18
	16	14	—	20	16	18	20	

[1]Two deer were taken for each date, with the exception of October, when only one deer was taken.

monoterpenoids.

The major evidence that big sagebrush monoterpenoids could suppress digestion in mule deer comes from in vitro studies (Nagy et al. 1964, Nagy and Tengerdy 1968). Our main concern is that concentrations of monoterpenoids used in some of those studies were four to eight times higher than those found in big sagebrush tissues (Welch and McArthur 1981). Nagy et al. (1964) prepared their substrates by adding 0.1 gm and 0.2 gm of essential oils (monoterpenoids) to 1 gm of oil-less big sagebrush dry matter. This would result in a concentration (9.1 and 16.7%) of monoterpenoids four to eight times greater than that found in big sagebrush (Welch and McArthur 1981). The apparatus used in the Nagy et al. (1964) experiment was a closed system which does not allow for escape of the monoterpenoids. Loss of monoterpenoids from the rumen through mastication, eructation, rumination, or absorption was found by Cluff et al. (1982) to be substantial (also see White et al. 1982). Cluff et al. (1982) found a reduction of 80% of the monoterpenoids in the forages consumed (see Van Soest 1981).

Another point that concerns us is that pouring the essential oils (monoterpenoids) into the culture exposes the microorganisms to the full concentration of the monoterpenoids, whereas, in the rumen the exposure is less concentrated because a portion of the monoterpenoids are trapped inside the coarse pieces of big sagebrush. The trapped monoterpenoids are not released to interact with rumen microorganisms until after further grinding, and further grinding through mastication may result in loss of monoterpenoids (White et al. 1982). In other words, the procedures used to demonstrate possible suppression of digestion by monoterpenoids were conducted under severe conditions—conditions probably not encountered by mule deer on winter ranges. To this point, Carpenter et al. (1979) found in their grazing study that wintering mule deer started gaining weight at the peak of big sagebrush consumption (see Welch and Pederson 1982).

In addition, Tueller's (1979) study lends support to the idea that high levels (35% or more) of big sagebrush in the diet of mule deer does not adversely affect digestion. He reported that the diet of mule deer wintering (12/66, 3/66, 12/67) in the Fox Mountain area of Nevada contained 69% big sagebrush, whereas, the diet of mule deer wintering (12/66, 3/66, 12/67) in the White Rock area contained only 28%. While the amount of big sagebrush in the diet of the deer from the two areas differ by a factor of 2.5, the amount of tail fat (an indicator of body condition) was almost the same between the two deer herds (32.4% for Fox Mountain deer and 29.1% for White Rock deer). The values listed are means of the three sampling dates. Mule deer with a tail fat of 30% is considered to be in good physical condition. It should be reiterated that 69% big sagebrush in the diet of Fox Mountain deer is well above the

level considered safe by some workers (Nagy et al. 1964, Wallmo et al. 1977, Carpenter et al. 1979).

The Tueller (1979) and Carpenter et al. (1979) reports support the idea that big sagebrush does not, under range conditions, cause adverse effects on wintering mule deer.

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