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A Study of the Effects of Aureomycin Upon the Microflora of the Ovine Rumen

Milton Allison

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A STUDY OF THE EFFECTS OF AUREOMYCIN UPON THE MICROFLORA OF THE OVINE RUMEN

by

Milton J. Allison

Submitted to the Graduate Faculty of South Dakota State College of Agricultural and Mechanic Arts in Partial Fulfillment of the Requirement for the Degree of Master of Science November, 1954
A STUDY OF THE EFFECTS OF AUREOMYCIN
UPON THE MICROFLORA OF THE OVINE RUMEN

By
Milton J. Allison

This thesis is approved as a creditable, independent investigation
by a candidate for the degree, Master of Science, and acceptable
as meeting the thesis requirements for this degree; but without
implying that the conclusions reached by the candidate are neces­
sarily the conclusions of the major department.
ACKNOWLEDGMENT

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Acknowledgment is made to Dr. L. B. Embry and the Department of Animal Husbandry for the use and care of test animals needed to carry out this investigation.

Appreciation is also expressed to Marjorie Allison, wife of the author, for her assistance in preparation of figures and for her encouragement during the writing of this thesis.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title Page</td>
<td>1</td>
</tr>
<tr>
<td>Approval Sheet</td>
<td>ii</td>
</tr>
<tr>
<td>Acknowledgment</td>
<td>iii</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>iv</td>
</tr>
<tr>
<td>List of Tables</td>
<td>v</td>
</tr>
<tr>
<td>List of Figures</td>
<td>vi</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Review of Literature</td>
<td>3</td>
</tr>
<tr>
<td>Methods</td>
<td>13</td>
</tr>
<tr>
<td>Results and Discussion</td>
<td>20</td>
</tr>
<tr>
<td>Conclusion and Summary</td>
<td>28</td>
</tr>
<tr>
<td>Literature cited</td>
<td>30</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1. Composition of the Basic Ration ...................... 13
Table 2. Average Feed Consumption Expressed as Pounds
Consumed Daily .................................................. 20
LIST OF FIGURES

Figure 1. Apparatus used to obtain the rumen samples ...... 16
Figure 2. Brewer anaerobic jar set up .......................... 16
Figure 3. Anaerobic growth in shake-culture tubes .......... 19
Figure 4. Numbers of organisms cultured on endos agar ...... 23
Figure 5. Numbers of organisms cultured on eosin methylene blue agar ........................................ 24
Figure 6. Numbers of organisms cultured aerobically on medium "A" ............................................... 25
Figure 7. Numbers of organisms cultured anaerobically on medium "A" ............................................... 26
INTRODUCTION

Growth stimulation in domestic animals due to dietary supplementations of antibiotics has received a great deal of attention in the last few years. Moore and co-workers in 1946 (35) observed that the addition of streptomycin to the diet of chicks led to increased growth responses and in 1948 Harned et al. (17) administered aureomycin to chicks and he also noted an increased growth rate. Little significance was attached to these discoveries and the present interest in antibiotics as feed supplements is the result of investigations by Stokstad and co-workers in 1949 (40) with cultures of *Streptomyces griseus* and *Streptomyces aureofaciens* as sources of vitamin B₁₂. These workers found that growth promotion using these cultures was not entirely due to the presence of vitamin B₁₂ because accelerated growth was observed when the basal diet contained adequate amounts of that vitamin. Other workers became interested and their experimental work soon demonstrated the economic practicability of antibiotic supplementations in the diets of certain farm animals.

The diets of growing pigs and poultry at the present time are quite generally supplemented with antibiotics. Improved feed efficiency, an increased rate of gain and a reduction in the incidence of scour have been demonstrated through numerous experiments in which antibiotics have been administered to calves.

There is considerable disagreement regarding the economic practicability of antibiotics in the rations of older ruminants.
Several workers have observed definite deleterious effects as a result of antibiotic supplementation; while others have been able to detect no change and still others report increased rates of gain and greater feed efficiency when older ruminants are fed antibiotics.

