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# Ruminal Variation in pH, Temperature, Bacterial Flora, and In vitro Activity as Affected by Ration Change and Season

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# RUMINAL VARIATION IN pH, TEMPERATURE, BACTERIAL FLORA, AND *IN VITRO* ACTIVITY AS AFFECTED BY RATION CHANGE AND SEASON

BY

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RUMINAL VARIATION IN pH, TEMPERATURE, BACTERIAL FLORA AND *IN VITRO* ACTIVITY AS AFFECTED BY RATION CHANGE AND SEASON

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 necessarily the conclusions of the major department.

Thesis Adviser

Head of the Major Department

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N. T. M.

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# INTRODUCTION

The outstanding importance of the vast number of microorganisms in the rumen to the nutritional physiology of the ruminant makes it imperative that the factors influencing the numbers and types of these organisms be thoroughly understood. It may be possible for dietary needs of the ruminant to be assessed if the different fluctuations associated with any change in microbial population are known. A knowledge of when these ruminal fluctuations occur and their causes, whether it be due to external environment or dietary change, would be of significant value in the preparation of rations which could stimulate the ruminal flora to their maximum efficiency and in turn receive maximum feed efficiency from the animal.

These fluctuations are accentuated by ration change, and to some extent seasonal variation of the weather. To determine the extent of any of the various fluctuations known to occur in the rumen, periodic measurements would have to be made. It has been along these lines that this study has been based.

The specific objectives of this project are as follows: (1) to make periodic checks of the *in vivo* rumen temperature, (2) to make periodic determinations of *in vivo* rumen pH, (3) to make periodic rumen flora activity checks with the use of the artificial rumen, *in vitro*, (4) to graphically illustrate how these determinations were affected by any of the various factors, (5) to attempt to grow culturely, rumen microorganisms to determine any periodic concentration change which may occur.

# REVIEW OF LITERATURE

## Ruminal pH

Extensive work has been reported on the different elements affecting the rumen bacterial population. This work has varied in scope from physical and environmental factors to variable components attributed by chemical changes occurring in the rumen environment. One of the environmental conditions of prime importance is rumen pH. Considerable work has been reported as to its variation and conditions affecting these variations.

Kick et al. (1938) reported a pH range from 5.5 to 7.7 depending on the type of ration being fed. It was reported that when alfalfa alone was fed, the ingesta tended to be more alkaline and as the amount of corn was increased in the ration the pH became more acidic. These workers reported that diurnal variation occurred according to the time of feeding. After feeding the pH would become more acidic for a period of approximately eight hours. When the animals were fed again, the pH again would lower, at a slower rate, for about eight hours. The pH would then begin to rise until a peak was reached just before feeding the following morning. Monroe and Perkins (1938) also reported an alkaline pH just prior to feeding in the morning, and a drop in pH after feeding proceeded by a gradual rise reaching a peak immediately prior to the evening feed.

Phillipson (1942) reported that the pH of the ingesta varies in a regular manner; the speed and extent of this variation depends on the

type of ration being fed. He reported that these changes reflect the fluctuations in the quantities of organic acids that accumulate in the ingesta as a result of fermentation of food in the rumen. It was also stated that the fermentation rate in the rumen depends upon the diet being fed. It was most rapid, in this particular case, when mangolds and cabbages were fed in addition to roughage. It was less rapid when hay alone was fed. Myburgh and Quin (1943) agreed with previous work in reporting increased acidity with the first four to six hours following feeding, after which it steadily reverted back to its previous level.

Since differential readings are known to occur in different areas of the rumen at certain times, it is essential that any in vivo pH determinations be made with this in mind. Also of importance is the problem of saliva contamination encountered while using the stomach pump method for obtaining rumen fluid to be used for pH determinations. Monroe and Perkins (1939) also reported a series of various pH readings which were dependent upon the location in the rumen the determinations were made. The front portion of the rumen was found to be slightly more acidic than the posterior part. Determinations made at deeper positions showed the entire lower portion of the rumen to have a relatively constant pH of approximately 6.9.

Smith (1941) ran similar determinations which were considerably lower than in vivo pH determinations reported by other workers. The front portion of the rumen was found to be slightly more alkaline than the posterior part. The determinations made at deeper positions showed the entire lower portion of the rumen to have a relatively constant pH

of approximately 6.13.

It was reported that these low pH determinations may be due to the fact that *in vitro* samples taken to the laboratory for the determinations will tend to lose carbon dioxide making them considerably more alkaline. To prove this Smith made the following *in vitro* and *in vivo* comparison.

TABLE I. COMPARISONS OF *IN VITRO* AND *IN VIVO* pH DETERMINATIONS OF RUMEN INGESTA

| | Front | Deep Front | Middle | Deep Middle | Rear | Deep Rear |
|---|---|---|---|---|---|---|
| Animal No. 1 | | | | | | |
| *in vitro* | 6.90 | 6.55 | 6.57 | 6.43 | 6.50 | 6.57 |
| *in vivo* | 6.32 | 6.32 | 6.32 | 6.28 | 6.28 | 6.25 |
| Animal No. 2 | | | | | | |
| *in vitro* | 6.88 | 6.49 | 6.61 | 6.23 | 6.17 | 6.10 |
| *in vivo* | 6.35 | 6.40 | 6.10 | 6.02 | 5.83 | 6.16 |

Both *in vitro* and *in vivo* readings were taken at the same locations in the rumen. The *in vitro* determinations were made 30 minutes after the sample was taken. From these reports it can be easily seen there can be no set pH value placed on any specific section of the rumen. The more thoroughly the ingesta is mixed the more uniform will be the pH values.

To make determinations *in vitro* that will vary as little as possible from *in vivo* readings, it is imperative that the pH be determined as soon as possible after extraction from the rumen. Myburgh and

Quin (1943) reported a constant rise in the pH of the ingesta in relation to the length of time it was exposed to air. Blake et al. (1957) reported that rumen samples collected orally were consistently higher than those collected via rumen fistula. Clark and Lombard (1951) reported that this would be due largely to the basic reaction of saliva encountered via the stomach tube.

A diurnal pH fluctuation was observed by Nottle (1956) in which the pH changed in a manner similar to the diurnal fluctuation of rumen bacterial concentrations. He also reported that there was a seasonal trend in which the pH also followed the bacterial concentration trends of the rumen.

## Ruminal Temperature

Rumen temperature is an important intraruminal variation which must be given due consideration in determining what may affect rumen microorganisms. Using thermocouples and a recording potentiometer, Dale et al. (1954) reported that in vivo rumen temperature was 4° F. above the rectal temperature when feed was always available to the animal. When feed was removed for 24 hours, the rumen temperature was only 1.5° F. above the rectal temperature. When four pounds of hay were fed to the fasting animal, however, the temperature of the rumen increased (within 15 minutes after the cow began eating) from the prefeeding level of 38.3° C. to the fed level of almost 40° C. The normal temperature of the rumen in this experiment was approximately 39° C.

Mangeroni (1954) confirmed the fact that the ingestion of food

raises the intraruminal temperature. However, the extent that the temperature was raised cannot be considered to be of such a magnitude as to cause digestive disturbances. The theory that overeating may lead to detrimental effects due to excessive heat produced in the rumen, seems to be without foundation, according to the results obtained from Mangeroni's experiment. From the review thus far it seems reasonable to assume that with a change in microflora activity there may be a concurrent change in rumen temperature and acidity.

## In Vitro Determination of Cellulose Digestion

Studies on the role of rumen microorganisms in the nutrition of ruminant animals have led to the development of the "artificial rumen" or in vitro fermentation technique. This method of determining digestion coefficients has been widely used in recent years as a method of measuring the cellulose digestion of rumen microorganisms. Recent work by Hershberger et al. (1959) has shown a correlation coefficient of + 0.97 between in vivo and in vitro cellulose digestion, using ovine rumen microorganisms, all-glass fermentation flasks, and a 24-hour fermentation period for the in vitro analysis. Other work reported by LeFevre (1958) also showed a very high correlation between in vivo and in vitro cellulose digestion. Quicke et al. (1959) has reported that no significant difference was observed between results obtained in vitro and in vivo with grass hays, however, in some of the legume hays, cellulose digestibilities, as measured by the two methods, were significantly different. It was further explained that results obtained with the

*in vitro* technique showed good reproduction ability and, in general, variances were less than those calculated for the corresponding sheep trial data. Pigden and Bell (1955) have demonstrated that total digestable nutrient values obtained after a forty-eight hour fermentation period (*in vitro*) compared favorably with the values obtained with sheep (*in vivo*). Hueter et al. (1958) also reported a close agreement qualitatively between the *in vivo* and *in vitro* methods of determinations.

Washed rumen bacteria, purified diets, and whole filtered rumen liquor, have all been used for the *in vitro* determination of cellulose digestion. Barnett (1957) reports that the use of whole filtered rumen liquor may be used to greater advantage in obtaining greater correlation between *in vivo* and *in vitro* trials on the assumption that microflora used by this method are kept more active for a more suitable length of time. It was also reported in this experiment that good agreement was obtained between cellulose digestion *in vitro* and the digestion of the crude fiber of silages, as measured in sheep trials.

There are many variable factors known to affect the cellulolytic breakdown by the rumen microorganisms. It is a well known fact that the breakdown of cellulose and hemicelluloses is through enzymatic action. Since cellulose splitting enzymes are not secreted in the digestive tract of the ruminants, these enzymes must be produced by symbiotic microorganisms present in the rumen. Kitts and Underkofler (1954) reported that the cellulose degradation enzyme may be a "celloglucosidase" which hydrolyzes individual glucose units from the ends of cellulose chains. Gill and King (1957) reported that enzymes which catalyze the

hydrolysis of carboxymethylcellulose are made up of at least three protein species.

Of the many factors which may affect cellulose digestion by the rumen flora, ration components may well have the greatest effect. Louw and Van Der Wath (1943) reported that the addition of easily digestible carbohydrates such as starch, cane sugar, or glucose to the ration of cattle or sheep reduced the digestibility of the fiber. Hoflund et al. (1948) reported that small amounts of a readily available carbohydrate aided cellulose digestion, whereas large amounts inhibited it. This was substantiated by Hunt et al. (1954). It was also reported that optimal cellulose digestion is necessitated by a balance between readily available carbohydrates and protein. Hueter et al. (1958) later reported that carbohydrate enhances ammonium utilization. Burroughs et al. (1949) who further verified this, also reported an increase in need for protein or protein supplement in order to maintain efficient roughage digestion. Burroughs et al. (1950) reports that there are two groups of factors affecting rumen flora activity. The first represents those conditions which are more or less fixed or regulated by the physiology and anatomy of the host animal. Such variations as temperature, moisture, salts contained in saliva, anaerobiosis, absorption of organic acids through the rumen wall, motion of stirring, and possibly the exclusion of light would fall in the first group of influential conditions.

The second group represents conditions subject to variations that are related to the environment of the host animal. This would include

the types of organisms present in the rumen or in the inoculum of the fermentation flask, the nutrients available to the bacteria present, and certain properties of the medium such as pH, total acidity, oxidation reduction potential, total salt concentration and possibly symbiotic relations between the different types of microorganisms.

Quin et al. (1951) reported that the affect of fasting also decreased cellulose digestion in the rumen. Hall et al. (1953) and Bentley et al. (1954a) have reported that in vitro cellulose digestion was stimulated by certain B-vitamins, particularly, biotin, paraamino-benzoic acid (PABA), vitamin $B_{12}$, vitamin $B_6$, and pantothenic acid, as shown by an increased cellulose digestion and volatile fatty acid production. Macleod and Murray (1956) reported that optimum in vitro cellulose digestion occurred over a comparatively narrow range of nitrogen concentration in the medium and above this range, inhibition occurred.

