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Simple Method to Remove Completely Ciliate Protozoa of Adult Ruminants[1]

A. R. ABOU AKKADA,[2] E. E. BARTLEY, R. BERUBE, L. R. FINA, R. M. MEYER, D. HENRICKS, AND F. JULIUS

Division of Biology and Department of Dairy and Poultry Science, Kansas State University, Kansas

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Dioctyl sodium sulfosuccinate (Aerosol OT) has been used in a remarkably short period to obtain rumen ciliate-free cows. Two 30-g doses of Aerosol OT given on consecutive days appeared to effectively eliminate all types of rumen ciliate protozoa without harming the health of the host animal. Measurement of the rumen metabolic parameters of gas production, carbon dioxide to methane ratio, *p*H value, volatile fatty acids, ammonia, and in vitro cellulose digestion, along with total amylolytic streptococci counts of the rumen contents, showed that conditions in rumens of defaunated animals are normal 4 days after such treatment. It appears that such animals may be used in ruminal studies that require defaunated animals.

Defaunation provides animals free of ciliate protozoa. Such animals can be used to test the essentiality of the ciliate protozoa for ruminants (1, 11). Defaunation also provides a method of growing selected types of protozoa (9) in the rumen in attempts to study the contribution to the host of each component species of rumen microfauna (6).

Existing methods of defaunation have certain disadvantages. Defaunation by prolonged starvation and copper sulfate administration (4) is fairly effective, but it drastically affects both ruminal processes and the animal's health. Heating rumen contents to 50 C is safe, but it does not remove all of the small oligotrichs *Diplodinium dentatum* and *Entodinium* sp. (9). Isolating newborn animals from adult ruminants (1, 5, 8) provides an effective and safe means of defaunation, but it is time consuming and not applicable to adult animals.

A simple, chemical method that effectively defaunates ruminant animals without adversely affecting their health is described here.

MATERIALS AND METHODS

Experimental animals and sampling. Two fistulated adult Jersey cows (13R and 36D), each weighing approximately 800 lb, were used in all studies except those involving measurement of gas ratios. In the latter study, two fistulated adult Jersey cows (34D and 37D) of similar weight were used. They were fed a ration that contained 27.2% ground corn, 35% ground sorghum grain, 22% dehydrated alfalfa pellets, 11% soybean oil meal, 2.8% urea, 1% dicalcium phosphate, and 1% salt.

Samples were collected with a tin can inserted deep at various locations in the rumen. Average weight of the rumen contents was 55 lb.

Methods of defaunation. (i) Two liters of rumen contents were removed via the rumen cannula and mixed with 30 g of Aerosol OT (courtesy of American Cyanamid Co., Princeton, N.J.; Aerosol OT is dioctyl sodium sulfosuccinate; tradename, Sur-ten) in a plastic container. The mixture was then returned to the rumen and the contents were stirred by hand for about 5 min. The procedure was repeated on two consecutive days. (ii) Aerosol OT (30 g) was administered per oz by the use of gelatin capsules and a balling gun. Administration of the capsules was also repeated on two consecutive days.

Defaunation was at 8:30 AM before the morning feeding. Then the animals were offered a highly palatable ration of 30% ground corn, 30% ground sorghum grain, 15% wheat bran, 12% soybean oil meal, 1% urea, 1% dicalcium phosphate, 1% salt, and 10% cane molasses. This ration was fed twice daily at 9 AM and 6 PM. Rumen samples were withdrawn daily at 8 AM and examined microscopically, before Aerosol OT was administered, to determine protozoal populations. The defaunated animals were then kept in separate pens to prevent contact with other ruminants. They were handled and fed by one person who had no contact with other ruminants (1, 5).

Isolating and counting Streptococcus. The typical rumen starch fermenting *Streptococcus* was isolated by a method similar to that described by McPherson (13). Rumen samples were diluted serially with buffer (2) under anaerobic conditions in a helium box. One-ml

[1] Contribution 971, Division of Biology, and 689, Department of Dairy and Poultry Science, Kansas Agricultural Experiment Station, Manhattan, Kansas.

[2] Permanent address: Department of Animal Production, Faculty of Agriculture, University of Alexandria, Alexandria, Egypt.

TABLE 1. *The effect of Aerosol OT on the rumen ciliate protozoa*[a]

Animal no.	Composition of protozoal population[b]		
	Before treatment	After treatment	
		1st dose	2nd dose
36D	*Entodinium* sp. +++	+	−
	Isotricha intestinalis ++	−	−
	Isotricha prostoma ++	−	−
	Epidinium caudatum +	−	−
	Diplodinium minor +	−	−
	Eudiplodinium maggii +	−	−
13R	*Entodinium* sp. +++	+	−
	Isotricha intestinalis ++	−	−
	Isotricha prostoma ++	−	−
	Dasytricha ruminantium +	−	−
	Ophryoscolex caudatus ++	−	−

[a] Each experiment was replicated once.

[b] Presence of protozoa: −, none; +, small number; ++, moderate number; +++, large number.

