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Rumen Microbial Ecology in Mule Deer[1]

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Mule deer rumen microbial populations from animals in the natural habitat in Utah and from captive deer fed various rations were studied. The microorganisms were characterized on the basis of morphology and Gram reaction. Rumen samples contained 13 identifiable types of bacteria and one genus of ciliate protozoa (*Entodinium*). Highest rumen bacterial populations were produced on rations containing barley. No differences in proportions of ruminal bacteria in the various morphological groups could be detected when animals were fed either natural browse plants or alfalfa hay. The total numbers of bacteria were similar for animals feeding on controlled diets of browse or hay and those in the natural habitat. Numbers of some bacterial types were directly related to ciliate protozoal numbers, whereas others were inversely related. Highest rumen ciliate protozoal populations were observed on rations containing barley. No differences in protozoal populations were noted between diets containing only browse or hay. Seasonal variations were noted in ciliate protozoal numbers from deer feeding in the natural habitat. The total number of ciliate protozoa decreased in the fall and winter and remained low until spring. There were indications that salt in the deer diet favorably affected rumen ciliate protozoa. Rather than revealing direct deer management applications, this study serves to stimulate and illuminate new approaches to research in range and wildlife nutrition.

Winter is a crucial time for game animals in colder climates because of low nutritional levels. The limited available forage has low nutritive value, and the animals are under stress conditions from the weather. Nutrition of ruminants is partly dependent upon the rumen microorganisms, which convert and supply many nutrients for the normal body activities of these animals.

The mule deer (*Odocoileus hemionus*), an important big game animal, has been studied widely; its rumen microbiology, however, has apparently been neglected. This study was undertaken to obtain some understanding of the microbial ecology of the deer rumen. Specific objectives were: (i) to distinguish members of the microbial populations and (ii) to determine their relative densities and, also, the variations in these densities with diet. Since ruminology in wildlife is relatively new, studies in domestic animals were used as a guide for the present investigation.

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MATERIALS AND METHODS

Animals and diets. Three captured mule deer were pen fed with several diets: (i) bitterbrush [*Purshia tridentata* (Pursh) DC], (ii) curlleaf cercocarpus (*Cercocarpus ledifolius* Nutt.), (iii) alfalfa hay, and (iv) alfalfa hay plus 1 lb (453.6 g) per day of barley. All deer were fed the same diet for a 16-day period, similar to deer feeding by Smith (10), who fed animals ad libitum.

Forty-one free-ranging mule deer in the natural habitat were killed between August 1961 and April 1962 to examine their rumen microbes. Deer were killed at nine locations from six counties in Utah. Four locations were in Cache County: (i) near Avon, (ii) Blacksmith Fork, (iii) Hardware Ranch, and (iv) North Cache County. Other counties where deer were killed included Box Elder, Morgan, Rich, Sevier, and Sanpete.

Feed available for deer in the natural habitat varied between August and April. Although alfalfa fields in the strictest sense are not a natural habitat, deer were often killed there during late summer and fall. Since snow often completely obscured herbaceous vegetation during the winter season, the diet consisted primarily of browse species. Important browse in areas

where deer were killed included bitterbrush, curlleaf cercocarpus, birchleaf cercocarpus (*Cercocarpus montanus* Raf.), cliffrose (*Cowania stansburiana* Torr.), oak (*Quercus gambelii* Nutt.), sagebrush (*Artemisia tridentata* Nutt.), and juniper (*Juniperus osteosperma* Torr. Little). Although rumen contents were not quantitatively analyzed, a definite trend was observed in types of food eaten by deer between August and April. The diet consisted predominantly of forbs and browse during late summer and fall, browse only during winter, and grass in early spring.

Based on dates killed, deer from the natural habitat were grouped for analytical purposes into five seasons: (i) late summer (August 31–September 15); (ii) early fall (October 5–13); (iii) late fall (October 21–November 3); (iv) winter (January 13–17); and (v) early spring (April 2). Since these seasons involved diet changes in plant maturity and plant species, seasons and diet in the natural habitat will hereafter be used interchangeably.