## Bacterial Types and Concentration

Conditions which affect cellulose digestion are in turn directly related to the activity and concentration of the rumen microorganisms. It was reported by Gall et al. (1949) and Baker (1943) that species and breed within species do not play a major role in influencing the rumen flora, also the miscellaneous factors found in a change of location did not appear to influence the kinds or numbers of bacteria present in the rumen. Gall et al. (1948) found the only variable studied which did seem to influence the bacterial population was ration and here changes were

not so much qualitative as quantitative. A change in an animal's ration seems to affect the rumen flora to some extent as well as having, in some cases, adverse affects on the animal itself. Hoflund (1948) reported that even death may result in individual cases where there has been an abrupt change from a low to a high protein diet. Hungate et al. (1952) reported that frequent fatal indigestion can be enduced in sheep on a diet of hay by changing the diet to grain. It was explained that the probable reason for this is that when a ration is changed it causes significant fluctuations in the microbiological activities. The feed, subjected to immediate fermentation, will have adverse effects on the host. It has been well established that there are different concentrations as well as different types of organisms for high concentrate rations and high roughage rations. Gall et al. (1948) and (1949) reported that animals on a high grain ration showed more fast growing organisms and higher growth than the low grain or pasture animals. It was also reported the kinds and number of bacteria in both cattle and sheep on winter rations did not appear markedly different. Hungate et al. (1952) also reported that a marked change in the rumen organisms occurred when an excess of grain or glucose was introduced into the rumen. It was found that the cellulolytic bacteria are greatly decreased in numbers, protozoa diminish and the relative number of gram positive bacteria increases. Contrary to this report, Bryant and Burkey (1953) reported that the level of feeding of alfalfa hay-concentrate ration appears to have little effect on the groups or numbers of bacteria and that the numbers of cellulolytic bacteria present per ml of rumen

ingesta was not markedly influenced by the amount of fiber in the ration because the number of these bacteria found when the concentrate ration was fed was of the same magnitude as when rations of higher fibers were fed. Bortree et al. (1946) reported that bacterial count variations were not markedly influenced by a change in quality of roughage ration. Likewise, when the animals were changed from hay to pasture the changes in the counts were not great. However, when three pounds of glucose was added to the usual feeding of hay, the counts were about 100 percent greater than those observed on hay alone. Administering starch in a similar manner produced bacterial counts which were only slightly different from those obtained from hay alone.

While the rumen population seems to vary with different rations, there has also been work reported on diurnal variations affected not only by the ration itself but as to the time of feeding and the length of time it is being digested in the rumen. Johnson et al. (1944) reported the greatest number of bacteria and the fewest protozoa one hour after feeding. At succeeding intervals the numbers of bacteria decreased while the numbers of protozoa increased more or less regularly for 16 hours. Bortree et al. (1946) had taken rumen samples prior to feeding and at two-hour intervals after feeding for periods of 10 to 12 hours. Results show a rapid increase in the number of organisms present in the rumen two hours after the animals have been fed, and that these counts are maintained or increased for several hours, then gradually return to the range observed prior to feeding. Nottle (1956) reported significant diurnal fluctuations in both bacterial concentrations and

rumen pH, as was mentioned previously, and that a marked difference occurred in the nature of the diurnal curve for bacteria in sheep which were fed two diets identical except for the protein source in the concentrate portion of the ration. Shehata (1958) gave the following summary as to diurnal flora variation in the rumen.

> Differences should be expected in diurnal variations of bacteria in sheep fed on different feeds. Assuming all conditions, particularly volume of rumen contents, were similar in experiments, then these differences may be attributed to chemical and physical changes or properties of the feed substance. These properties will affect the rate of availability of nutrients to the rumen bacteria and consequently influence the diurnal change. If most of the nutrients are readily available one should expect a rapid increase of bacterial count which would be followed by a rapid decrease. However, if only a part of the nutrients are readily available later on due to chemical decomposition, to changes in physical properties, or to symbiotic action of different bacteria in the rumen, then the early rapid increase in bacteria may be maintained at a high level for several hours after feeding.

Another influential condition affecting bacterial variations was reported by Nottle (1956) which pertained to a seasonal variation. He reported that the pH of the rumen fluctuated seasonally as well as daily, in a manner similar to the seasonal fluctuations of rumen bacterial concentrations. Moir (1951) also reported this seasonal fluctuation but suggested that it was due to the source and availability of nitrogen.

It would seem, from the review thus far, that the conditions of pH, temperature, and activity may be affected, either directly or indirectly, by the type of ration being fed. Although there seems to be some controversy on the subject of bacterial concentration and the type of organisms present in the rumen, it seems that these variations will depend largely

on the ingredients of the ration being fed. Evidence has been shown that an increase in bacterial concentration occurs when readily available carbohydrates are fed, as well as a change in the types of organisms present. With this bacterial change there seems to be a change in the degree and rate of fermentation in the rumen, which would appear to promote a change in the rumen temperature. With the possibility of excess fermentation, there also would be an increase in the formation of volatile fatty acids which would in turn lower the acidity of the rumen. The work reviewed has shown when and possibly why, in some cases, the reason these variations occur.

## Culture of Rumen Organisms

The cultural growth of these organisms has been attempted by various workers, with success in some phases of the work. There have been various culturing techniques used with Doetsch et al. (1952) reporting a fairly successful technique using roll tubes inoculated with dilutions of rumen ingesta. Gall et al. (1947) reported a very successful method of determining rumen bacterial counts. This method was accomplished by the use of nigrosine, a negative stain, which colors the background but leaves the bacteria unstained. This method of staining was used by Moir and Williams (1950) and Nottle (1956) with good results.

Cultural counting of the total concentration of rumen micro-organisms is a technique in which results will vary exceedingly between culture plates or tubes. Huhtanen et al. (1952) and Wilson and Briggs (1955) reported that cultural counts involving inoculum of single tubes

of media using 10-fold dilutions, commonly show 10- and 100- and sometimes 1000-fold differences even when samples are obtained under a single standard set of conditions.

Due to the vast complexity of rumen microbiology, various phases of the work has yet to be successfully accomplished, particularly in the area of culturing rumen microorganisms to obtain total counts.

It is clear from the current review that no simple explanation can be given for results obtained by studying the effect that different rations may have on the individual animal. To control the nutritive levels of ruminants, the nutritive requirement for the microorganisms in the rumen must be known, as well as environmental conditions for which these organisms are best adapted, for optimum, nutritional efficiency. Two of these conditions, thought to affect the microbial efficiency in the rumen, are the acidity of the ingesta containing the organisms and the temperature evolved from the fermentation reactions occurring in the rumen. In this project it was hoped to obtain information on how the variability of these two conditions would affect or be effected by the cellulose digesting ability of the microorganisms, as tested by in vitro determinations. Information on total rumen microorganism concentration, was attempted to further substantiate any variations that may occur during the test period.

# METHODS OF PROCEDURE

## Experimental Animals and Rations

The various determinations needed for this project were obtained from grade Hereford twin steers and four wether feeder lambs, predominantly of Rambouillet breeding. One of the steers had been fitted with a rumen fistula, while the other had been cannulated. All four wethers were cannulated. Figures 1 and 5 illustrate two of the cannulated animals. At the beginning of the test period, the steers weighed approximately 1250 pounds with the cannulated steer being the heavier of the two. The four wethers weighed approximately 160 pounds each with little variation between them.

Rations for the animals consisted of a high roughage and a high concentrate ration. The high roughage ration consisted of 3 parts roughage and 1 part concentrate, with the concentrate ration being made up of 3 parts concentrate and 1 part roughage. The concentrate portion of both rations consisted of 50 percent ground No. 2 yellow corn and 50 percent ground whole oats. Medium quality alfalfa hay made up the roughage portion for both rations. A protein analysis made of the rations showed them to be quite similar as to protein content, with the roughage ration having 12.47 percent protein and the concentrate ration having 14.22 percent (moisture free basis).

It was essential for this type of project, to keep the quality of the ration ingredients as closely the same as possible throughout the test period. This is especially true for the roughage portion of these

rations, since quality of forages may vary considerably. To insure fairly constant quality of the roughage, a large amount was set aside in the hopes of lasting throughout the test period. However, the latter three weeks of testing had to be supplemented with a higher quality of roughage than had been previously used.

The entire ingredients of the rations were ground, mixed and sacked at the college feed unit to insure proper mixing of each ration.

Each experimental animal was fed twice a day, at approximately 8:00 a.m. and 5:00 p.m. The steers were fed 28 pounds each per day, which later was found to be an excessive amount. These rations were decreased to 20 pounds per steer which sufficed for the remainder of the test period. The four wethers received 4.5 pounds each per day which also was reduced to approximately 4 pounds depending on the individual animal.

The steers and two of the wethers were kept on the high roughage ration throughout the test period, except for the inadvertant feeding of concentrate to the wethers supposedly on the roughage ration. The two remaining sheep were switched during the 11th week of the trial from the high roughage ration to the high concentrate ration. They remained on this ration for 14 weeks and were then returned to the original roughage ration.

All animals with the exception of one wether, receiving the high concentrate ration, remained on feed throughout the entire period. All animals had access to salt and trace mineral mix at all times.

## In Vivo Rumen pH

In determining the variations in rumen pH and temperature over a designated period of time, a workable schedule of making these determinations had to be made in which representative observations could be obtained of the daily and weekly fluctuations within the rumen.

The pH determinations were made on Tuesday and Thursday of each week during the test period and three times during each of these days, on all animals used in the experiment. Morning pH observations were taken prior to the morning feeding, with determinations again made at approximately 11:00 a.m., three hours after the feeding. The evening readings were made just prior to the evening feed.

For the first two weeks of the experiment a Beckman portable pH meter, model 180, was used for making the determinations, as pictured in Figure 2. Due to the small openings in the cannulas, pH readings had to be made from a flask after extraction of the rumen fluid. A Thompson suction pump fitted with a suction flask was used to obtain the rumen fluid for these observations. This is illustrated in Figure 4. The pH readings were made immediately upon obtaining the fluid from the rumen.

Due to fluctuation in readings of the portable Beckman meter and delicacy of the electrode, it was decided to make the pH determinations in the main laboratory with the use of the more accurate Beckman meter, model 42, shown in Figure 6. There seemed to be little variation between observations made immediately after extraction of the rumen fluid and

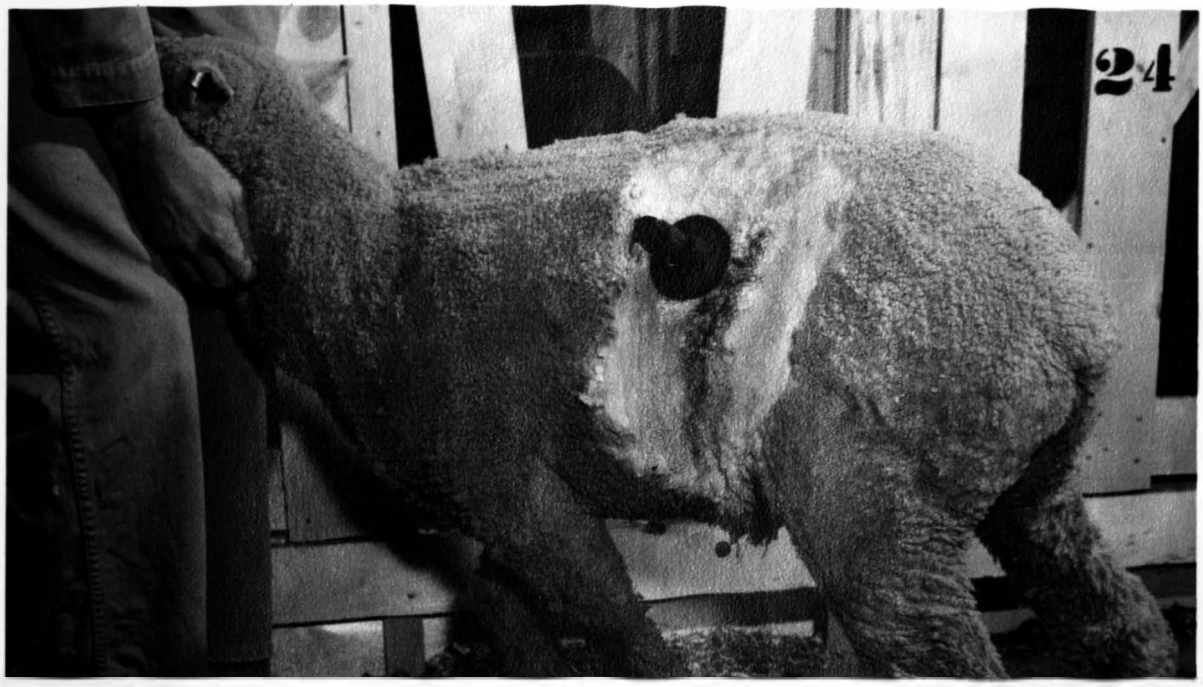


Figure 1. A view of a cannulated sheep.

Figure 2. A view of the determination of pH using a portable pH meter.

observations made in the laboratory with the larger pH meter.

To keep the pH as constant as possible during transportation to the laboratory, pre-heated, stoppered quart and pint thermos bottles were used, as shown in Figure 5. All determinations were made within one hour after extraction of the rumen fluid with very little variation in pH occurring during this time. A usual sample of fluid consisted of between 400 and 600 ml depending on the container being used. The pH of each sample was taken immediately upon reaching the laboratory, prior to straining of the fluid in preparation for the artificial rumen.

## In Vivo Rumen Temperature

Rumen temperature observations were made at the time rumen fluid was extracted for the pH determinations. In this way both pH and temperature of the rumen could be observed before feeding in the morning, at noon, following the feeding, and just prior to the evening feed.

A centigrade thermometer was used for these determinations, which was graduated in 0.1 degrees.