Nutritionists interested in ruminant antibiotic supplementation are confronted with several problems which have not been completely solved. There are the problems of evaluating the usefulness of the antibiotics in relation to milk production in cattle, wool production in sheep, growth, carcass quality, reproductivity and hardiness of offspring in both species, and establishing the most practical level of antibiotic in relation to basal diet, size and species of animal.

Many have speculated and some have attempted to determine experimentally the mode of antibiotic action in feed supplements. Since ruminant nutrition basically depends on bacterial synthesis, antibiotics might be expected to alter or affect this synthesis by changing the number or kinds of microorganisms present. This research was begun to determine whether or not such changes do occur when a given level of antibiotic is included in the diet of an adult sheep.
REVIEW OF LITERATURE

Zuntz (46) in 1891 suggested that the bacteria of the rumen were symbionts rather than commensals. This idea, called the "Hypothesis of Zuntz", has been vindicated many times as research workers have studied rumination and the physiology of ruminant bacteria. Hastings (18) in a review of ruminant digestion stated that "The transformation of food into microbes is so great that some have been led to say that the ruminant lives not on the material ingested but upon the mass of microorganisms that has been formed from that material."

The ruminant stomach consists of four compartments: the rumen, the reticulum, the omasum and the abomasum. The first three of these cavities or compartments are merely dilations of the esophagus and they secrete no digestive juices. The rumen is the largest of these compartments as it accounts for about 80 per cent of the stomach capacity.

The physical and chemical conditions which exist in the rumen are conducive to the growth of large numbers of microorganisms. Studying these conditions Burroughs and co-workers (4) found that continuous recorded temperatures in cattle through rumen fistulas gave an average value of 40°C. Also, the moisture content of the rumen presented a condition highly favorable for microbial growth in that it usually varied between 85 and 90 per cent. The food or substrate necessary for microbial maintenance appears to be present in adequate or even optimal amounts and microbial products are constantly removed so as to prevent any inhibitions which might
result if they accumulated. McDougall, (31) stated that the saliva of the ruminant provides the basal media for the microorganisms present in the rumen; it is well suited for this purpose considering its buffering capabilities. The saliva contains a considerable quantity of sodium bicarbonate which maintains the reaction very near neutrality.

Cellulose, an important source of ruminant energy, is utilized only through the action of symbiotic microorganisms since the saliva of the ruminant contains no cellulase nor does the rumen secrete this enzyme. The useful products of cellulose breakdown are mainly short chain fatty acids, especially propionic acid. The mechanisms of this degradation are complex if only because of the many different kinds of microorganisms involved.

Direct microscopy and cultural methods have been employed in the study of cellulose fermentation with the cultural observations made by Hungate being particularly outstanding. In 1944 (23) he reported the isolation of Clostridium cellulosporum, a Gram positive, spore forming anaerobe that fermented cellulose. In 1950 (25) he described an organism which he called Bacteroides succinogenes, a Gram negative, non-spore forming, anaerobic rod also capable of fermenting cellulose. He found that cellulose digesting bacteria existed in numbers as high as 100 million per gram of rumen contents. He considered a colorless coccus to be the most important cellulolytic organism in the rumen because of the rapid rate at which it digested cellulose.
Heald (20) studied the fermentation of xylan in the rumen of sheep. The results he obtained suggested that a sheep on pasture might ferment a nutritionally significant quantity of xylan— as much as 60 to 80 grams in 24 hours.

Loosli et al. (29) demonstrated that when sheep are fed a purified diet in which urea is the only source of nitrogen, the 10 amino acids essential for rat growth are synthesized. Reed and co-workers (39) fed growing rats rumen bacterial protein and they found that as far as the rat is concerned this protein must be regarded as low in digestibility, relatively high in biological value, but mildly deficient in methionine.

The proteolytic action of rumen microorganisms was shown by McDonald (30). El Shazly (12) made a determination of the fate of the free amino acids and he found the main reaction products from casein are ammonia, carbon dioxide and volatile fatty acids.

