STUDIES OF VARIATION IN pH AND VOLATILE FATTY ACID CONCENTRATION WITHIN THE RETICULUM AND RUMEN OF A GRAZING COW

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INTRODUCTION

The ruminant is but one component of the great cycle of energy utilization, a cycle which derives its energy from that of the sun and enables man not only to exist, but also to flourish.

The complex and comprehensive nature of the cycle does not belittle the role played by ruminants - the conversion of high fibre feedstuff of no direct value to man into a whole array of products, many of which are now considered essential to human nutrition and welfare.

Their ability to perform this feat is attributable to the symbiotic relationship which exists between the microorganisms of the rumen and the host animal. This remarkable example of symbiosis has always elicited man's interest even though the interest initially arose from an inherent curiosity rather than a specific need.

However, over the last few decades the resources of workers from diverse fields of science have been directed towards an understanding of the activities within the rumen and their importance to the nutrition of the host animal. This upsurge of interest has arisen from the demand for more efficient agricultural production which, from the animal side, can be achieved by maximizing the effeciency with which the ingested food material is converted to the animal products. Inherent in this concept is the prevention and correction of the metabolic disorders which tend to accompany the intense and unnatural exploitation of the ruminant.

The outcome is that many of the activities within the rumen are no longer a mystery to the biologist and chemist.

Thus it has been shown that the amounts and proportions of the individual volatile fatty acids produced within the rumen vary according to the diet. The

amount and proportions of these acids have also been shown to influence the value of the food in meeting the requirements of maintenance, growth and fattening. Similarly, in the lactating cow, they influence the yield and composition of the milk.

Most of this work has been done in countries where the reliance on direct consumption of pasture by grazing animals is less than in this country. The result is that New Zealand workers have been faced with the study of problems that are not so important elsewhere. Although a considerable amount of local work has been done, particularly on some metabolic disorders and deficiencies, much still remains. Very little information is available concerning the effect on the fermentation processes of feeding pastures of varying botanical and chemical composition and the different grazing practices used in their utilisation. The normal variability in the concentration of fermentation end products that can be expected with grazing animals is particularly lacking in definition.

In the programme of work originally planned, it was hoped that some information on the normal state of microbial activity in the rumen of the grazing animal would be obtained. However, the literature revealed that one of the features of the rumen contents is a marked heterogeneity in the distribution of ingested food materials. Furthermore, a few reports indicated that in the bovine rumen this heterogeneity was reflected in the fermentation end products in the form of concentration gradients or stratification. The impression was gained that this effect may be particularly marked in grazing animals.

Thus, although the analytical work involved limited the scope of the study, an attempt was made to obtain a clearer understanding of the nature and magnitude of this stratification. Such a study was believed necessary for the intelligent selection of a sampling site in any studies on the microbial activity within the rumen of the grazing bovine.

Two feeding systems were chosen. The first involved a limited period of grazing after a period of fasting, a system often used in comparing the effect

of different diets on the fermentation processes. The second system consisted of free grazing similar to that which would prevail in any survey work.

In preliminary trials it was found that in some instances an analytical procedure commonly used for the determination of volatile fatty acid (VFA) concentration in rumen liquor failed to give reproducible results. Differences in VFA concentration of up to 30% were found between determinations on the same sample of rumen liquor. Further, the number of rumen samples in the experiments as planned necessitated their storage for a period of several days. It was therefore deemed advisable not only to compare some of the commonly used procedures for determining the VFA concentration in rumen samples but also to examine the stability of the VFA's during storage.

Part 1 is a report of this study and although it is complete in itself it is considered an integral part of the whole thesis as entitled. Each feeding system is dealt with separately in Chapters II and III of Part II. In Chapter IV the pertinent findings are discussed in relation to possible causes of stratification and the effect it has on sampling the rumen contents. A general summary of the results and conclusions for both experiments is presented in the final chapter.

PART I

ESTIMATION OF THE VOLATILE FATTY ACID CONCENTRATION BY STEAM DISTILLATION

1 REVIEW OF LITERATURE

Recognition of the full importance of volatile fatty acids (VFA) in ruminant nutrition was not made possible until the development of reliable methods for the analysis of VFA mixtures. Following the introduction of partition chromatography by Martin and Synge (1941), several reliable column partition chromatographic techniques have been reported (Elsden, 1946; James and Martin, 1952; Wiseman and Irvin, 1957).