All temperature observations were taken directly from the rumen ingesta. The thermometer was inserted approximately 8 to 10 inches into the cannula opening and held in this position for two minutes, as illustrated in Figure 5. This allowed ample time for thermometer reading to rise to that of the rumen temperature. It was found that more accurate readings could be obtained if the thermometer was lifted out of the cannula just enough to observe the reading. If completely removed before the reading was observed, the temperature would tend to

be lower than the actual temperature of the rumen, due to the lower temperature of the atmosphere.

It was found that the thermometer could be inserted into the fistulated animal by slightly adjusting the fistula and inserting the thermometer directly beside it, without its removal. This proved to be a better method, with more accuracy and less discomfort to the animal.

To avoid the difficulty of observing abnormally low temperature due to water intake, animals were given limited access to the water on days observations were made. At other times water was given free choice.

The animals were quartered in the animal husbandry nutritional research building. Separate pens being used for the sheep, as shown in Figure 3. The steers were stantioned prior to the evening feed and released the following morning, immediately after feeding. However, on days observations were made, they were kept stantioned until after the noon readings were taken.

## In Vitro Rumen Flora Activity

In making in vitro rumen activity checks, it was again necessary to make the determinations at a time which would give the best representative information for the test period. Due to the time consuming procedure of making artificial rumen runs, it was only possible to make two activity checks of each animal per week. These determinations were made simultaneously with morning, noon, and evening pH and temperature observations. The inoculum for the first weekly in vitro activity determination was taken in the morning, prior to feeding. The following Thursday, an

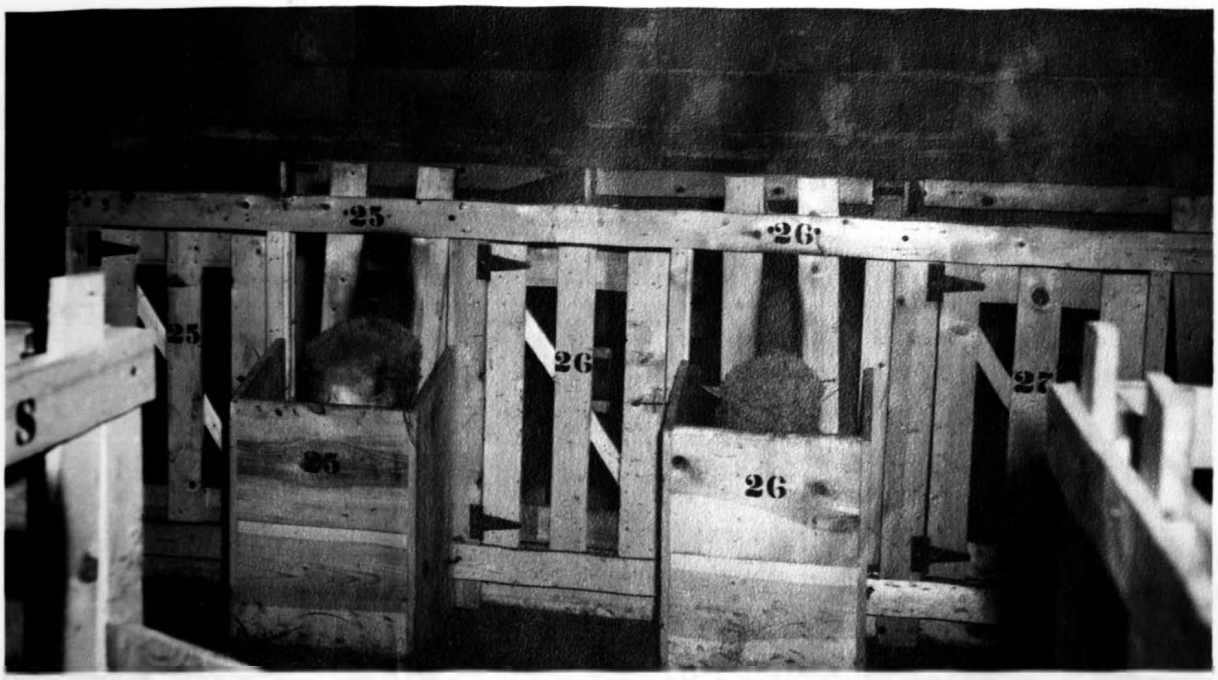


Figure 3. A view of the pens housing the cannulated sheep.

Figure 4. A view of the Thompson suction pump fitted with a suction flask.

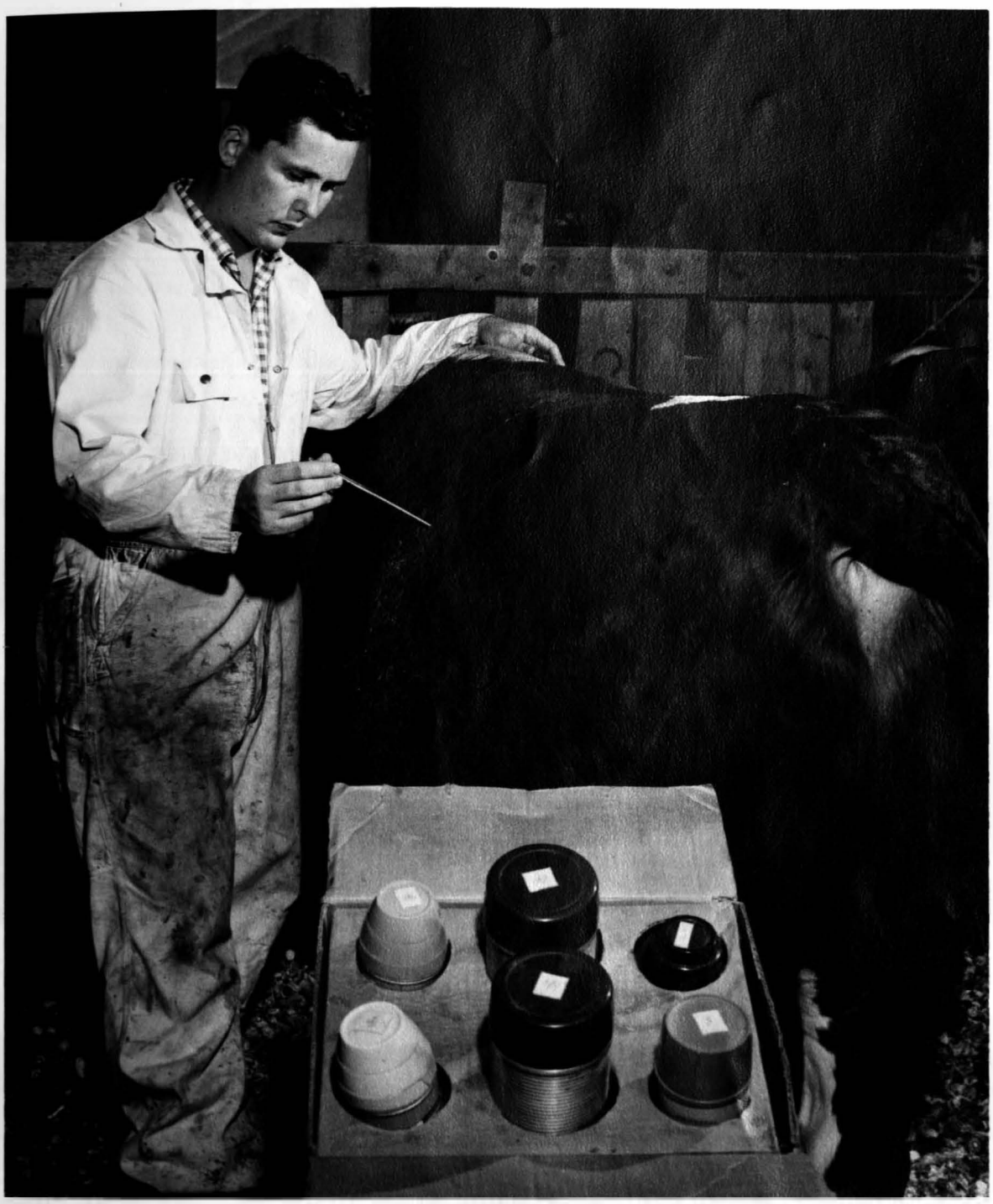

Figure 5. A temperature determination being made from a cannulated steer, also stoppered thermos bottles used to transport rumen fluid.

*in vitro* determination was made of rumen fluid obtained during noon observations. The first determination of the following week was then made of rumen flora sampled during the evening observations. This pattern of making *in vitro* determinations was used throughout the entire period.

The rumen fluid was transported to the laboratory in the same manner as for that of the pH determinations.

The *in vitro* fermentation method used for obtaining cellulose digestion coefficients was carried out according to the procedure of Burroughs et al. (1950) as modified by Bentley et al. (1954a). Hershberger et al. (1955) reported a rapid volumetric method for the determination of cellulose, which was used in this experiment.

Solka floc, a purified wood cellulose relatively free of lignin, was the source of cellulose used for the rumen microorganisms, during *in vitro* fermentation. The composition of the basal medium used in all *in vitro* fermentation trials is listed in Table II. After preparation of the fermentation flasks, they were placed in the pre-heated thermostaticly controlled water bath (38.0° C. $\pm$ 0.2° C.) and individually gassed with carbon dioxide, as shown in Figure 6. Two-hole rubber stoppers were fitted with two glass tubes, one going below the surface of the medium in the fermentation flasks, thoroughly flushing the flask with carbon dioxide, and the other acting as an escape route for excess carbon dioxide. The carbon dioxide was passed through the medium of each flask at a slow rate, keeping the flasks as anaerobic as possible and giving the medium a slight swirling action.

Prior to the inoculation of the fermentation flasks, the rumen fluid was strained through four layers of cheesecloth. Immediately

TABLE II. COMPOSITION OF BASAL MEDIUM FOR THE IN VITRO RUMEN FERMENTATION

| Constituent | Grams/100 Milliliters |
|---|---|
| Solka Floc[1] | 1.00 |
| $Na_2HPO_4$ | 0.113 |
| $NaH_2PO_4$ | 0.109 |
| $NaH_2PO_4 \cdot H_2O$ | 0.125 |
| KCl | 0.043 |
| NaCl | 0.043 |
| $MgSO_4 \cdot 7\ H_2O$ | 0.01164 |
| $Na_2SO_4$ | 0.015 |
| $FeCl_3$ | 0.0044 |
| Glucose | 0.40 |
| Urea | 0.168 |
| | **Micrograms/100 Milliliters** |
| Biotin | 20.00 |
| PABA[2] | 50.00 |
| Valeric Acid | 10.00 |
| | **Milliliters/100 Milliliters** |
| Inoculum[3] | 40.00 |

[1]purified wood cellulose

[2]Paraaminobenzoic Acid

[3]Whole rumen fluid strained through four layers of cheesecloth.

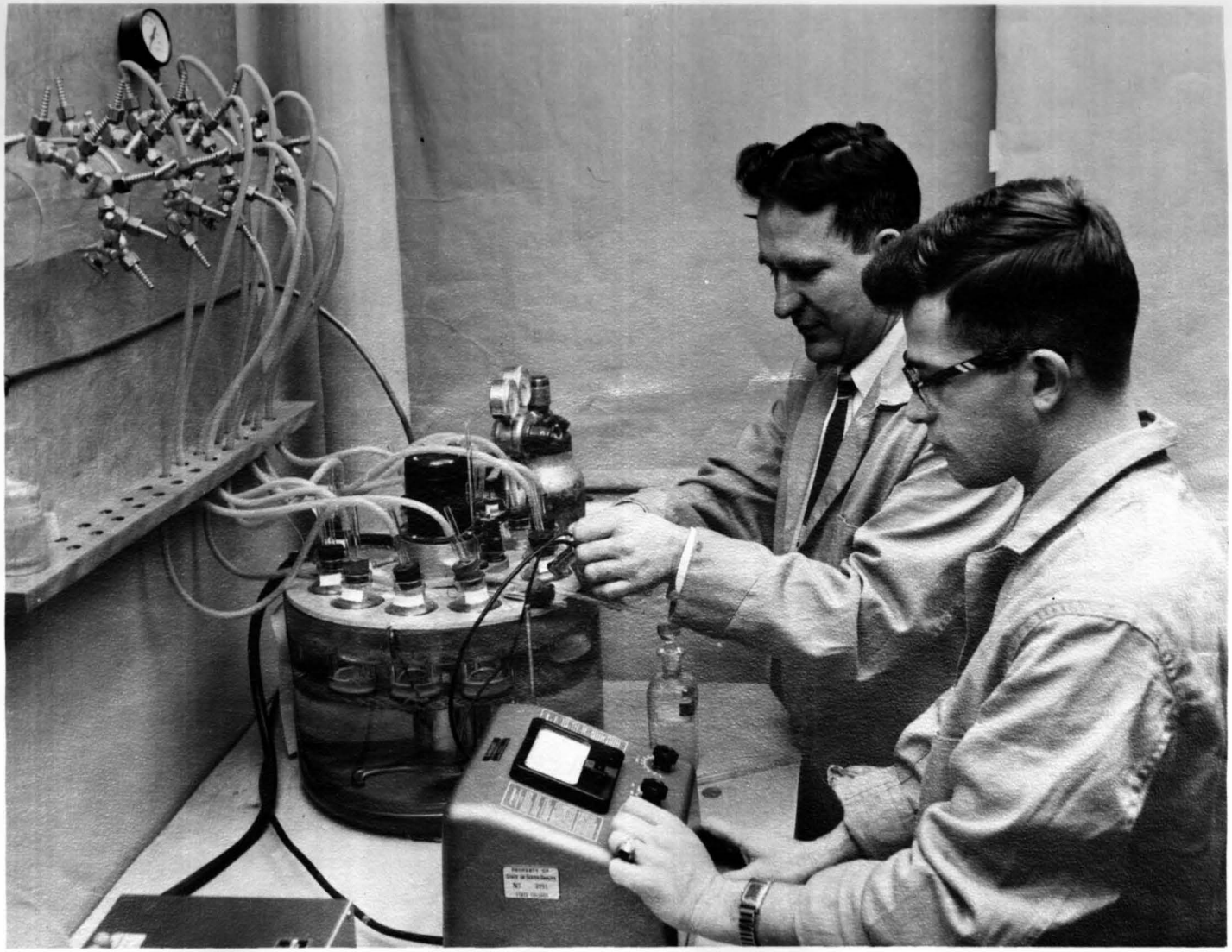

Figure 6. A view of the apparatus utilized for in vitro digestion.

after straining, 40 ml of the fluid was added to each of the duplicate flasks corresponding with the animal from which the rumen fluid was obtained. Glassware was thoroughly cleaned and new cheese cloth used after straining the rumen fluid from each individual animal.