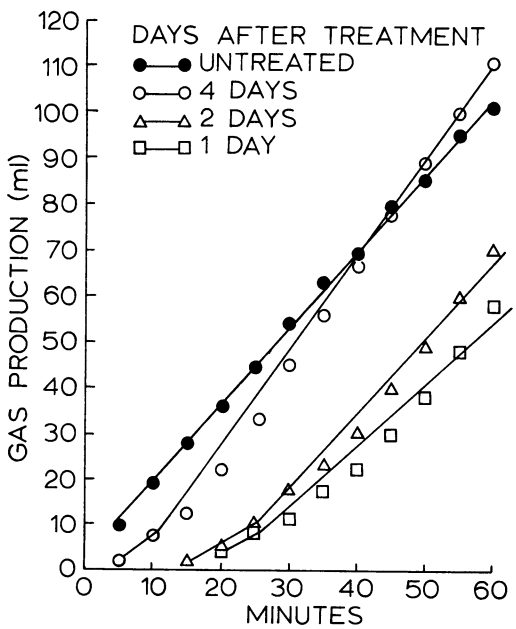

FIG. 1. *Effect of Aerosol OT on gas production in the rumen. Each value is an average of two samples from each of two animals.*

samples of each dilution were spread over the surface of starch agar plates (13) with glass spreaders. The plates were incubated under CO_2 atmosphere for 48 hr at 39 C, and then sprayed with Gram's iodine solution. Colonies with a clear halo of hydrolyzed starch were counted. Standard plate counts were determined for each rumen sample.

Rumen fermentation rate measurements. Rumen contents (50-ml quantities) were transferred into prewarmed jars, and the rate of gas production was measured for 60 min by the el-Shazly and Hungate method (14).

Ratio of carbon-dioxide to methane in rumen gas. Rumen gas was obtained through a sampling port positioned in the cap of a rumen fistula plug. At each sampling, 50 ml of gas was collected in a gas-tight syringe from the free gas space in the dorsal rumen. Gas was analyzed for carbon dioxide and methane by gas-liquid chromatography, by the use of Varian Aerograph A-90-P. The column was 0.84 cm of 10% PEG (15 to 20 M) on gas-chrom Z 80/100. Helium gas carrier with a flow rate of 55 ml/min was used. The separation was obtained at room temperature (25 to 30 C).

In vitro cellulose digestion. The ability of rumen microorganisms to digest cellulose was determined by incubating 25 ml of rumen fluid with 0.5 g of air-dried alfalfa hay for 48 hr, using the simplified artificial rumen procedure of Baumgardt et al. (3).

Total volatile fatty acids and ammonia were determined in the rumen contents by methods described previously (1). After samples of rumen fluid were

TABLE 2. *Ruminal activities of cows 4 days after defaunation with Aerosol OT*[a]

Ruminal activities	Cow 13R	Cow 36D
*p*H	6.58	6.72
VFA (μmole/100 ml)	10.65	11.50
Ammonia (mg/100 ml)	33.4	30.4
In vitro cellulose digestion (%)	49.35	44.60
Streptococcus counts	2.73×10^8	3.01×10^8

[a] Samples of rumen contents were obtained 3 hr after feeding and 4 days after treatment with Aerosol OT.

TABLE 3. *Ratio of carbon dioxide to methane in rumen gas of cows 2 days before and 5 days after defaunation with Aerosol OT*[a]

Cow no.	Carbon dioxide to methane ratio[b]					
	Days pretreatment		Days posttreatment			
	1	2	1	2	3	5
34	1.48	2.06	2.00	2.19	3.17	3.10
	1.44	2.07	2.02	1.04	3.15	3.14
37	1.87	1.83	1.79	1.83	1.99	2.47
	1.79	1.78	1.74	1.82	2.03	2.52

[a] Samples of gas were obtained 3 hr after feeding. Each value is an average of duplicate determinations.

[b] Methane = 1.

collected, *p*H value was determined immediately with a glass electrode.

RESULTS AND DISCUSSION

Effect of Aerosol OT on the rumen ciliate protozoa. Protozoal populations of the two Jersey cows, before and after Aerosol OT was administered, are indicated in Table 1. One dose obviously killed all types of rumen protozoa except for a few sluggish entodinia, which disappeared from rumen contents after the second dosing. Larger oligotrichs, *Epidinium caudatum* and *Ophryoscolex caudatus*, seem to be more susceptible to Aerosol OT than is the smaller oligotrich *Entodinium* sp. Administering Aerosol OT through the cannula or by capsule was equally lethal to rumen ciliate protozoa. Aerosol OT offers obvious advantages over previously used antiprotozoal agents that are effective only against holotrich protozoa (7).

Effect of Aerosol OT on rumen activity. Rate of gas production in rumen contents 1, 3, and 4 days after Aerosol OT treatment was measured (Fig. 1). Treatment depressed total rumen fermentation for 3 days after treatment, but rumen fermentation rate was normal on the 4th day. Treated animals remained healthy throughout the experiment except for going off feed for 2 to 3 days after treatment, which was overcome by feeding a highly palatable ration.

Values of *p*H, volatile fatty acids, ammonia and in vitro cellulose digestion of rumen contents obtained from defaunated cows 4 days after treatment are given in Table 2 . The values are normal compared to those previously reported (3, 10, 12). Ratio of carbon dioxide to methane appeared to be not unduly disturbed by Aerosol OT treatment (Table 3).

Isolating typical rumen starch-fermenting amylolytic streptococci similar to *Streptococcus bovis* (Table 2) from the microbial system after the death of all rumen ciliates indicates little deviation from a normal concentration of rumen bacteria 4 days after Aerosol OT treatment. Furthermore, previous studies (10) gave 10^7 per gram as an average figure for *Streptococcus* in the rumen.

Aerosol OT appears to be an effective and safe defaunative. It leaves conditions in the rumen normal shtorly after defaunation, which permits the use of defaunated ruminants in ruminal studies. When extreme care is taken to keep animals isolated, it has been possible to maintain animals in a ciliate-free condition for at least 2 weeks (duration of the experiment). Animals defaunated with Aerosol OT are now being used to compare ruminal activities of faunated and defaunated cows that are maintained under various feeding regimes.

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