Before the chromatographic procedure can be applied to the separation and identification of VFA in biological fluids, their isolation from other acidic substances is generally required. This isolation is most commonly achieved by steam distillation; titration of the distillate giving a measure of the total volatile acids. Methods that eliminate the need for steam distillation have been developed (Neish, 1949; Wiseman and Irvin, 1957).

As many organic acids other than fatty acids are volatile in steam but to a lesser extent than the lower fatty acids, the conditions of distillation are adjusted in an endeavour to eliminate from the distillate, all but the fatty acids.

Of the numerous procedures available, the double distillation method of Friedemann (1938) has proved the most reliable. In the second distillation, use of mercuric salts and magnesium sulphate quantitatively removes pyruvic acid, reduces lactic acid to less than 0.2% of the quantity in the original sample and oxidises formic acid (Baker, 1957).

McAnally (1944) found that with blood, the method of Friedemann gave results that were dependent on the time taken for distillation indicating that decomposition of labile substances had occurred. Precipation and subsequent removal of protein from a diluted sample of blood, besides giving a good

recovery of added acids, gave a result unaffected by time taken for distillation. This method, like that of Friedemann's, is based on the observations of Olmsted, Whitaker and Duden (1929) that high concentrations of $Mg.SO_4.7H_2O$ greatly increases the rate of distillation of the VFA. This method has been applied to the analysis of rumen fluid by many subsequent workers, the essential steps being the precipation and removal of protein, acidification and steam distillation in the presence of $Mg.SO_4.7H_2O$.

In rumen fluid where the levels of lactic and pyruvic acids are considered negligible under normal conditions, the simpler and less tedious method of McManally is usually preferred to the accurate, but time consuming method of Friedemann. Many modifications to these methods have been reported (Opperman _etal_, 1957; Johns, 1955; Barnett and Reid, 1957). The most widely used steam distillation apparatus is that of Markham (1942).

II MATERIALS AND METHODS

A large volume (2 l.) of rumen liquor was collected from a fistulated cow by expressing a portion of the rumen ingesta through a single layer of cheese cloth. Representative samples of the liquor were treated as follows.

Procedure A (Johns, 1955). Preserved by addition of 2% (V/V) $HgCl_2$ as a saturated solution. Aliquots were distilled in the presence of 1ml.10N H_2SO_4 saturated with $Mg.SO_4.7H_2O$.

Procedure B. As for Procedure A but the rumen liquor was divided into eight portions (one portion analysed each day) and stored at $-10^{\circ}C$.

Procedure C. 20 ml.10N H_2SO_4 were added in the proportion of 20 ml. to each 100 ml. of rumen liquor. The resulting suspension was stored and used for subsequent analysis.

Procedure D. As for Procedure C except that immediately prior to distillation, an aliquot was centrifuged at 500 g. to remove most of the particulate material. The resulting supernatant was used for analysis.

Procedure E (Annison, 1954). The sample was mixed with an equal volume of N H_2SO_4 saturated with $Mg.SO_4.7H_2O$, allowed to stand for 30 minutes at room temperature to denature protein which was then removed by centrifuging at 500 g. for 5 minutes. The supernatant was stored for subsequent analysis.

Procedure F (Balch and Rowland, 1957). The sample was centrifuged (500 g. for 5 minutes) to remove suspended solids and the supernatant diluted with four volumes of water. Equal volumes of diluted fluid and N H_2SO_4 saturated with $Mg.SO_4.7H_2O$ were mixed and left overnight to denature the protein. The precipitate was centrifuged off and the supernatant stored for analysis.

To determine the recovery of the VFA's, the same procedures were applied to further samples of rumen liquor to which was added a known amount of a standard solution containing 10.22 mM/100 ml. of acetic, propionic and butyric acids in the proportions 6 : 3 : 1 :

With the exception of Procedure B, storage was at 5°C. On each of eight successive days, the VFA concentration in samples representing all procedures was determined in duplicate. The order in which the ten samples were analysed was randomised by drawing ten numbered marbles from a bag.