Adjustments of the pH to 6.9 for each flask was made at four-hour intervals during the first 12 hours of fermentation and every 10 to 12 hours thereafter. A saturated solution of $Na_2CO_3$ was used to adjust the pH.

At the termination of the fermentation period of 22 hours, the flask contents were diluted to 100 ml with distilled water. Duplicate 10 ml aliquots were taken for the cellulose analysis, after a thorough mixing of the medium. The cellulose determinations were made according to a rapid volumetric method of analysis, as explained by Hershberger et al. (1955).

## Culturing of Rumen Microorganisms

Rumen microorganisms were cultured in the Bacteriology department laboratory, with the assistance of personnel from that department.

Rumen flora used for inoculations were transported to the laboratory by means of the stoppered thermos bottles used for the previous determinations. All thermos bottles containing strained rumen fluid for inoculation purposes were first thoroughly flushed with carbon dioxide before transporting to the laboratory for culturing.

It was found that several different methods of culturing as well as different types of media had to be experimented with, in the hopes

of finding a suitable method for determining total counts of each of the individual animals.

The first attempt at culturing the organisms involved a plating technique in which sterile petri dishes were used. The larger top of the dish was inverted and a small designated amount (0.3 to 0.5 ml) of undiluted rumen fluid was placed in the center of it. Immediately following this was 15 ml of the culture media which had been autoclaved for 15 minutes at 120° C. and cooled to approximately 40° C. After thoroughly mixing the culture with a swirling motion, the bottom of the petri dish was placed flush with the surface of the media. To insure anaerobic conditions, melted beeswax and paraffin were poured along the edges of the plate. The media used for this technique was described by Mahtanen (1952), to which was added 0.2 percent cellobiose for a bacterial carbon source.

Because of a lack of consistency in the results obtained from this method of culturing and excessively lower counts than normal, it was felt a new method should be employed.

A roll tube technique used by Doetsch et al. (1952) was used as the next method of rumen culture. Screw cap test tubes were used to insure anaerobic conditions throughout the fermentation period. By the use of these tubes (15 mm x 125 mm), blown caps could be avoided, which occurs frequently with this type of culturing. This is due to the large amounts of gas usually produced by certain gas producing microorganisms present in the rumen. A commercial media was used with this method, known as Bacto MacConkey Agar. Its constituents are listed in Table III.

TABLE III. COMPOSITION OF BACTO MacCONKEY AGAR, A COMMERCIAL MEDIA USED FOR THE CULTURING OF MICROORGANISMS.

| Constituent | Grams/liter |
|---|---|
| Bacto-peptone | 17.0 |
| Proteose-peptone, Difco | 3.0 |
| Bacto-lactose | 10.0 |
| Bacto Bile Salts No. 3 | 1.5 |
| Sodium Chloride | 5.0 |
| Bacto Agar | 13.5 |
| Bacto Neutral Red | 0.03 |
| Bacto Crystal Violet | 0.001 |
| Cellobiose (carbohydrate source for rumen organisms.) | 0.200 |

By the use of a sterile micropipette, 0.01 ml of undiluted inoculum was placed in a sterile tube, immediately followed by 3 ml of autoclaved media previously cooled to 40° C. After securing the cap, the media was solidified in a thin film about two-thirds of the way up the walls of the test tube. This was accomplished by rotating the tube under a stream of cold water. The cap was then removed and the tube flushed with carbon dioxide. After the cap had been quickly replaced, the tube was placed in an incubation oven set at 39° C. The incubation time for the bacteria, varied from 15 to 20 hours. Colony counts were made of each of the three duplicate tubes prepared for each animal being used.

Again due to a lack of consistency in the bacterial colony count and being abnormally low, a new method of determining bacterial counts was adopted.

A strain method of counting was attempted, using the nigrosine smear technique as described by Gall et al. (1947).

The inoculum from a concentrate fed sheep and a roughage fed sheep were used in hopes of determining any difference in rumen bacterial concentration of the animals on these two different rations.

The rumen contents were diluted in a 1 percent sterile glucose solution to 1:1000. After the dilution was made, the bottles were thoroughly mixed on an automatic shaker at 240 oscillations per minute. After mixing, 0.01 ml of the diluted inoculum was transferred to a slide by the use of a standard inoculating loop graduated to 0.01 ml. An equal amount of one-half saturated methyl alcohol solution of nigrosine

was thoroughly mixed with the inoculum and spread evenly over a 4 $cm^2$ area. The slide was then immediately flame dried and cooled. Counts were then made with a Spencer microscope under oil immersion. The bacteria appeared white on the dark blue background and were easily counted, however, a large number of artifacts in the slide gave inaccurate counts and a new method had to be used.

For two weeks following the test period, gram positive and negative stains were made from rumen fluid obtained from two sheep. One of the animals, having been on roughage for about two weeks prior to the time these samples were taken, was immediately changed over to a high concentrate ration. The other sheep, acting as a control animal, was kept on its original diet of roughage.

Morphological changes of the rumen microorganisms were to be observed during this ration change. Daily checks were made and slides were kept to show any variation in the types of organisms present. During this experimental period, it was attempted to photograph some of the significant changes which appeared, with a Spencer photo-microscope. Pictures were also taken of the live organisms, most of which were protozoa, with the use of a cover slip placed over a drop of rumen fluid on a glass slide. This method of observing live organisms is similar to the "hanging drop" method commonly used in Bacteriology. All pictures were taken with K 135 color film.

## RESULTS AND DISCUSSION

The main objective of this thesis was to compare some of the variable factors that affect cellulose digestion in the rumen. Since six animals have been used for the experiment, all six having different pH, temperature, and activity variations, it is essential to break the problem down into segments. The first results will be given for the steers which were on continuous roughage rations, followed by the results from the two sheep also on roughage rations. The results determined for the two animals receiving a ration change will then be given. To further classify this, each individual animal had a corresponding number which will be used during the explanation of the problem. The fistulated steer was No. 11, with the cannulated steer being No. 12. The four sheep were numbered 1, 2, 3, and 4. Sheep No. 1 and 2 were on continuous high roughage, and sheep No. 3 and 4 were fed both concentrate and roughage, intermittently.

### Steers Fed a High Roughage Ration

#### Animal Differences

Both steers were kept on the forage ration the entire length of the trial. Neither one went off feed during this period and left very few orts after feedings. The mean values obtained for pH, temperature, and activity variations showed only slight differences between the two steers, during the entire period. As shown in Table IV, the mean pH determined from the morning, noon, and evening readings of steer No. 12

(6.73) were slightly lower than that obtained from steer No. 11, (6.84). Figure 14 shows this lower pH tendency in graphic form for steer No. 12. Only slight temperature differences were observed for these two animals. Steer No. 12, having the lower mean pH, had a higher mean temperature (39.81°C.) than observed for that of steer No. 11, (39.62° C.) This low pH and high temperature relationship may indicate that a higher degree of fermentation is occurring in the rumen having the lower acidity and higher temperature. A lower pH would probably be due to an increased amount of organic acids being produced in the rumen as reported by Phillipson (1942). If a higher degree of fermentation were occurring, resulting in a higher production of organic acids, rumen temperature would probably rise slightly due to the heat produced during the fermentation reactions.

Table IV also shows the similarity of *in vitro* cellulose coefficients, with steer No. 11 having a slightly higher mean activity (47.92%) than that obtained from steer No. 12, (45.90%). The digestion of cellulose was used as an indication of activity.

It can be observed in Figure 11 that the patterns formed by the weekly variations of pH, temperature, and activity of steer No. 11, follow quite closely the patterns formed by steer No. 12, as shown in Figure 12.

As pointed out earlier, these animals are twins which may account for closeness of observations between these two animals in pH, temperature, and activity. The similarity of digestion and metabolism between twin animals has been reported by Kelkar and Gullickson (1950).

Differences in Morning, Noon, and Evening Determinations of pH and Temperature

The two animals No. 11 and 12, although variable from day to day, followed similar patterns for morning, noon, and evening readings of pH and temperature. For example, it will be noted in Table IV and Figures 11 and 12, that the morning pH of both animals, taken prior to feeding, was the most alkaline of the three readings observed. The noon determinations, taken approximately three hours after feeding, were the most acidic, with the evening pH, taken just prior to the evening feed, being generally slightly more acidic than the morning reading and less acidic than the noon determination, e.g. Steers No. 11 and 12 had a morning reading of 6.98, 6.88; a noon reading of 6.73, 6.61; and an evening reading of 6.82, 6.71, respectively.

The results of these pH observations are in complete accordance with reports by Kick et al. (1938), Phillipson (1942), Myburg and Quin (1943), and Nottle (1956). Diurnal variations were reported to occur, depending on the time of feeding, after which the pH would lower for approximately eight hours. It was also reported that a peak in pH occurred just prior to the morning feed. Diurnal fluctuations observed for these two animals follow quite closely these same fluctuations.

The rumen temperature of the two steers (No. 11 and 12) varies with the time of day but in an inverse manner to that of pH. The temperature readings which were taken simultaneously with pH readings (Table IV and Figures 11 and 12) show that the mean noon temperature to be the highest of the three readings taken. The morning temperature is

quite similar to that of the evening temperature.

Nangaroni (1954) has reported that following a feeding there is a slight rise in rumen temperature followed by a gradual decline to the pre-feeding levels, as reported here. The time it takes for the temperature to return to its lowest level would seem to depend on the time of the last feeding and total feed intake.

A period of approximately nine hours would elapse between morning and evening feeds. From the evening feed to the following morning feed, is approximately 15 hours. During this 15-hour interval, rumen temperature has sufficient time to lower to its pre-feeding morning level. However, with only the nine-hour interval between morning and evening feeds, the rumen temperature in the evening will only occasionally equal the lower morning temperature.

Temperature and pH readings were the only determinations observed three times each testing day, for all the animals involved. Since approximately 30 hours were required in making activity determinations, only two such checks could be made weekly.

Related pH, Temperature, and Activity Fluctuations

During certain periods of this trial, there seems to be related fluctuations between temperature, pH, and activity. It will be noted in Figures 11 and 12, during the fifth week, there was a decline in activity values for both steers, corresponding with a sharp decrease in temperature. Immediately following this, activity and temperature increased simultaneously. Rumen acidity, in this instance, seemed to remain quite

constant. Although this seems to be the only week that this occurs during a short period of time, it would appear that any decrease in activity should result in a decrease in rumen temperature. It will be noticed, however, that temperature usually varies very little, as compared to a change in activity over the same period. Although weekly temperature and activity fluctuations show little relationship, over the entire trial there does seem to be a gradual corresponding temperature and activity change.

Figures 11 and 12 also show that both animals had an increase in pH values from approximately 6.6 to 6.9 and a decrease in activity from about 45 percent to 30 percent, simultaneously, during the final three weeks of the trial. This seems to be the only part of the experiment where a pH and activity relationship is accentuated, for these animals. This relationship seems to be more readily observed when activity drops to a very low level, as it did during this period.