Sampling procedure. Rumen contents from captive deer were sampled three times at weekly intervals. The first sample was taken approximately 40 hr after the initial feeding of each diet. Samples were obtained by means of a 3/8-inch (0.95 cm) stomach tube approximately 16 hr after placing fresh feed before the animals. Anatomical location of sampling in the rumen could not be controlled with the stomach tube. Approximately 25 ml of the rumen contents was removed and placed in a sterile, covered beaker. The samples were taken to the laboratory from 15 min to 1 hr after collection. The fluid was separated from the solids by squeezing the contents through several layers of sterile cheesecloth. A part of this fluid was used to make Gram-stained microscopic slides for bacterial grouping purposes, and the remainder was preserved in an equal volume of 10% Formalin for total counts of microorganisms.

Samples of rumen contents from mule deer killed in the natural habitat were obtained by opening their rumens. Rumen contents were mixed, and 25 ml of rumen fluid was immediately squeezed through cheesecloth into sterile plastic bottles containing an equal volume of 10% Formalin. A smear was made immediately for Gram staining.

Microscopic counts. Clumps of bacteria and individual ciliate protozoa were counted by means of the hemacytometer (11, 14). Instead of individual bacteria, clumps were counted, since this value more closely describes results obtained from counts of colonies (2). For counting purposes, bacteria were stained with 0.01% methylene blue solution. Gram, geisma, and iron-hematoxylin stains were used for identifying morphological characteristics of bacteria and ciliate protozoa.

Bacterial classification. Preliminary comparison of mule deer and domestic animal rumen bacteria showed no important morphological differences. Therefore, the descriptions of rumen bacteria given by Breed, Murray, and Smith (*Bergey's Manual*, 7th ed.), Bryant (1), and Hungate (3) were used as a basis for a system that yielded 13 distinguishable types (Table 1). The bacterial types will be referred to hereafter as letter designations, which are defined in Table 1. This classification system is original to this research, although the articles mentioned provided criteria for descriptions. All 13 bacterial types were observed in most of the sampled animals.

TABLE 1. *Morphological classification of Gram-stained rumen bacteria found in mule deer*

Types of bacteria	Bacterial type designation[a]	Bacteria described in literature
Gram-positive cocci	PC	Peptostreptococci Ruminococci Streptococci
Small (diameter up to 0.5 μm), gram-negative cocci	NSC	*Veillonella*
Large (diameter greater than 0.5 μm), gram-negative cocci	NMC	*Neisseria*
Long (length greater than 1.5 μm), gram-positive bacilli	PLR	Lactobacilli Cillobacteria Propionibacteria
Short (length up to 1.5 μm), gram-positive bacilli	PSR	Eubacteria
Curved, gram-positive bacilli	PCR	*Lachnospira*
Sporeforming, gram-positive bacilli	PFR	Bacilli *Clostridium*
Long (length greater than 1.5 μm), gram-negative bacilli	NLR	*Escherichia*
Short (length up to 1.5 μm) gram-negative bacilli	NSR	*Bacteroides* Fusobacteria
Crescent or moon-shaped gram-negative, gram-variable bacilli	NMR	*Selenomonas*
Oval, gram-negative bacilli	NOR	*Succinimonas*
Curved, gram-negative bacilli	NCR	*Butyrivibrio* *Succinivibrio* *Desulfovibrio*
Spiral-shaped, gram-negative bacilli	NBR	*Borrelia*

[a] First letter: P, gram-positive, N, gram-negative. Middle letter: S, small cocci or short bacilli; M, large cocci or moon-shaped bacilli; L, long; C, curved; F, sporeforming; O, oval; B, spiral. Last letter: C, cocci; R, bacilli.