Vitamin synthesis has been studied by Kon and Porter (28), by McElroy and Goss (32) and others. It is known that the synthesis of the B complex group and vitamin K is accomplished by rumen microorganisms; however, results are mostly qualitative and amounts of synthesis have not been shown. Attempts have been made to identify specific microorganisms with the synthesis of vitamins but these have not been successful.

Phillipson (38) estimated that the lower fatty acids produced in the rumen would be sufficient to supply at least 40 per cent of the fasting energy requirement. These fatty acids which are produced through bacterial action are in part absorbed through the walls of the rumen. Barcroft et al. (1) found that
the amount of volatile fatty acid in the blood draining the rumen of sheep, expressed as acetic acid, ranged from one to five grams per hour.

Two methods have been used to remove samples from the rumen. A stomach tube attached to a vacuum pump is most practical when a number of animals are to be sampled because of its simplicity and because it requires no alteration of the test animal. The main disadvantage of this method is that it is impossible to know from what portion of the rumen the sample is being taken. If the ration has roughage such as corn cobs in it and if considerable quantities of such material are still in the rumen when the sample is taken the tube may become clogged. Doetah et al. (9) found the stomach tube impractical for this reason but were able to remove samples from the bovine rumen by "gentle up and down motion" of the tube without the aid of vacuum. Moir and Williams (34) used a stomach tube to obtain samples from sheep which were then immediately killed and further representative whole rumen samples obtained. Direct microscopic methods were used and they felt that the relatively close comparison of the total count of microorganisms between whole rumen and stomach tube samples justified the latter method of sampling. Many workers have used fistulated animals and simply withdrawn material through the opening. Using this method, the worker is able to tell from which part of the rumen the sample is obtained. The disadvantage of this method is the necessity for a surgical operation and the artificiality thus induced may influence the conditions in the rumen.
Van der Wath (42) found a seasonal variation of protozoa in the rumen of sheep. He found less than 100,000 per milliliter while the animals were grazing on winter pasture and up to 450,000 per milliliter while grazing on summer pasture. Hastings (18) reported that the number of protozoa found in bovine rumen fluid will range from 500 million to one billion per milliliter. Various workers have speculated regarding the importance of protozoa in ruminant digestion. Owen (37) reported a better utilization of food by lambs in which the protozoa had been killed by drenching with a 2 per cent CuSO₄ solution. Elsdon and Phillipson (11) stated that there is considerable evidence that protozoa play no vital role in ruminant digestion and when they were dispensed with no ill effects were experienced by the host.

The bacteria of the rumen have been studied by both cultural and direct microscopic methods. Many of the cultural methods were designed to study certain groups of organisms or certain mechanisms, for example, the methods employed by Hungate (23) (24) who studied cellulolytic action. Doetsch et al. (10) in a study of the nutritional requirements of bovine rumen bacteria found that no synthetic medium supported growth as well as a medium which contained rumen fluid but the essential factors in this substance have not been identified. He also stated that the rich nitrogenous materials ordinarily used to culture fastidious bacteria were not satisfactory, in fact some of these substances were inhibitory. Wasserman et al. (44) reported the stimulatory action of an unidentified factor present in crude materials upon certain anaerobic lactobacilli from the rumen. Adding to the medium 20 crystalline amino acids and
certain vitamins did not replace the stimulating factor in the crude materials.

Hunegate (25) and Ruhtanen et al. (22) considered it necessary to keep air away from the samples of rumen fluid to be cultured. They also emphasized the importance of speed, stating that samples should be processed within 20 minutes after they were removed from the animal. Doetsch et al. (9) found that these precautions were not necessary for they cultured the same number of organisms from samples that had been exposed to the air for six hours as they did from samples processed immediately.

Numbers of organisms have varied when cultural methods of counting were used. Doetsch et al. (9) using a non differential medium obtained colony counts of 10 to 80 million per milliliter. Using a liquid dilution series Gall et al. (15) found 50 to 100 billion organisms per gram. Other workers making total counts have usually arrived at figures between these values, but in no case have as many organisms been cultured on solid media as have been counted with liquid dilution procedures.