5 ml. aliquots were distilled in a Markham still. Two successive 50 ml. fractions of distillate were collected and titrated with 0.06N NaOH using phenolphthalein as indicator. During titration of the 50 ml. fractions, interference by CO_2 was reduced by using a sintered glass filter stick to bubble CO_2 free air through each fraction. Use of 0.06N NaOH obviated the extreme care required when dealing with more dilute (< 0.02N) alkali.

TABLE II

THE CONCENTRATION OF VFA IN THE RUMEN LIQUOR AFTER THE ADDITION OF 25 ml OF A STANDARD SOLUTION OF VFA (10.22 mM/100 ml) TO EACH 100 ml OF RUMEN LIQUOR. METHODS AS FOR TABLE I (mM/125 ml)

PROCEDURE

	A	B	C	D	E	F
Day 1	15.8	15.7	16.2	16.0	15.9	12.9
	15.8	15.7	16.1	15.9	15.9.	12.8
2	15.7	15.7	16.2	15.9	15.9	14.7
	15.7	15.6	16.0	15.9	15.9	14.8
3	15.7	15.8	16.0	16.0	15.7	13.2
	15.7	15.8	16.1	16.0	15.7	13.2
4	15.7	15.7	16.0	16.0	15.8	13.6
	15.7	15.7	16.0	16.0	15.8	13.6
5	15.7	15.7	16.0	16.0	15.7	13.4
	15.7	15.8	15.9	16.0	15.8	13.3
6	15.7	15.8	16.0	16.0	15.9	13.3
	15.7	15.8	16.0	16.0	15.9	13.3
7	15.7	15.8	16.0	16.0	15.8	13.3
	15.7	15.8	15.9	16.0	15.9	13.3
8	15.7	15.7	16.0	16.0	15.8	13.4
	15.7	15.7	16.0	16.0	15.8	13.6

ANALYSIS OF VARIANCE

SOURCE	d.f.	M.S.	F.	
Procedures	4	0.3180	42.4	**
Days	7	0.0043	< 1	
Interaction	28	0.0075	5.00	**
Determinations	40	0.0015		

** ($P < 0.01$) Highly significant

Data for Procedure F excluded from statistical analysis

TABLE 1

THE VFA CONCENTRATION IN RUMEN LIQUOR ON EACH OF EIGHT SUCCESSIVE DAYS AS DETERMINED IN DUPLICATE BY FIVE PROCEDURES (mM/100 ml)

	PROCEDURE					
	A	B	C	D	E	F
Day 1	13.2	13.3	13.6	13.5	13.3	11.6
	13.2	13.3	13.6	13.5	13.3	11.7
2	13.1	13.3	13.6	13.4	13.3	11.0
	13.2	13.3	13.6	13.6	13.3	11.0
3	13.2	13.3	13.6	13.5	13.2	11.0
	13.3	13.3	13.6	13.6	13.2	11.0
4	13.2	13.0	13.5	13.5	13.2	11.1
	13.2	13.0	13.5	13.5	13.3	11.2
5	13.2	13.2	13.6	13.6	13.2	11.1
	13.2	13.2	13.6	13.5	13.3	11.4
6	13.2	13.3	13.5	13.6	13.3	11.7
	13.2	13.3	13.5	13.6	13.3	11.7
7	13.2	13.3	13.5	13.6	13.3	10.8
	13.2	13.3	13.5	13.6	13.3	10.8
8	13.1	13.3	13.6	13.6	13.2	11.1
	13.1	13.3	13.6	13.6	13.2	11.0

ANALYSIS OF VARIANCE

SOURCE	d.f.	M.S.	F.	
Procedures	4	0.515	68	**
Days	7	0.010	1.33	NS
Interaction	28	0.0075	6.00	**
Determinations	40	0.00125		

NS Not significant ($P > 0.05$)
** ($P < 0.01$) Highly significant

Data for Procedure F excluded from statistical analysis

III RESULTS

The VFA concentration in the rumen liquor, and rumen liquor plus added VFA, for the eight days are presented together with the analyses of variance in Tables I and II respectively. Because of their obvious inconsistency, the results obtained by using Procedure F were excluded from the statistical analyses.