RESULTS AND DISCUSSION

Rumen bacteria in animals with controlled diets. Numbers of bacteria in the rumen of captured deer varied among the diets (Table 2). Deer on alfalfa hay plus barley had the highest total count of bacteria (19.2 billion per ml of rumen

TABLE 2. *Average number of rumen bacteria (billions per milliliter of rumen fluid) from three pen-fed mule deer on selected diets*

Types of bacteria[a]	Diet			
	Bitterbrush	Curlleaf cercocarpus	Alfalfa hay	Alfalfa hay plus barley
PC	5.89	4.73	4.73	6.30
NSC	0.43	0.57	0.29	0.69
NMC[b]	0.55	0.71	0.33	1.13
PLR[b]	0.12	0.16	0.13	0.39
PSR[c]	0.45	0.47	0.42	0.56
PCR[c]	0.11	0.13	0.18	0.66
PFR	0.03	0.03	0.01	0.03
NLR	0.73	0.57	0.47	0.61
NSR[c]	3.92	2.43	2.78	5.17
NMR[b]	0.69	0.52	0.56	0.98
NOR[b]	0.99	0.47	0.59	1.19
NCR	0.52	0.55	0.40	0.57
NBR	0.39	0.28	0.60	0.54
Total[c]	14.82a[d]	11.62a	11.13a	19.22

[a] Bacterial designation given in Table 1.
[b] Significance at the 0.05 level.
[c] Significance at the 0.01 level.
[d] Value followed by letter "a" is significantly different at the 0.01 level from values not followed by "a."

fluid), followed by bitterbrush (14.8 billion), curlleaf cercocarpus (11.6 billion), and alfalfa hay (11.1 billion).

The increase (1.72 times) in total bacterial numbers when barley was added to the alfalfa hay diet was similar to bacterial responses found in domestic animals. Maki and Foster (6) found two to three times more bacteria when concentrates were fed to cattle. Short (8) recorded the highest content of volatile fatty acids in rumen of deer on an alfalfa hay-concentrate diet, thus indicating high fermentation rates and high numbers of microorganisms.

Numbers of seven individual types of bacteria were significantly different among the controlled diets (Table 2). All seven types were more numerous while the animals were feeding on the diet containing barley. No significant differences in individual types of bacteria or total counts were found among deer. Although the first ruminal sample from the captive deer followed the initial feeding of each diet by a period of 40 hr and other samples were taken 1 and 2 weeks later, no significant differences between counts and proportions of bacteria were found among sample periods.

Since some specific types of bacteria fluctuated in numbers, whereas others remained relatively constant with diet changes, an apparent individual bacterial specificity for a particular kind of diet or nutrient is indicated. Although bacterial disappearance was not observed, adverse conditions could certainly cause one or more specific bacteria to disappear from the rumen flora. This could conceivably prevent digestion of some particular nutrient and hinder the animal's use of certain feeds. Warner (15) suggested that even 3 to 4 days of starvation may cause loss of some microorganisms from the rumen of domestic animals, and a new source of inoculation is needed to reestablish the species.

Natural habitat. Counts of bacteria from rumen contents of deer feeding in the natural habitat varied among individual deer, but no differences related to location, sex, or age of the animals were detected.

Although total rumen bacterial numbers were not different among seasons (Table 3), numbers of seven individual types of bacteria showed significant seasonal fluctuations. Each type responded differently to seasons. For example, numbers of gram-positive cocci (type PC) were highest in late summer and lowest during fall and spring, whereas numbers of short, gram-negative bacilli (type NSR) were highest in fall and lowest in late summer.

The individual responses of rumen bacteria from deer feeding in the natural habitat probably reflected changes in the diet or changes in

TABLE 3. *Average number of rumen bacteria (billions per milliliter of rumen fluid) from mule deer feeding in the natural habitat during various seasons of the year*

Types of bacteria[a]	Late summer	Early fall	Late fall	Winter	Spring
PC[b]	4.97	3.14	3.32	4.38	3.32
NSC	0.45	0.56	0.54	0.69	0.57
NMC[c]	0.49	0.39	0.68	0.45	0.49
PLR	0.21	0.22	0.22	0.21	0.30
PSR[c]	0.63	0.67	0.95	0.62	0.92
PCR[b]	0.19	0.25	0.33	0.23	0.43
PFR	0.01	0.02	0.05	0.01	0.02
NLR	0.60	0.74	0.79	0.71	0.68
NSR[b]	2.29	3.61	3.70	2.96	2.56
NMR[b]	0.68	0.78	1.14	0.76	0.52
NOR	0.61	0.88	0.55	0.64	0.57
NCR[b]	0.31	0.62	0.84	0.74	0.63
NBR	0.32	0.36	0.30	0.30	0.31
Total	11.76	12.24	13.41	13.66	11.30

[a] Bacterial designation given in Table 1.
[b] Significance at the 0.01 level.
[c] Significance at the 0.05 level.