## Sheep Fed a High Roughage Ration

### Animal Differences

Sheep No. 1 and 2 were also fed the high roughage ration used for the two steers. However, during the 16th week, these animals were fed the high concentrate ration inadvertently, resulting in some pronounced changes, as shown in Figures 7 and 8.

Values obtained for the mean pH, temperature, and activity, again have shown only slight differences between the two animals. Sheep No. 1 had a slightly lower mean pH (6.60) than that obtained for sheep No. 2

(6.75), as shown in Table IV. These differences are graphically illustrated in Figure 14. Rumen temperature for the two animals varied less than 0.1° C., with sheep No. 1 having a slightly higher mean temperature (39.77° C.) than for sheep No. 2, (39.68° C.) As previously observed with the steers, the animal having the lower pH (sheep No. 1) also has a slightly higher rumen temperature. Although these differences are very slight, it may indicate, as previously mentioned, a higher degree of fermentation occurring in the rumen. Both animals had very similar mean activity values, with sheep No. 1 having a lower value (33.83%) than observed for sheep No. 2 (36.34%) as noted in Table IV.

As shown for the two steers, the patterns formed by the weekly variations of sheep No. 1, follow quite closely, the patterns of sheep No. 2, (Figures 7 and 8).

Differences in Morning, Noon, and Evening Determinations of pH and Temperature

These two animals (sheep No. 1 and 2) seem to show a similar pattern for morning, noon, and evening pH and temperature readings, as previously reported for steers No. 11 and 12. The morning pH determinations, from both sheep, were the most alkaline of the three observations. The noon readings were the most acidic, with the evening determinations being generally slightly more acidic than the morning readings, and less acidic than the noon determinations, e.g. Sheep No. 1 and 2 had a morning pH of 6.75, 7.03; a noon pH of 6.48, 6.58; and an evening pH of 6.58, 6.65, respectively. These observations also correspond with results reported by the various workers, mentioned previously.

The temperature values, taken simultaneously with the pH determinations, varied with the time of day, but in an inverse manner to that of pH. The mean noon temperature was the highest of the three observations made, with the morning temperature being quite similar to that of the evening determinations. These results are also similar to those obtained from the two steers, (No. 11 and 12), and are in accordance with the work reported by Mangaroni (1954).

As noted in Figure 8, the noon temperatures, for the first five weeks, were unusually low for sheep No. 2. There were also unusually low temperatures observed, at certain instances during the trial, for some of the other animals tested.

A controlled watering procedure was followed as closely as possible throughout the entire period. However, there were instances when the animals were watered inadvertently, possibly resulting in abnormally low rumen temperatures, if the water was consumed prior to the temperature observations.

Related pH, Temperature, and Activity Fluctuations

One of the most pronounced relationships of pH and activity, for these two animals, occurs during the 16th week (Figures 7 and 8) at the time of the inadvertent feeding of the high concentrate ration. There is a distinct decline in pH values for both animals (sheep No. 1 and 2) and a corresponding decline in ruminal activity, (as determined by *in vitro* cellulose digestion). Sheep No. 1 had a more pronounced activity decline (35% to 15%) as well as a distinct recovery period, (15% to 32%).

Although the activity for sheep No. 2 declined slightly (45% to 38%), there was no recovery observed until the 17th week.

During the last three weeks of the trial, these two sheep responded to the roughage change similarly to the change observed for the two steers. In the 25th week there was a drop in activity values, for sheep No. 1, from 40 percent to less than 10 percent in a two-week period, followed by an increase to nearly 30 percent within the last week of the trial. Sheep No. 2 declined from 35 percent to less than 10 percent, during this same two-week period, with no recovery observed. During these activity declines, pH values for both animals increased from approximately 6.7 to 6.9.

As shown for steers No. 11 and 12, there is little weekly relationships that can be observed for pH and activity fluctuations of these two animals. However, over the entire test period there seems to be gradual temperature decrease that corresponds with a slight average decrease in activity. This can be observed in Figures 7 and 8 during the last eight weeks of the experiment.

## Sheep Fed Roughage and Concentrate Ration Intermittently

### Animal Difference

Sheep No. 3 and 4 had a ration change during the 11th week from a high roughage ration to a high concentrate ration. This was continued until the 23th week, when the animals were returned to the original roughage ration. Sheep No. 3 went completely off feed during the 18th

TABLE IV. MEAN MORNING, NOON, AND EVENING pH AND TEMPERATURE DETERMINATIONS, WITH MEAN ACTIVITY VALUES OF ANIMALS ON HIGH ROUGHAGE RATION THE ENTIRE TEST PERIOD.

| Time of Readings | Steer No. 11 | Steer No. 12 | Sheep No. 1 | Sheep No. 2 |
|---|---|---|---|---|
| pH | | | | |
| Morning | 6.98 | 6.88 | 6.75 | 7.03 |
| Noon | 6.73 | 6.61 | 6.48 | 6.58 |
| Evening | 6.82 | 6.71 | 6.58 | 6.65 |
| Ac. Mean[4] | 6.84 | 6.73 | 6.60 | 6.75 |
| Temperature | | | | |
| Morning | 39.40 | 38.64 | 39.81 | 39.58 |
| Noon | 40.07 | 40.64 | 39.94 | 39.78 |
| Evening | 39.39 | 40.17 | 39.57 | 39.68 |
| Ac. Mean | 39.62 | 39.81 | 39.77 | 39.68 |
| Mean Activity | 47.92 | 45.90 | 33.83 | 36.34 |

[4]Accumulative mean of morning, noon, and evening determinations

week resulting in some extreme pH fluctuations, as noted in Figure 9. This animal was rather sluggish and tended to be slightly off feed for the balance of the trial, which seemed to result in the extensive pH fluctuations. Sheep No. 4 remained on feed throughout the entire test period, leaving few orts after each feeding.

The mean values obtained for pH, temperature, and activity generally showed only slight differences between the two animals. As shown in Table V, the mean pH of sheep No. 4 (6.41) is slightly higher than for sheep No. 3 (6.34), over the entire 28-week period. During the initial feeding of the roughage ration (first 11 weeks), the sheep (No. 3 and 4) had similar pH values, (6.54 and 6.55, respectively). These values were lower for the period the concentrate ration was fed, with sheep No. 3 having a slightly lower value (6.12) than observed for sheep No. 4, (6.25). When the animals were changed back to the original roughage ration (during the 25th week), they again had almost identical mean pH values for the three week period, (sheep No. 3, 6.68 and sheep No. 4, 6.69).

Mean temperature differences between animals, during the entire test period, were usually less than 0.08° C., with the exception of the last three weeks of the trial when roughage was again being fed. The rumen temperature for both sheep (No. 3 and 4) decreased during this period (39.68° C. and 39.78° C., respectively), from a level of 40.05° C. and 40.10° C., respectively, when the animals had been fed the high concentrate ration. For the 28-week period, sheep No. 3 had a slightly lower mean temperature (40.02° C.) than observed for sheep

No. 4, (40.04° C.)

Ruminal pH is considerably lower for these two sheep than previously observed for the other animals (steers 11 and 12, sheep 1 and 2), and rumen temperatures are generally higher. Kick et al. (1938) has reported that as the amount of concentrates are increased in the ration, the rumen acidity will also increase. Phillipson (1942) has stated that the fermentation rate of the rumen depends on the diet fed, being most rapid when a readily available carbohydrate was added. These low pH and high temperature determinations observed for these two animals (sheep No. 3 and 4) are probably again the result of increased fermentation, with a higher production of organic acids, when the concentrate ration was being fed.

Activity fluctuations for these two animals were generally quite similar throughout the test period, as noted in Table V. During the entire 28-week period sheep No. 3 had a higher mean activity value (28.31%) than observed for sheep No. 4, (23.02%). Higher activity values were observed, for both animals (sheep No. 3 and 4) during the initial feeding of the roughage ration (37.32% and 26.20%, respectively) in the first 11 weeks. During the 14-week period immediately following, when the concentrate ration was fed, mean activity values declined for both animals (Table V), with sheep No. 3 having a higher mean value (22.84%) than for sheep No. 4, (13.13%). Activity values for the last three weeks of the trial, when the animals were returned to the roughage ration, showed a rise in mean activity for sheep No. 4 (22.43%) and a slight lowering in activity value for sheep No. 3 (22.06%) from the

previous period.

Both animals have shown a considerable decrease in activity when changing from the roughage to concentrate ration. Reasons for this have been indicated by Louw and Van Der Wath (1943), Hoflund et al. (1948), and Hunt et al. (1954) who have reported that increased readily available carbohydrates in the rumen, decreases cellulose digestion. Sheep No. 3, however, had only a slight decrease in activity when being changed from concentrate to the roughage ration. By all indications of work reported and results obtained from sheep No. 4, the animal should have had an increase in cellulose digestion during this change. It's believed that the animal's poor eating habits (going off feed) may be a contributing factor affecting these particular results.

It can be observed in Figures 9 and 10 that the patterns formed by the variations of sheep No. 3 generally do not follow those formed by the variations of the other animal. However, both show the characteristic activity rise and decline immediately after changing to the concentrate ration. There also seems to be higher temperatures for both animals, during the summer months, as observed for the other animals tested.

Differences in Morning, Noon, and Evening Determinations of pH and Temperature

The results for the pH and temperature evaluations of these two sheep, show a pattern similar to that obtained from the other test animals used in this experiment. The most alkaline pH reading was observed in the morning, with the noon determination being the most

acidic. The evening readings again were usually slightly more acidic than the morning observations, and more alkaline than the noon readings, (Table V and Figures 9 and 10).

Temperature evaluations for these animals varied with the time of day, but in an inverse manner to that of pH. The morning temperature was usually the lowest, with the noon temperature being the highest observed. The evening temperature was generally not as low as that observed in the morning, and seldom as high as the noon temperature.

Related pH, Temperature, and Activity Fluctuations

Probably one of the most pronounced changes in pH, temperature, and activity in the entire experiment can be observed from the results obtained from sheep No. 3 and 4, fed concentrate and roughage rations, intermittently, as noted in Table V and Figures 9 and 10. During the 14 weeks the concentrate ration was being fed (11th to the 25th week), there was a distinct decline in activity and pH values, with a corresponding rise in temperature. This pattern of fluctuation holds true for both animals. It will be noted that immediately following the ration change (roughage to concentrate) there was a pronounced rise in activity, followed by the decline to the lower activity levels. As reported by Hoflund et al. (1948), small amounts of readily available carbohydrate aids cellulose digestion in the rumen, whereas large amounts inhibit it. This seems to be indicated for these two animals (sheep No. 3 and 4).

When the concentrate was first fed, cellulose digestion increased,

then it seems as the microorganisms became better adjusted to the new ration, the readily available carbohydrates were fermented, leaving the more complex cellulose to be digested later or not at all. As mentioned in Hoflund's report, the fermentation rate increases with an increase in readily available carbohydrates. As mentioned previously, this would probably account for the higher rumen temperatures and lower ruminal acidity observed for these two animals. In the 25th week (Figures 13 and 14) when the roughage ration was again being fed, there was an increase in pH values from approximately 6.2 to 6.9 in a two-week period, followed by an abrupt leveling off to the end of the trial. Corresponding with these pH fluctuations is the decrease in activity (25% to 18%). Due to completion of the trial at this time, no recovery period could be determined for these fluctuations.

## Effect of Roughage Quality

Prior to the beginning of the experimental period, all of the test animals were being fed a high quality roughage (leafy, green alfalfa). With the beginning of initial readings of pH, temperature, and activity, a much lower quality of alfalfa had to be fed because of its availability throughout the entire test period. However, during the final three weeks of the trial a change of roughage was again necessary. The forage used during this period appeared to be of higher quality than had been previously fed.