Direct microscopic methods have enabled the investigators to study the rumen population qualitatively in that morphology and cytochemical staining reactions have been observed. Quantitative studies have also been made using a method which involved dilution of the sample and placing a measured quantity on a measured area of a glass slide. Organisms were stained and counted using a microscope which had been calibrated for this purpose. Counts have also been made using a Petroff Hauser counting chamber. Numbers of organisms counted using the micro-
acope vary significantly. Moir (33) found bacteria in numbers ranging from 32 to 88 billion per cubic centimeter of material from the rumen of sheep, while Walter (43) found from 10 to 42 billion per milliliter of bovine rumen fluid. Gall and co-workers (13) reported counts averaging about 150 billion organisms per gram of rumen contents from both cattle and sheep.

The newer methods of ruminology are in vitro studies which include manometric techniques using washed suspensions of rumen bacteria and studies with the artificial rumen. There are certain disadvantages to these methods but when properly used valuable information can be obtained.

Although some of the bacteria of the rumen have been isolated and classified, the taxonomic position of most of the organisms is not known. Workers have usually classified the groups of organisms as to their morphology, Gram reaction and their ability to live in the absence or presence of oxygen. Huhtanen and Gall (21) isolated nine rods, both Gram negative and Gram positive, all of which had the qualifications considered necessary to play major roles in the digestion of the ruminant.

Bortree and co-workers (3) were among the first to point out that the ration has an influence upon the microflora of the rumen of cattle. Using a direct microscopic procedure and counting only the organisms which stained a deep blue with iodine, they found that there is a rapid increase in the number of these organisms within two hours after the animals have been fed. High
counts of organisms were maintained or increased for a period and then gradually returned to the range observed prior to feeding. The addition of glucose to the usual feeding of hay resulted in a 100 per cent increase in numbers. Feeding starch plus hay resulted in only a slight difference from that observed when hay was fed alone.

Gall et al. (13) studied both cattle and sheep on summer and winter rations. They obtained higher total counts from animals on winter rations than they did from animals on pasture. Gram stains were very similar, with greater numbers of cocci from animals on a high grain diet. The presence of Sarcina and star-shaped organisms was noted from animals on pasture but were seldom if ever, found in animals on winter rations. In later work, Gall et al. (14) showed that a high grain diet increased the numbers of organisms already present rather than producing an entirely different flora. In contrast to the results obtained previously, animals on pasture had higher total counts than those on winter rations. Bauman (2) also reported cocci in large numbers from cattle on a high grain ration. In experiments feeding purified diets to sheep and lambs, Gall et al. (16) showed that the ration had a pronounced effect on both the kind and number of bacteria present in the rumen. Three groups of animals were fed the same basic purified rations; a group which had its ration supplemented by urea plus sulfur and a group with rations supplemented with casein showed about double the total bacterial population supported by the animals whose basic diet was supplemented by urea without sulfur.
The growth rate of fattening lambs was depressed when they were fed aureomycin, penicillin, or streptomycin at a rate of 100 milligrams per day per animal by Colby et al. (8). Lambs receiving aureomycin went "off feed" and lost 0.16 pounds per day while control animals gained 0.52 pounds per day. Decreased fiber digestion and anorexia resulting from aureomycin supplementation were also noted. At levels of 6 to 12 milligrams of aureomycin daily, Jordon and Bell (27) found that fattening lambs on a corn alfalfa ration, required 20 to 22 per cent less concentrate per 100 pounds gain. Twenty one lambs fed 6 milligrams per day gained 0.49 pounds daily while controls gained 0.39 pounds. In a second trial lambs fed 10.8 milligrams per day gained 0.40 pounds while the controls gained 0.36 pounds per day. In a later experiment Jordon (26) fed lambs at a rate of 7.2 to 14 milligrams per day. It was noted that the higher levels decreased the body weight gains. In one of the trials the antibiotic reduced the incidence of enterotoxemia.