TABLE 4. *Number (millions) of ciliate protozoa per milliliter of rumen fluid from pen-fed mule deer*

Deer no.	Age[a]	Bitter-brush	Curlleaf cerco-carpus	Alfalfa hay	Alfalfa hay plus barley	Mean
408	F	0.47	0.29	0.43	1.09	0.57a
422	M	0.41	0.63	0.51	1.15	0.68a
417	F	0.81	0.85	0.83	1.83	1.08
Mean[b]		0.57a	0.59a	0.59a	1.36	

[a] M, mature; F, fawn.

[b] Mean followed by letter "a" is significantly different at the 0.01 level from those means not followed by "a."

the nutritive value of the diet during the season. Maturity causes changes in nutritive value of plants (12), and, as the plants change, specific rumen bacterial types would be expected to change.

Animal age is not considered important from the rumen bacterial standpoint, since fawn, yearling, and adult deer had similar kinds and numbers of bacteria by August.

Comparison. All 13 types of rumen bacteria were found in animals feeding on controlled diets and in the natural habitat. Relative numerical proportions of each bacterial type were similar in all samples, controlled diets or natural habitat. For example, gram-positive cocci (type PC) and short, gram-negative bacilli (type NSR) were always higher than the other types of bacteria, and sporeforming gram-positive bacilli (type PFR) were lowest. Deer fed the diet containing barley had higher total rumen bacterial counts than those fed other diets or those killed in the natural habitat.

It is concluded from this study that the numbers of bacteria changed with the diet, although all morphological types were present regardless of diet.

Rumen ciliate protozoa. Ciliate protozoa observed in rumen contents of deer included only the genus *Entodinium*. According to descriptions given by Zielyk (16), *E. dubardi* and *E. longinucleatum* were present. These two species were identified by Zielyk in mule deer from Utah and Wyoming and in white-tailed deer from Pennsylvania and New York. These species were also found subsequently in white-tailed deer from Texas (7). Several genera of ciliate protozoa have been observed in other animals (4).

Controlled diets. An average of 1.36 million ciliate protozoa was counted per ml of rumen fluid in animals feeding on the alfalfa hay plus barley diet. This was statistically higher than for the other pen-fed diets (Table 4). Other diets had 0.57 to 0.59 million ciliate protozoa per ml of rumen fluid.

The increase in ciliate protozoal numbers when barley was added to the deer diet is possibly due to the increased starch supply. Most species of the genus *Entodinium* digest starch (4, 13).

Rumen protozoal numbers differed significantly among animals (Table 4), from 0.57 to 1.08 million. Factors responsible for these differences are unknown. The age of the animal was not considered a determining factor, since significant differences were found in rumen protozoa among animals of the same age.

More ciliate protozoa were found when captive deer had salt added to their alfalfa hay diet (Table 5). Statistically significant differences occurred only in deer presumably starved of salt on the winter range. These deer (408 and 423) were captured during the winter and probably had little or no salt for several months prior to being captured, except for salt received from the forage. Although salt content varies with the type of forage (12), the salt intake of deer on the winter range apparently was inadequate. When salt was provided during captivity, the rumen environment rapidly became more adapted for protozoal growth. Rumen protozoa from animals (417 and 422) with a history of salt in their diets did not decrease significantly, however, even after the animals were placed on a salt-free diet for 42 days.