Similar conclusions were drawn from the results of both analyses of variance. The significant interactions indicate that with some Procedures, there was a significant difference in VFA concentration between days. The difference required to reach significance at the 5% level was 0.17 m M/100 ml. for both tables. The significant interaction was apparently caused largely by the VFA concentration as determined by Procedure B on Day 4 (Table I) and the concentration as determined by Procedure C on Day 1 (Table II). For any one procedure there was no trend in VFA concentration with days. On the basis of these observations it was concluded that for Procedures A, B, C, D and E, no change of practical importance occurred during the eight days of storage.

The Procedure means for both sets of VFA concentration determinations are presented in Table III together with standard errors and the detectable differences required for significance at two levels.

TABLE III

The means of the VFA concentration of rumen liquor with and without added VFA over a storage period of eight days as determined by five procedures.

	Procedure	Mean	General Mean	S.E.	Difference required dθ.05	dθ.01
Without added VFA	A	13.19	13.36	± 0.02	0.06	0.08
	B	13.25				
	C	13.56				
	D	13.55				
	E	13.26				
With added VFA	A	15.71	15.86	± 0.02	0.06	0.08
	B	15.74				
	C	16.03				
	D	15.98				
	E	15.83				

This table illustrates that Procedures C and D gave higher estimates of VFA concentration than any of the other procedures.

The analysis of variance of the recovery data (Table IV) showed that the procedures did not differ in their ability to recover added VFA's. The average percentage recovery of 98% was considered satisfactory.

TABLE IV

THE CONCENTRATION OF THE STANDARD SOLUTION OF VFA ADDED TO THE RUMEN LIQUOR AS CALCULATED FROM THE AVERAGE OF DUPLICATE DETERMINATIONS IN TABLES I AND II BEFORE THEIR CORRECTION TO ONE DECIMAL PLACE. (TRUE CONCENTRATION-10.22 mM/100 ml)

(mM/100 ml)

PROCEDURE

	A	B	C	D	E	F
Day 1	10.3	10.0	10.2	9.9	10.7	4.8
2	10.4	9.5	10.0	9.6	10.4	15.0
3	10.0	10.2	10.0	9.8	10.0	8.4
4	10.0	11.1	9.8	10.0	10.0	9.8
5	10.0	10.4	9.4	10.0	10.0	8.4
6	10.0	10.0	10.2	9.8	10.4	6.4
7	9.8	10.0	9.6	9.8	10.3	10.0
8	10.2	9.4	10.0	9.8	10.4	9.8

ANALYSIS OF VARIANCE

SOURCE	d.f.	M.S.	F.	
Procedures	4	0.2275	2.21	NS
Days	7	0.0586	0.57	NS
Error	28	0.1029		

NS Not significant (P > 0.05)

Data for Procedure F excluded from statistical analysis

IV DISCUSSION

The procedure of Balch and Rowland (1957) proved unsatisfactory when used under the existing experimental conditions. As this procedure involved dilution of the rumen liquor, the titration of the steam distillates required only small volumes of 0.06N alkali (approx.1 ml.). For this reason it was considered that the unsatisfactory results obtained were a reflection of the relative magnitude of the titration errors rather than an indication of an inherent fault in the procedure.

The procedures did not differ in their ability to recover added VFA's but there was a significant difference in their estimation of the initial VFA concentration in the rumen liquor. However, the percentage difference between the mean estimates (Table III), suggests that although the difference was statistically significant, it may not be of practical importance, for in rumen studies, sampling errors are usually large (Williams and Christian, 1956).

It was concluded that the VFA concentration of the rumen liquor, as estimated by five procedures did not show an alteration of practical importance over the storage period of eight days. This finding appears incompatible with the inconsistent results that were obtained using Procedure A in preliminary trials. However, the results reported here provide no grounds for assuming that any of the five procedures would give similar results on other rumen samples. The observation was made during the preliminary trials that samples which failed to give consistent results with Procedure A, gave consistent results with Procedure D. Besides indicating that it was unlikely that it was the experimentor at fault, this also suggests that a Procedure X Sample interaction may occur.

In view of this, it was concluded that for subsequent work, procedures involving denaturation and removal of protein would be the least likely to cause

trouble. Of the two appropriate procedures, the higher titre required in titration of the Procedure D distillates, makes this method preferable to Procedure E.

In ascertaining the validity of methods for determining VFA concentration in biological fluids, complete recovery of added VFA serves as a valuable check on possible losses associated with deproteinization and distillation procedures provided the assumption is accepted that the recovery of VFA originally present is as good as that of the added VFA. However, doubt still remains as to the nature of the acidic substances estimated.