Hatfield and Garrigus (19) found that the daily gains were higher for lambs on rations supplemented with aureomycin at a rate of 10 milligrams per pound of concentrate mixture but no significant differences were noted as far as feed efficiency was concerned. Turner and Hodgett (41) administered aureomycin to three sheep at the rate of 23.6 to 27.1 milligrams per kilogram of body weight. It was found that this relatively high level of antibiotic caused total bacterial numbers in the rumen to be decreased by 75 per cent within two hours. Fermentation within the rumen was greatly depressed and the appetites of the sheep were reduced.
In an experiment conducted by Neuman et al. (36) yearling beef heifers on a fattening ration were fed 2 milligrams of aureomycin per pound of air dry feed. There were no important physiological changes and the rate of gain was unaffected. It was determined that the antibiotic brought about no change in total counts, but the types of organisms found in the animals receiving aureomycin were less diverse.

A series of articles were published by Chance et al. (5) (6) (7) regarding the effect aureomycin has upon rumen function and upon rumen microorganisms. Crystalline aureomycin hydrochloride was administered to two year old fistulated steers at the rates of 0.5 gram per day for one 15 day test period followed by one gram per day for a similar period. Bacteriological studies indicated that the lower level of antibiotic stimulated bacterial action because total counts were higher than controls when the 0.5 gram level was fed. Both levels of antibiotic brought about a reduction in numbers of streptococci. Coliform counts were inconsistent since aureomycin brought about an increase when administered to one of the two steers, but failed to change the counts in the other.

Although antibiotics had proved their value as growth stimulators when administered to certain non-ruminant animals, it was conjectured that they might interfere with the nutrition of ruminant animals by destroying necessary rumen microorganisms. The truth of this supposition has not been proved when antibiotics are used at relatively low levels and workers in the field are not in agreement with respect to their nutritional value. The research which has been done presents a picture which is as yet incomplete.
## METHODS

Six yearling wethers predominately of Rambouillet breeding were furnished by the Animal Husbandry Department of South Dakota State College to be used as test animals in this project. They were paired and each of the three groups was fed on a different ration.

### TABLE 1.
**Composition of the Basic Rations**

<table>
<thead>
<tr>
<th>Ration No.</th>
<th>Animals Fed</th>
<th>Composition</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7 and 8</td>
<td>Chopped alfalfa and</td>
<td>84.5 per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bromegrass hay</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ground oats</td>
<td>7.5 per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ground shelled corn</td>
<td>7.5 per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salt</td>
<td>0.5 per cent</td>
</tr>
<tr>
<td>2</td>
<td>9 and 10</td>
<td>Chopped alfalfa and</td>
<td>39.0 per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>bromegrass hay</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ground oats</td>
<td>30.0 per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ground shelled corn</td>
<td>30.0 per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Limestone</td>
<td>0.5 per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salt</td>
<td>0.5 per cent</td>
</tr>
<tr>
<td>3</td>
<td>11 and 12</td>
<td>Ground corn cobs</td>
<td>40.0 per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ground shelled corn</td>
<td>50.0 per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Corn gluten meal</td>
<td>7.0 per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urea</td>
<td>1.0 per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bone meal</td>
<td>0.5 per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Limestone</td>
<td>1.0 per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salt</td>
<td>0.5 per cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitamin A</td>
<td>1,000 units per lb.</td>
</tr>
</tbody>
</table>

The animals were their own controls, as they were observed experimentally for a 12 week period before having their diet supplemented with aureomycin, and for 10 weeks after the antibiotic was removed from the ration. The aureomycin was fed for a 15
week period in the form of Aureofac 2A* at a level supplying approxi-

mately 10 milligrams of aureomycin per pound of total ration.

Samples were taken in the afternoon seven to eight hours after
feeding. Variations in bacterial numbers resulting from the intake
of feed, as shown by Bortree et al. (3), should be at a minimum
after this length of time. All samples were obtained using a
stomach tube which consisted of a section of rubber tubing with a
bore diameter of 0.8 centimeters and an outside diameter of 1.8
centimeters. This tube was about 2 meters long and was smooth on
the outside. Small holes were bored into the tubing near one end
and a rubber stopper was placed in the tip. When the first samples
were taken, the tube was inserted through a short piece of heavier
rubber tubing in the animal's mouth to keep it from biting the
stomach tube. Later it was found that this precaution was unnecessary,
if the tube was moved over to the side of the mouth after it had
started down the esophagus. Care was taken to avoid forcing the tube
into the trachea.