The roughage change in the first week of the trial was reflected by an intensive fluctuation in activity (as determined by <u>in vitro</u>

TABLE V. MEAN MORNING, NOON, AND EVENING pH AND TEMPERATURE DETERMINATIONS, WITH MEAN ACTIVITY VALUES OF ANIMALS SUBJECTED TO INTERMITTENT CONCENTRATE AND ROUGHAGE RATIONS.

| Weeks | Morning | Noon | Evening | Accumulative mean | Morning | Noon | Evening | Accumulative mean |
|---|---|---|---|---|---|---|---|---|
| pH | Sheep No. 3 | | | | Sheep No. 4 | | | |
| 1-28[5] | 6.56 | 6.13 | 6.35 | 6.34 | 6.79 | 6.04 | 6.42 | 6.41 |
| 1-11[6] | 6.81 | 6.37 | 6.44 | 6.54 | 6.85 | 6.31 | 6.49 | 6.55 |
| 11-25[7] | 6.32 | 5.85 | 6.21 | 6.12 | 6.73 | 5.71 | 6.31 | 6.25 |
| 25-28[8] | 6.79 | 6.59 | 6.67 | 6.68 | 6.87 | 6.58 | 6.64 | 6.69 |
| Temperature | | | | | | | | |
| 1-28 | 39.88 | 40.29 | 38.89 | 40.02 | 39.85 | 40.28 | 40.01 | 40.04 |
| 1-11 | 40.01 | 40.20 | 40.04 | 40.07 | 40.11 | 39.77 | 40.27 | 40.04 |
| 11-25 | 39.83 | 40.49 | 39.85 | 40.05 | 39.71 | 40.69 | 39.90 | 40.10 |
| 25-28 | 39.56 | 40.00 | 39.50 | 39.68 | 39.56 | 40.23 | 39.56 | 39.78 |
| Mean Activity | | | | | | | | |
| 1-28 | 28.31 | | | | 23.02 | | | |
| 1-11 | 37.32 | | | | 36.30 | | | |
| 11-25 | 22.56 | | | | 13.13 | | | |
| 25-28 | 22.06 | | | | 22.43 | | | |

[5]Values taken over the entire test period.

[6]Values taken during the first 11 weeks while sheep No. 3 and 4 were on the roughage ration.

[7]Values taken from the 11th week up to and including the 25th week while sheep No. 3 and 4 were on concentrate ration.

[8]Values taken during the last three weeks of the trial, when sheep No. 3 and 4 were returned to the roughage ration.

cellulose digestion), a slight decrease in temperature and higher pH values. These changes can be observed on the individual figures for each animal or in Figures 13 and 14.

Both the sheep and cattle showed similar pattern response during this time. The animals appeared to adjust to the roughage change during the period, as demonstrated by a gradual increase in activity, lower pH, and slight increases in temperatures.

In the 25th week the roughage change appeared to disrupt the normal curves of pH, temperature, and activity. It would be expected that since the hay quality was improved during the latter three weeks, that activity would rise. This, however, was not true and the activity tended to decrease for each animal tested, with the exception of sheep No. 3 and 4 which did not decrease until the 26th week. It could not be determined, however, whether the adjustment to the forage change would be made to a higher level than previously obtained, since the experiment was terminated at this point.

## Seasonal Fluctuations

There may be some pH, temperature, or activity variations occurring in the rumen that may be attributed to seasonal weather change. There appears to be a slight increase in both activity and temperature, for all of the animals involved in this experiment, during the warmer summer months of the trial. Of these two variations, temperature appears to be the most responsive to the seasonal change.

Prior to the warmer season of the year, and immediately following

this season, this experiment showed that rumen temperature appeared to be slightly lower for all animals involved in the trial, e.g. the total mean temperatures (morning, noon, and evening determinations) for all of these animals, over the different seasonal periods are: 1st to the 7th week, 39.45° C., 7th to the 22nd week, 40.20° C., and the 22nd through the 28th week, 39.45° C. No discernible fluctuation could be observed for pH that may have been attributed by a seasonal change. However, it seems that if a change in activity and temperature occurs, a corresponding change in pH would also occur.

Nottle (1956) has reported seasonal fluctuations in bacterial concentrations and pH of the rumen, which could not be related to any changes in composition of the rations fed. Moir (1951) also found a seasonal variation in rumen microorganisms, but attributed it to the quantity and quality of the herbage being grazed.

No work has been reported, as yet, as to seasonal temperature variation. Although very little work has been reported for any seasonal variations of the rumen, it seems it may be a contributing factor to pH, temperature, and activity fluctuations over a seasonal period.

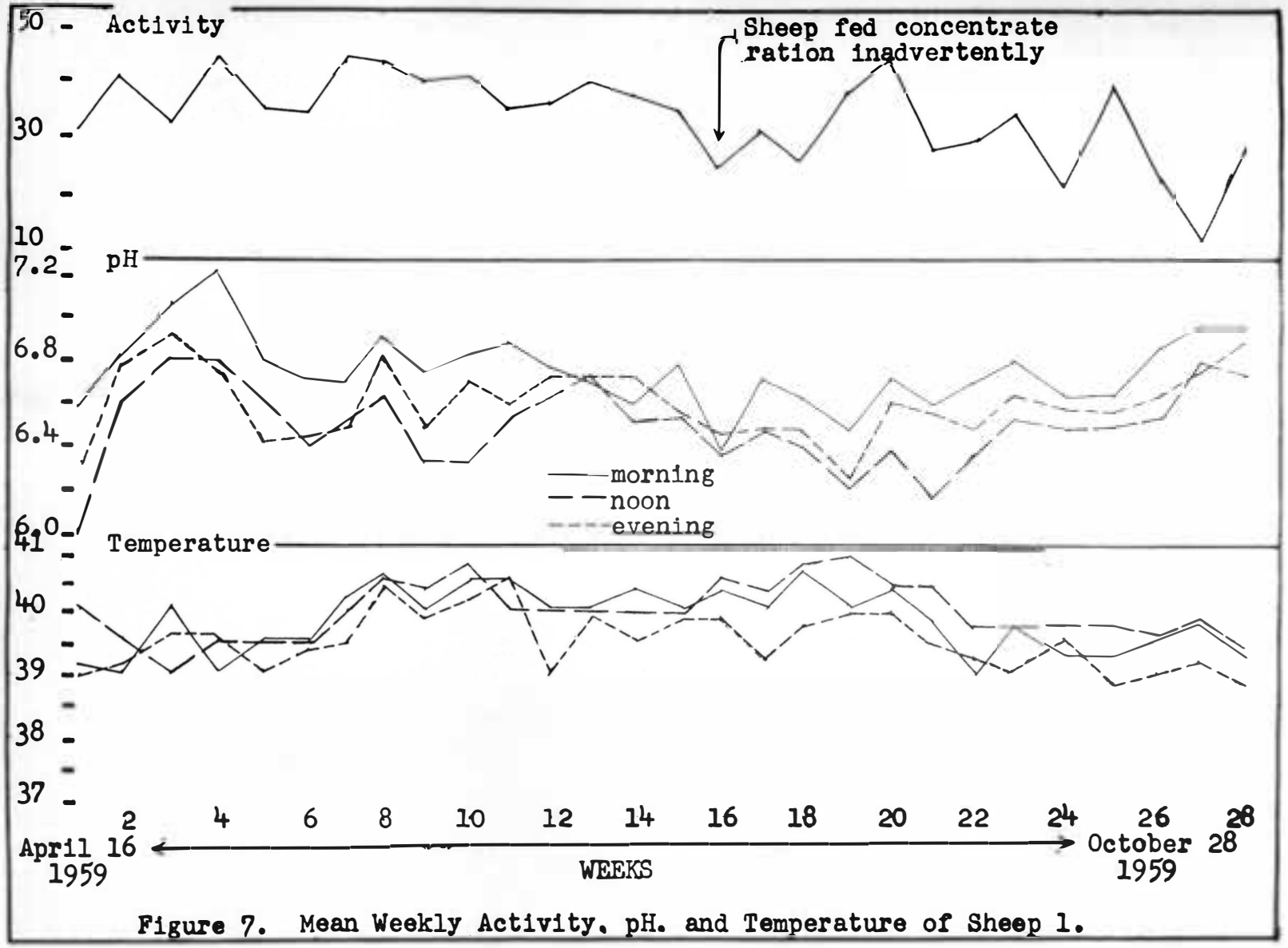


Figure 7. Mean Weekly Activity, pH. and Temperature of Sheep 1.