Findings in this study suggest that the quantity of ciliate protozoa in the rumen could be used to indicate feeding conditions of the deer. In several

TABLE 5. *Number (millions) of ciliate protozoa per milliliter of rumen fluid from captive mule deer with and without salt while on an alfalfa hay diet*

Deer no.[a]	Mean[b]	
	Salt	No salt
417	1.19a	0.91a
422	0.69a	0.36a
408	0.61a	0.19b
423	0.44a	0.07b

[a] Deer 417 and 422 were in captivity several months with salt in their diet before salt was removed from their diet for 42 days. Deer 408 and 423 were captured on the winter range and were sampled several weeks before the addition of salt to their diet.

[b] Means with same letter are not significantly different at the 0.01 level.

TABLE 6. *Number (millions) of ciliate protozoa per milliliter of rumen fluid from mule deer in the natural habitat*

Season	Ciliate protozoa[a]
Late summer	0.73
Early fall	0.21a
Late fall	0.35a
Winter	0.16a
Spring	0.37a

[a] Mean followed by letter "a" is significantly different at the 0.05 level from those means not followed by "a."

instances, rumen protozoa disappeared when wild deer would not eat after capture or injury, whereas the bacteria were still present. It can be concluded, therefore, that protozoa are more sensitive to feeding conditions than bacteria. Warner (15) reported similar responses in domestic animals.

Natural habitat. Numbers of rumen ciliate protozoa from free-ranging deer in the natural habitat were not affected by location, age, or sex of the deer (Table 6). However, their numbers differed significantly among seasons; they were highest in late summer. This variation in numbers of protozoa with seasons indicates a direct relationship between forage availability or nutritive value and rumen protozoal numbers. Rumen protozoa decreased with plant maturity and with shortage of feed caused by snow. White-tailed deer from Texas showed similar decreases in rumen protozoa with plant maturity (7).

Rumen ciliate protozoal numbers did not increase significantly during early spring, as did protozoal numbers in white-tailed deer from Texas. Possible reasons for the different responses would include differences in vegetative growing seasons and range condition. Utah has a cooler climate and a later growing season than Texas. Deer collected in April came from an area in Utah with poor range conditions, as indicated by an apparent lack of forage and "highlined" browse. When considering either or both explanations, the nutrient intake of deer in Utah apparently was not sufficient to give similar responses in protozoal numbers to those found in the Texas deer.

Studies with deer in Colorado (9) indicate low fermentation rates extending into April. This supports the seasonal findings in mule deer from Utah. Vegetative responses in Colorado, which result from climate, would be more similar to those in Utah than to those in Texas.

Hungate (3) estimated that protozoa account for 20% of the total fermentative activity in the rumen of domestic animals. If this is true in deer, low fermentation rates found in Colorado probably reflected low numbers of protozoa. Since fermentation rate was directly related to the available energy in browse and the protozoa in deer apparently digest starch, seasonal variations are conceivably related to fluctuations of this nutrient in the plant.

Winter range vegetation often has a marginal or submarginal protein content. With sufficient protozoa in the rumen, however, protein deficiencies could be partially overcome.

Relationship between rumen bacteria and ciliate protozoa. Total bacterial numbers were not significantly correlated with the rumen ciliate protozoa from deer in the natural habitat or on controlled diets. Numbers of several rumen bacterial types (PLR, PCR, and NMR) were positively related to the numbers of ciliate protozoa from deer in controlled conditions but not from deer in the natural habitat. Numbers of bacterial type NCR were inversely related to the numbers of ciliate protozoa from deer in natural habitats and in controlled conditions. Since the variance due to these relationships are quite low, remarks are only speculative. Possibly the positively related bacteria were consuming the same type of nutrient, which was sufficient in controlled conditions and not in natural habitats. On the other hand, these bacteria could be producing by-products used by the protozoa.

The inverse relationship between bacterial type NCR and ciliate protozoa possibly reflects: (i) competition for the food supply or (ii) consumption of this type of bacteria by the protozoa. A similar inverse relationship between total bacteria and ciliate protozoa from white-tailed deer rumen samples has been reported by Pearson (7). In domestic ruminants, it is evident that various bacteria are used as food by protozoa and that particular protozoa may select specific types of bacteria (3, 5). This might explain the relationship between protozoa and bacteria found in this study.

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