When the first samples were taken the sheep were held so they
sat on their haunches. However, it was found that samples could be
obtained more easily while the animals were standing with their
heads held in a stanchion. A sterile 500 milliliter suction flask
was attached by means of a rubber stopper to the end of the stomach
tube and vacuum was applied so that the sample could be drawn into
the flask. The vacuum pump, which was powered by an electric motor,

* Aureofac 2A is a residual fermentation product from the manufacture
of aureomycin.
was adjusted so that approximately 15 pounds of negative pressure developed. (Fig. 1, page 16).

Occasional difficulty was encountered while sampling the animals which had corn cobs in their ration because the tube became plugged. At such times the first action was to move the tube up and down slightly and if this did not help, the tube was removed and flushed out with water.

The material withdrawn from the rumen was mostly liquid with some solid particles. The pH of a number of samples was measured with a Beckman pH meter (Model G) and it was found to range between 6.9 and 7.3. The distilled water to be used as a diluent was buffered with 0.1 per cent each of KH₂PO₄, K₂HPO₄ and NaHCO₃ so that its pH was approximately 7.1. Ten milliliters of each sample were placed in bottles containing 90 milliliters of the sterile, buffered water giving a dilution of 1:10. Each bottle was shaken by hand 200 times and one milliliter of this solution was pipetted into a sterile, buffered 99 milliliter dilution blank to give a dilution of 1:1000. Several dilutions were made in this manner so that the final dilution had a concentration of 10⁻⁹.

Preparation and selection of media involved a considerable amount of trial and error. A number of types of prepared media were used initially and from these were selected those which appeared to give the most consistent counts. The prepared media used most extensively were Difco eosin methylene blue agar and endos agar. Several non selective types of media were tried; these were designed to support growth of a maximum number of the bacterial types which inhabit the
Fig. 1. Apparatus used to obtain the rumen samples.

Fig. 2. Brewer anaerobic jar set up.
rumen. A hay infusion, to which agar and a number of inorganic salts were added, permitted considerable growth, but a precipitate formed, which made it difficult to distinguish the colonies. A second medium, developed by Hungate (24), was also used. The organic matter in this medium was supplied as rumen fluid and to this fluid an inorganic salt solution, agar and a reducing agent (cysteine hydrochloride) were added. This medium was especially designed to promote the growth of cellulolytic bacteria, but total counts of organisms were not very high.

A medium developed by Huhtanen et al. (22) designated as medium "A", was used for both aerobic and anaerobic cultures. This medium consisted of 1 per cent each of peptone, tryptone, beef extract, and yeast extract, 0.1 per cent each of K$_2$HPO$_4$ and glucose, and 1.5 per cent agar. When anaerobic organisms were to be cultured, the air above the medium was displaced by CO$_2$ immediately after being autoclaved to prevent oxygen from becoming dispersed in the medium. The standard pour plate method of culture was used. Depending upon the rate of growth, incubation was from two to seven days at a temperature of 37°C.

Anaerobic cultural conditions were established with a Brewer anaerobic jar which used the combustion of illuminating gas to obtain the anaerobiosis. The cultures in inverted petri dishes were placed in the jar along with soda lime (to absorb excess carbon dioxide) and a methylene blue solution which acted as an oxidation-reduction indicator. Since the anaerobic growth was quite slow, the jars were incubated a week before colonies were counted. (Fig. 2, page 16.)
A technique was developed using agar shake tubes for culturing anaerobic organisms. Standard 16 x 150 millimeter culture tubes were filled with medium "A" and sterilized in an autoclave. Immediately after sterilization the small amount of air at the top of these tubes was displaced with carbon dioxide and sterile rubber stoppers were placed in them. When the medium had cooled to approximately 45°C, one milliliter of the desired dilution of rumen fluid was pipetted into the tube. Carbon dioxide was again used to displace any oxygen which might have entered and the sample was mixed throughout the medium by slowly inverting and rolling the tube. After incubation at 37°C for five to seven days growth was observed. The appearance of the colonies was much the same as those cultured in the Brewer anaerobic jar. Microscopic observation showed that their morphology and Gram reaction were also the same. The curvature of the tubes made it very difficult to count colonies and for this reason the method was not used for comparative counts. (Fig. 3, page 19).