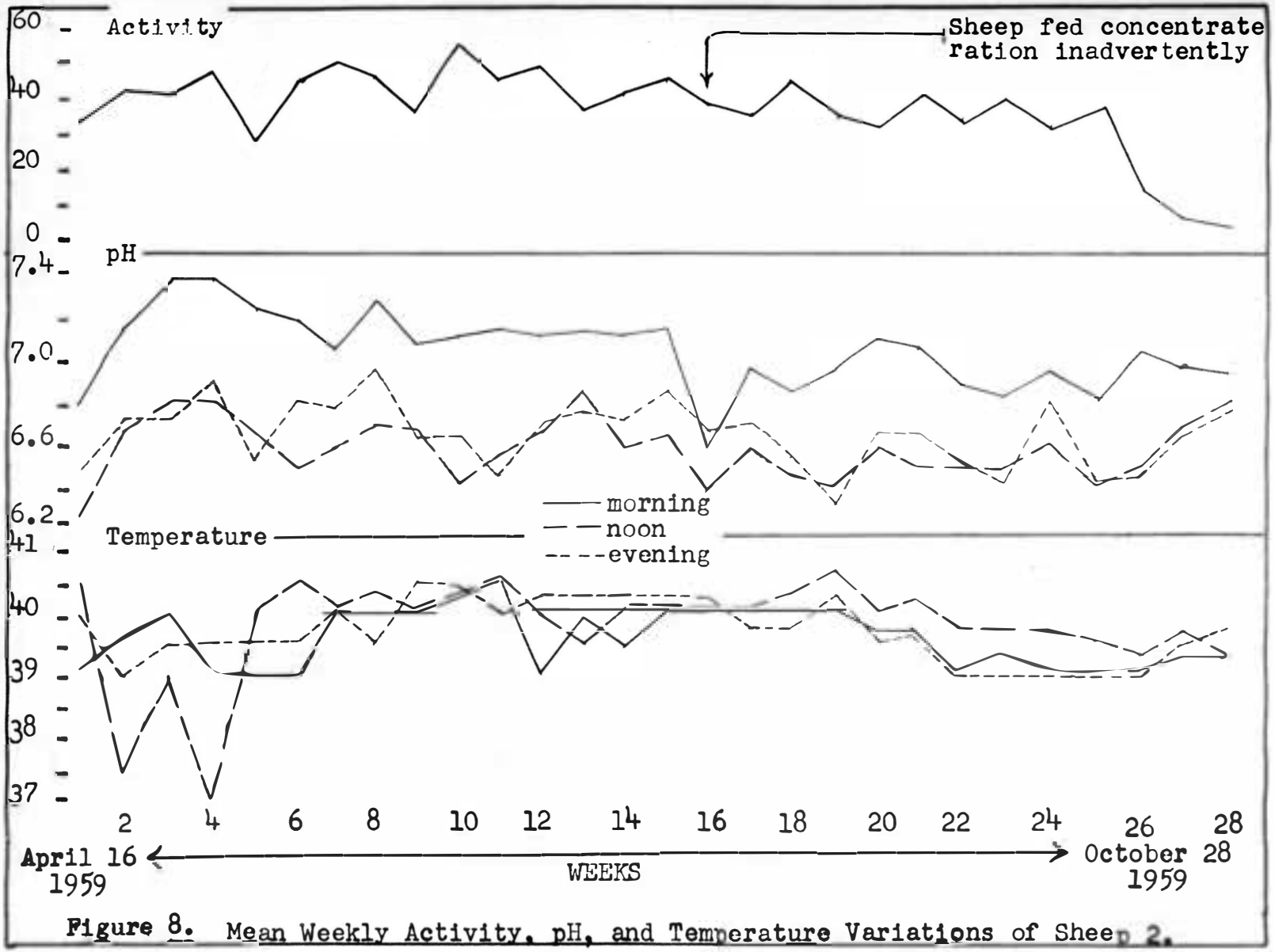


**Figure 8.** Mean Weekly Activity, pH, and Temperature Variations of Sheep 2.

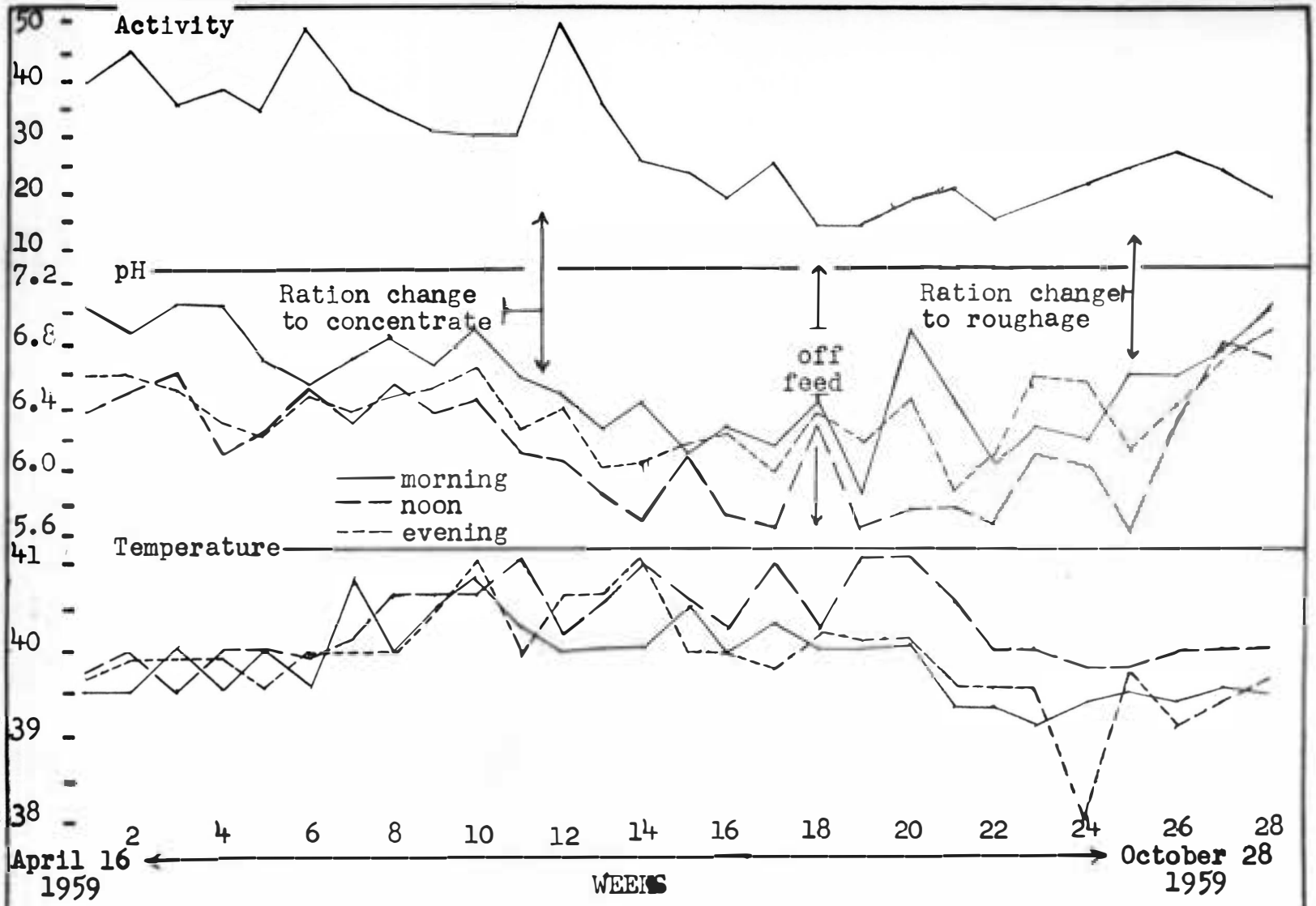


**Figure 9.** Mean Weekly Activity, pH, and Temperature Variations of Sheep 3.

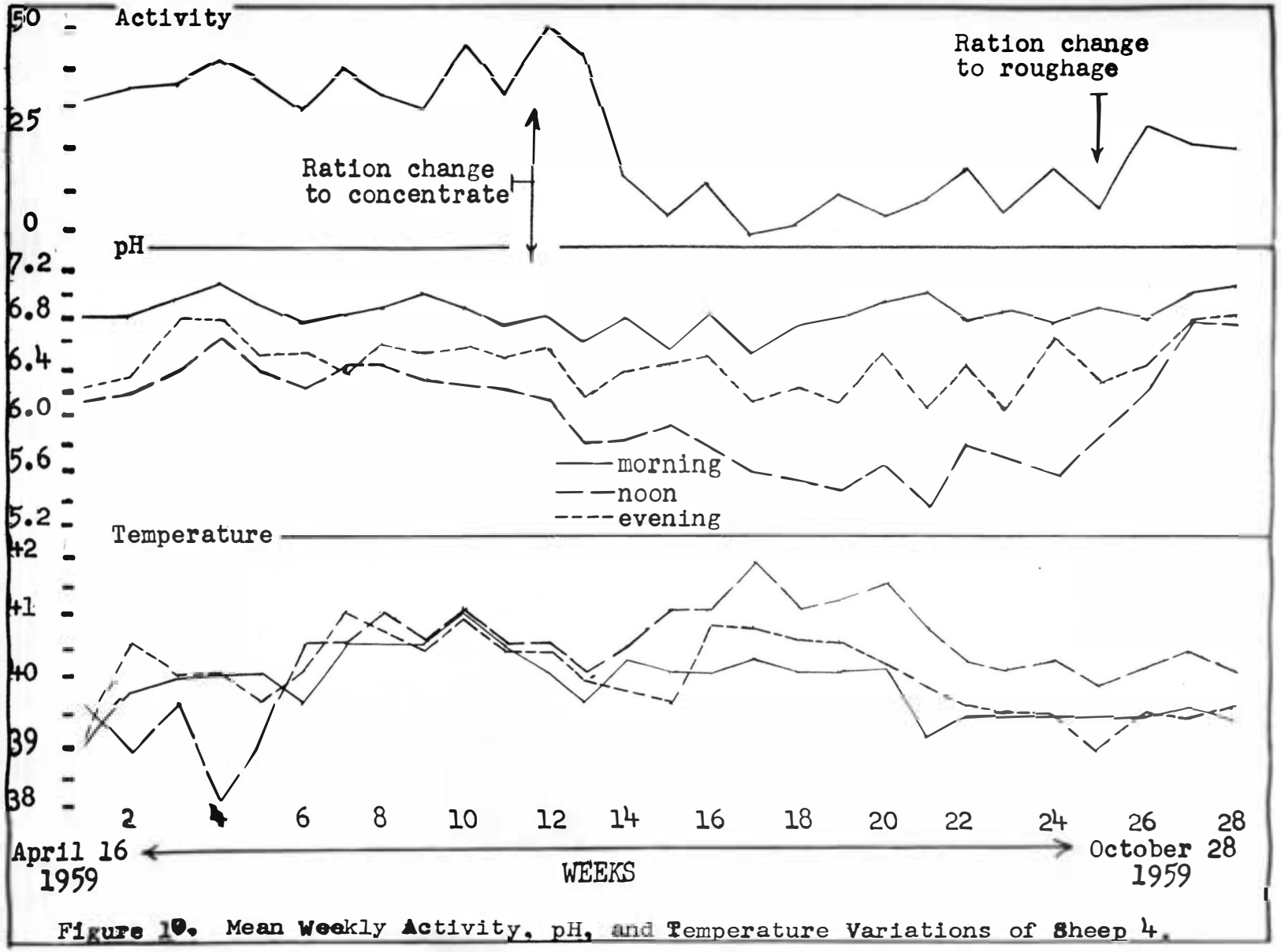


Figure 19. Mean Weekly Activity, pH, and Temperature Variations of Sheep 4.

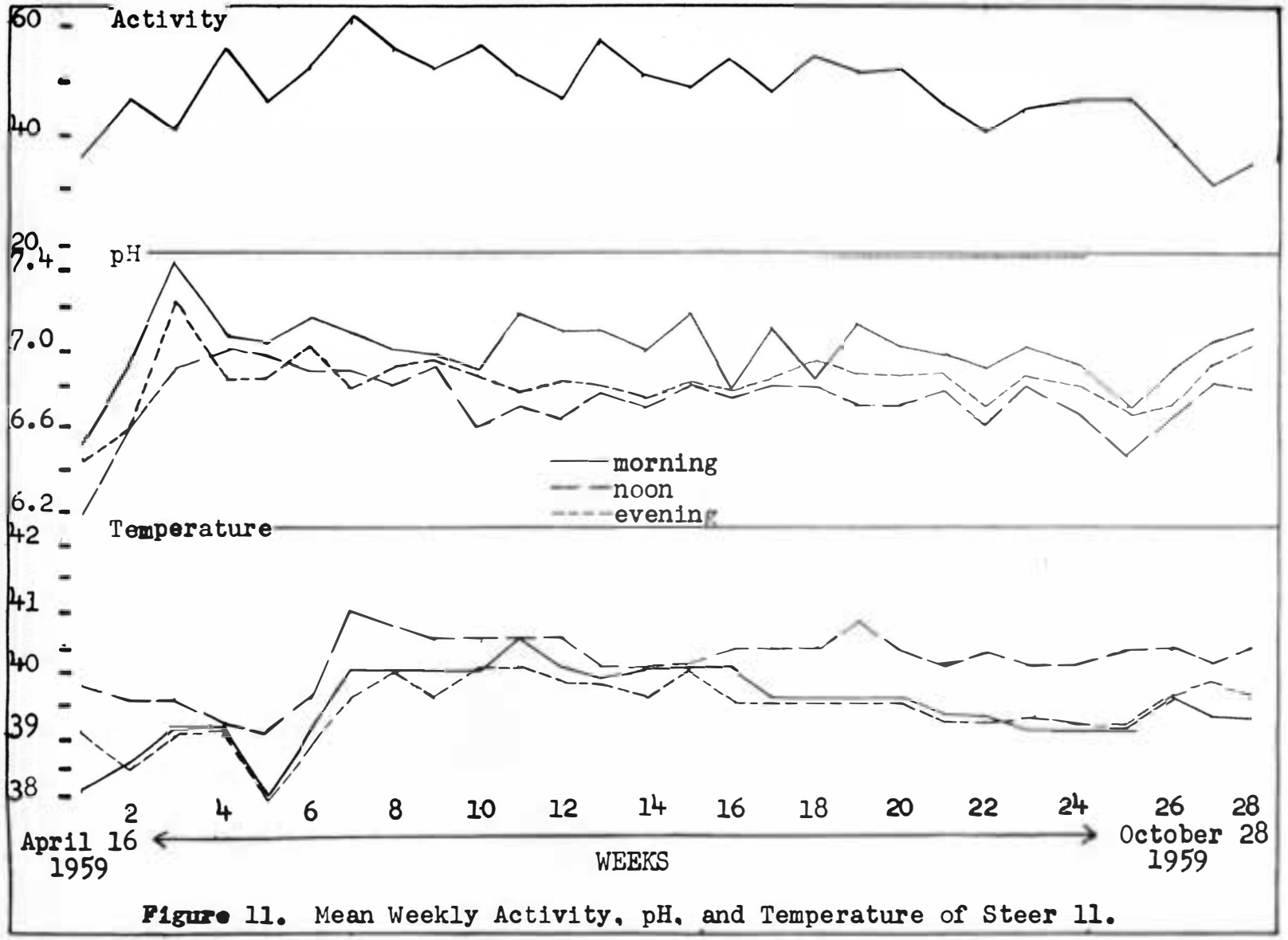


**Figure 11.** Mean Weekly Activity, pH, and Temperature of Steer 11.

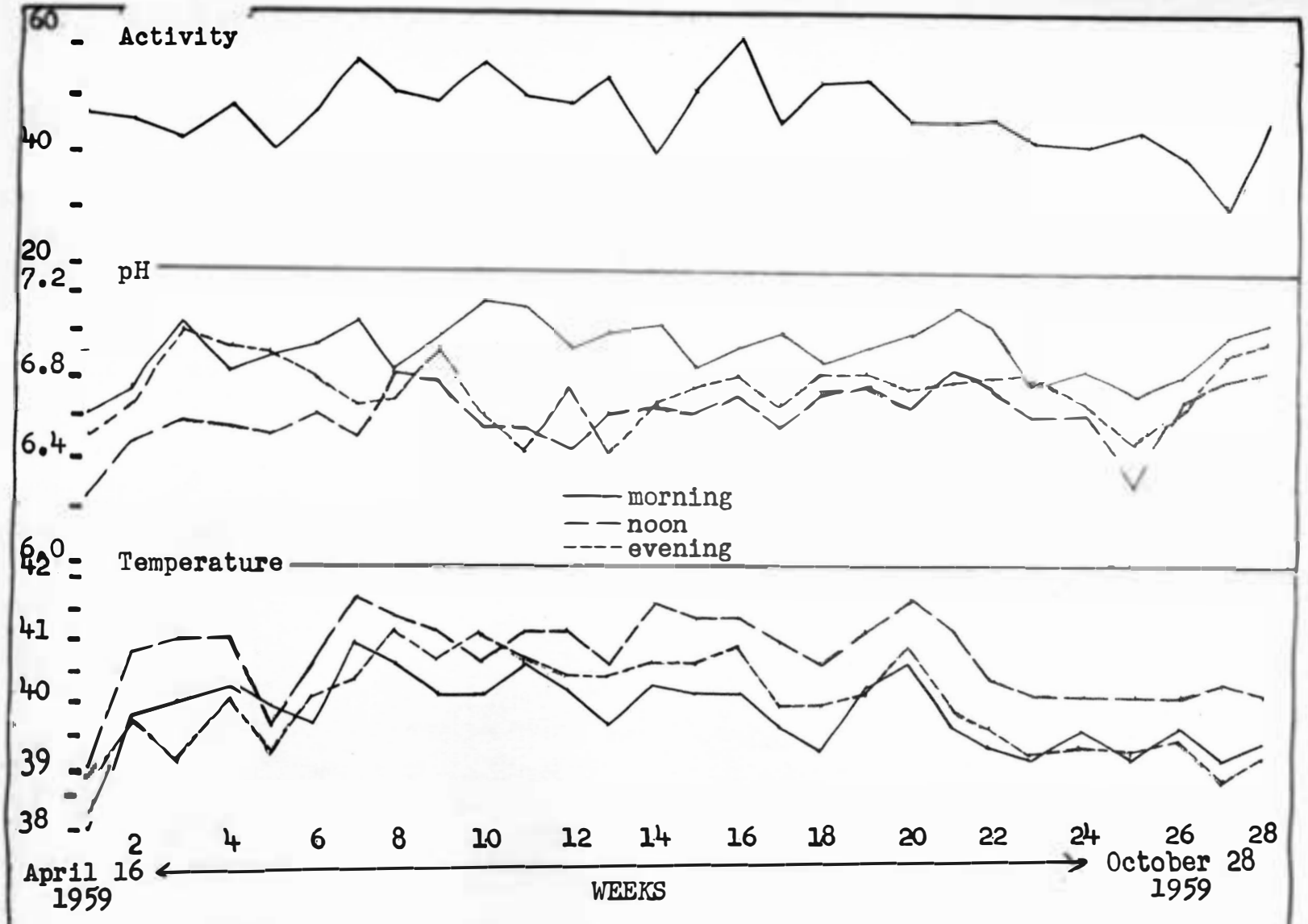


Figure 12. Mean Weekly Activity, pH, and Temperature of Steer 12.