Microscopic observations were made in conjunction with all the cultural work. Gram stains of colonies from the petri dish cultures were made at regular intervals as were Gram stains from 1:10 and 1:1000 dilutions of rumen fluid.
Fig. 3. Anaerobic growth in shake-culture tubes.
RESULTS AND DISCUSSION

A daily record was made of the amount of feed which was fed to each animal. At intervals of two weeks, the weight of the feed was totaled, from which the weight of the ords was subtracted so that the average daily feed intake might be calculated.

TABLE 2
Average Feed Consumption Expressed as Pounds Consumed Daily

<table>
<thead>
<tr>
<th>Period</th>
<th>Length of Time (weeks)</th>
<th>Animal No. 7</th>
<th>Animal No. 8</th>
<th>Animal No. 9</th>
<th>Animal No. 10</th>
<th>Animal No. 11</th>
<th>Animal No. 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Antibiotic Control Period</td>
<td>12</td>
<td>2.80</td>
<td>2.91</td>
<td>3.34</td>
<td>3.59</td>
<td>3.34</td>
<td>3.32</td>
</tr>
<tr>
<td>Antibiotic Period</td>
<td>15</td>
<td>2.53</td>
<td>2.76</td>
<td>2.76</td>
<td>3.13</td>
<td>2.78</td>
<td>2.63</td>
</tr>
<tr>
<td>Post-Antibiotic Control Period</td>
<td>10</td>
<td>3.00</td>
<td>3.37</td>
<td>3.00</td>
<td>3.24</td>
<td>1.95</td>
<td>1.77</td>
</tr>
</tbody>
</table>

Aureomycin was fed at a level averaging 28.31 milligrams per animal per day, supplying a daily average of 19.21 milligrams per 100 pounds of body weight. A decrease in the feed consumption of each animal was noted when aureomycin was included in the ration. The average daily feed consumption for animals 7, 8, 9 and 10 during the pre-antibiotic control period was 3.16 pounds, but when these sheep were fed aureomycin it dropped to 2.80 pounds, returning in the post-antibiotic control period to 3.15 pounds per day. Animals 11 and 12, which were on the corn-cob and urea diet, went "off feed" near the end of the antibiotic period, and the
relatively low rate of consumption continued throughout the post-
antibiotic control period.

When the animals were grouped as to the rations they were fed, it was found that sheep on ration No. 1 experienced a 7.37 per cent reduction in feed intake when the antibiotic was added to the ration. Feed consumption of animals on ration No. 2 was reduced by 14.98 per cent and that of animals on ration No. 3 was reduced by 18.62 per cent.

During the course of the 15 week antibiotic period, at least eight cultures on each medium were made from the rumen samples. A similar number were cultured during one or both of the control periods. Colony counts varied considerably, and in order to minimize the effect of the wide variation, logarithmic averages of the counts were computed. The numbers of bacteria which were observed during the test periods are shown on Figures 4, 5, 6 and 7.

Endos agar and eosin methylene blue agar are specialized media giving typical color reactions when coliform organisms are cultured on them. In this study only the colonies which gave these typical reactions were counted. Frequent microscopic checks were made confirming that the small Gram negative rods were being counted. The difference which exists between the numbers of organisms counted on the two media is due to a difference in media specificity. Apparently the eosin methylene blue agar is less specific than the endos agar as far as the coliform organisms of the rumen are concerned.
The results obtained show that the addition of aureomycin to the ration at the level which was fed brought about a definite lowering of the number of coliform organisms in the rumen. Animals on all three of the rations experienced this decrease. Cultures on both media showed that fewer coliform organisms were present in the rumina of both animals on the corn-cob urea ration; this was true during both control and antibiotic periods.