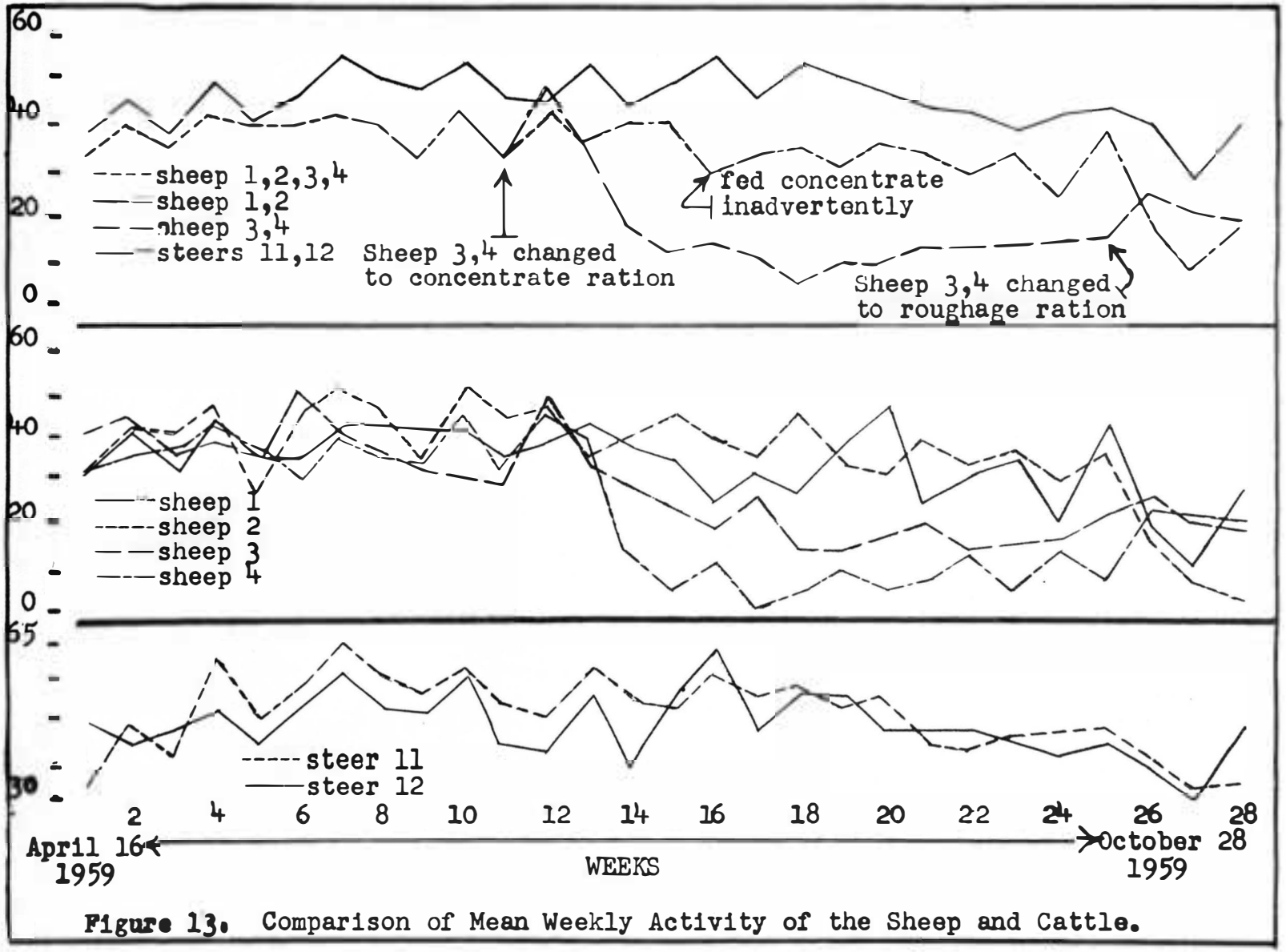


**Figure 13.** Comparison of Mean Weekly Activity of the Sheep and Cattle.

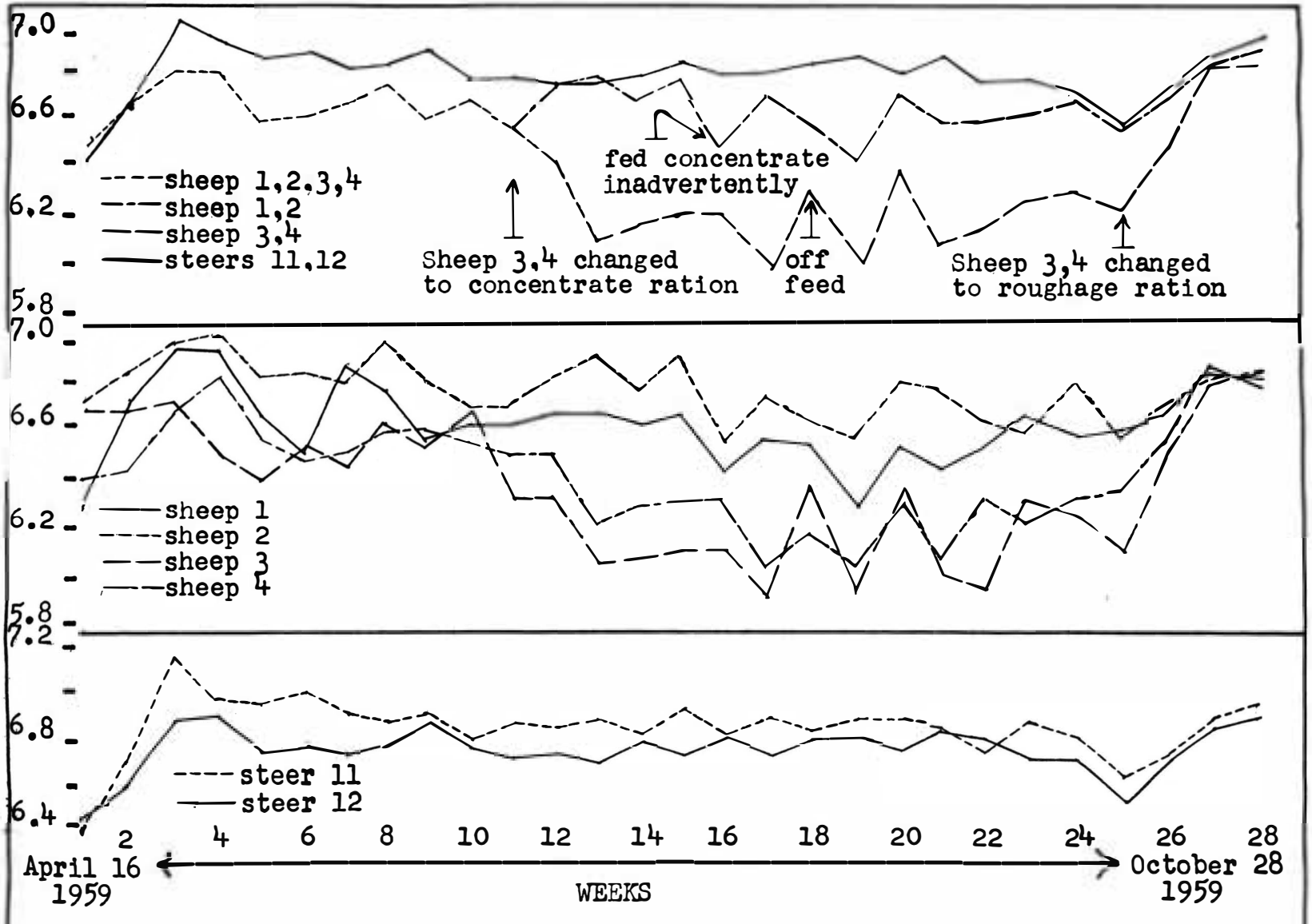


**Figure 14.** Comparison of Mean Weekly pH of the Sheep and Cattle.

## SUMMARY AND CONCLUSIONS

Work has been done pertaining to the fluctuation of *in vitro* rumen acidity, *in vivo* rumen temperature, and *in vitro* cellulose digestion by rumen microorganisms. These three factors were studied along with the attempted culturing of rumen organisms.

The primary objective was to determine if these three conditions fluctuate correspondingly with one another and if they are influenced by seasonal or ration change. Two steers and four wethers were used in this trial. Two of the sheep were subjected to ration changes, consisting of intermittent high roughage and high concentrate feeding.

Morning, noon, and evening pH and temperature determinations were made every Tuesday and Thursday for a period of 28 weeks. The trial began in April and was terminated the following October, extending through the spring, summer, and early fall months. *In vitro* activity determinations were made twice a week, each having a 22-hour fermentation period.

Temperature and pH observations of all animals used in the experiment appeared to produce a similar trend that indicated (1) that rumen pH determined in the morning, prior to feeding, was the most alkaline of the three readings, (2) the lowest pH determinations were generally at noon, taken approximately three hours after feeding, (3) evening rumen acidity values (prior to feeding) fell between the morning and noon determinations, usually being only slightly more acidic than the morning readings, (4) and rumen temperatures (taken

simultaneously with the pH readings) followed similar trends shown for the pH values, but in an inverse manner.

There was evidence shown in the experiment that certain fluctuations of pH, temperature, and activity were shown to have been due to a change in roughage quality. At the beginning of the trial when a poorer quality roughage was used, than had been previously fed, there was a pronounced fluctuation of activity, corresponding with an increasing rumen pH and a slight lowering of rumen temperature.

In the 25th week a high quality roughage had to be substituted for the forage being used in the rations. An activity increase would be expected with a change of this type, however, activity showed a decline after the change occurred. This drop in cellulose digestibility by the rumen microorganisms was immediate for the animals on the continuous high roughage rations. Two of the sheep which had been on concentrate were changed back to their original high roughage ration at the same time the quality change in roughage occurred. A distinct rise in activity followed this change to roughage. This higher cellulose digestion was followed by an activity decline similar to that shown for the continuous roughage fed steers and sheep in the trial.

In the 27th week the animals on roughage ration (two steers and two wethers), with the exception of one wether, showed a slight recovery in their activity values, while no recovery was indicated for the sheep which had just been changed back to a roughage ration. Due to the termination of the trial, it could not be determined what the extent of the recovery for the continuous roughage fed animals would be, or if a

recovery period would occur for the two wethers on the intermittent roughage, concentrate ration change.

Seasonal changes were quite evident in rumen temperature decreases during the spring and fall months of the trial. Slight decreases in activity observed for some of the animals during this same period seemed to correspond with the temperature decreases. However, pH fluctuations during this time did not seem to correspond with the temperature and activity changes.

Statistical correlations showing the relationship of pH, temperature, and activity fluctuations were anticipated prior to this experiment. Linear correlations were attempted with these three factors, correlating only two of the variables at one time. The results obtained indicated an abnormal relationship between the three factors, possibly due to the difference in the numerical values of the different figures. It seems that pH ($-\log_{10}$[illegible]), correlated with activity (percentage) or temperature (degrees) does not show a true relationship. A fluctuation of activity is not followed, to the same degree, by a corresponding fluctuation in pH and temperature. An increase in activity may result in only a slight increase in temperature and perhaps a sharp decline in acidity, all showing corresponding relationship but not the same degree of change. If all three of these variables (pH, temperature, and activity) could be expressed with workable numerical values, it appears that a multiple correlation of pH, temperature, and activity would show a definite relationship.

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