Under aerobic conditions larger numbers of organisms were cultured on medium "A" than on any of the other media. A wide variety of morphological types were found capable of growing on this medium. Gram stains showed the presence of Gram negative rods 1 to 2 microns long, singly and in chains, and Gram negative rods 0.5 microns long or smaller occurring singly in punctiform colonies. The latter did not stain very well and being extremely small, were difficult to discern. Gram positive organisms cultured included rods 1 to 2 microns long occurring singly and cocci appearing both in clusters and singly. Although no differential count was made, it was evident that the Gram positive organisms were more numerous than the Gram negative organisms. Many of the Gram positive rods were found in subsurface, circular, lens-shaped, opaque colonies (1 to 2 millimeters in diameter) with the edge entire or slightly irregular. This is the same type of colony that was cultured in the agar shake tubes.

Aureomycin brought about a more pronounced reduction of the numbers of bacteria which grew aerobically medium "A" than it
Fig. 4. Numbers of organisms cultured on endos agar. First bar of each group indicates pre-antibiotic control period, second bar indicates antibiotic period and the third bar indicates the post-antibiotic period.
Fig. 5. Numbers of organisms cultured on eosin methylene blue agar. First bar of each pair indicates antibiotic period and second bar indicates post-antibiotic control period.
Fig. 6. Numbers of organisms cultured aerobically on medium "A". First bar of each pair indicates antibiotic period while the second bar indicates the post-antibiotic control period.
Fig. 7. Numbers of organisms cultured anaerobically on medium "A". First bar of each pair indicates antibiotic period while the second bar indicates the post-antibiotic control period.
did on organisms cultured on the other media. The average reduction in this case was from 1,600,000 organisms per milliliter to 320,000 per milliliter, a reduction to about 20 per cent of the original number of organisms. The greatest average reduction of organisms cultured on other media was on eosin methylene blue agar where a reduction from 790,000 organisms per milliliter to 308,000 per milliliter, or to approximately 39 per cent of the original number was shown.

No variation in morphology or in colony appearance could be demonstrated as a result of the differences in rations or from the additions of aureomycin to the rations.

The results obtained indicate that during the control period just as many of the organisms, capable of growing on this type of medium, were present in the rumina of animals on the corn-cob urea ration as were found in the rumina of animals on the other rations. This is in contrast to the results obtained when coliform organisms were cultured.

The organisms which were cultured on medium "A" under anaerobic conditions could not be distinguished morphologically or by colony appearance from those which grew aerobically on the same medium. It is thought that many of these are facultative organisms and thus able to multiply under either aerobic or anaerobic conditions.

As was noted with the aerobic cultures of medium "A", the animals on Rations No. 3 supported a ruminal flora with a relatively high number of organisms as compared to the animals on the other rations and in contrast to the results obtained while culturing the coliform organisms.
CONCLUSIONS AND SUMMARY

Three groups of sheep on separate rations were observed to determine what effect the addition of aureomycin to the ration would have on the rumen microflora. Bacteriological observations were made on the rumen fluid during three periods, the first and last of which were control periods, while the aureomycin was fed during the second period.

A cultural method of study was used which made it possible to determine bacterial numbers. Coliform organisms were cultured aerobically on two media and it was found that the number of organisms decreased when aureomycin was fed at a level of 10 milligrams per pound of feed.

On a non differential medium a variety of morphological types of organisms were cultured under both aerobic and anaerobic conditions. The bacteria which grew on this medium under the conditions provided were fewer in number when aureomycin was included in the ration than they were during a control period.

It is therefore concluded that when the ration of an adult sheep is supplemented with a given level of aureomycin, a definite quantitative modification of certain rumen bacteria is experienced.

It is not intimated that all the bacteria of the rumen were counted, nor is it suggested that the addition of aureomycin affects all of the microfloral inhabitants of the rumen in the same manner it did those in this study.
A better basic knowledge concerning bacterial participation in ruminant digestion is needed before the action of substances which appear to modify this participation can be interpreted physiologically.
LITERATURE CITED