The lower monohydroxy and keto acids such as lactic and pyruvic acids are slightly volatile with steam (Baker, 1957). Decomposition to labile acidic substances may result in other acidic substances occurring in the distillate.

Lactic acid is the only non-fatty acid likely to be present in appreciable amounts in rumen liquor. Although usually present in trace amounts only, enhanced levels are encountered in the rumen when excessive amounts of readily fermented carbohydrates are consumed by the animal (Baloh and Rowland, 1957; Briggs et al, 1957). Even in such cases, it was accepted by these authors that a sufficiently accurate estimate of the VFA concentration was obtained by use of the simple steam distillation procedures similar to those outlined above.

That some error will be present is shown by the work of Elsden (1946) and Reid and Lederer (1951).

In order to determine what portion of the titratable acidity was due to fatty acids with the exception of formic, Elsden performed a series of analyses on sheep rumen fluid in which the steam distillates obtained using the procedure of McAnally were collected after titration and re-distilled according to the method of Friedemann. On the assumption that the acids collected by the latter procedure were fatty acids only, the method of McAnally over-estimated the VFA

concentration by 10-15%, a figure that has possibly been reduced since the introduction of the Markham still and a blank titration.

Reid and Lederer devised a paper partition chromatographic procedure for the separation, identification and estimation of individual VFA from C_2 - C_7. As formic and lactic acids could not be separated from acetic acid by this method, their removal by preliminary steam distillation was necessary. They found however, that with rumen liquor containing up to 5 m.mole/100 ml. of lactic acid, the amount of lactic acid which distilled over when using McManally's method caused an error of only 3 - 5% in the determination of acetic acid. The error caused by a single distillation can therefore be neglected in all instances except those where unusually high levels of lactic acid (e.g. Briggs et al, 1957) are encountered.

Thus it is concluded that the high levels of VFA present in the ruminal contents can, for routine analysis, be estimated sufficiently accurately by the steam distillation procedures outlined. However, in view of comments made above, use of procedures that involve the removal of protein may be advisable. More accurate but time consuming procedures similar to those used for blood are obviated by the high levels of VFA and low levels of interfering substances found in the rumen.

V SUMMARY AND CONCLUSIONS

1. A preliminary investigation of the stability of the VFA concentration as determined by steam distillation was carried out on stored rumen liquor samples. Six procedures of preparation were used and their abilities to recover added VFA's were compared. Duplicate determinations for each sample were performed on each of eight days.

2. One procedure gave variable estimates of the VFA concentration of the rumen liquor.

3. The remaining five procedures differed significantly in their abilities to estimate the initial VFA concentration of the rumen liquor. It was suggested that this difference may not be of practical importance.

4. All procedures except one did not differ in their abilities to recover added VFA's. The recoveries achieved were considered satisfactory.

5. No change of practical importance was found in the VFA concentration during the eight day storage period.

6. It was concluded that the VFA concentration of rumen liquor could be determined with an accuracy suitable for routine analysis by the procedures outlined. However, certain reservations were made.

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PART II

VARIATION IN THE pH AND VOLATILE FATTY ACID CONCENTRATION WITHIN THE RETICULO-RUMEN OF A GRAZING COW

I REVIEW OF LITERATURE

A. Variation in the distribution of ingesta components.

In recent years, workers studying various aspects of rumen function have observed an apparent heterogeneity within the ingesta of the reticulo-rumen. Although only two studies specifically on variation have been reported (Smith, Sweeney, Rooney, King and Moore, 1956; Lampila, 1955), the problem of obtaining a representative sample of the ingesta has prompted various workers to conduct preliminary trials, the reports of which are scattered throughout the literature and are reviewed here.

(1) Ingested Food Material

After a period of eating much of the newly ingested food is found in the dorsal sac of the rumen. Where fibrous food has been consumed, the mass in the dorsal sac is relatively dry and tightly packed (Balch, 1950) and may contain over 15% of dry matter (Balch, 1959). In the reticulum and ventral regions of the rumen there is a more fluid ingesta containing 5 - 8% dry matter (Balch, 1959). The more liquid portions contain smaller food particles which have been in the rumen for a longer period than the more recently ingested material (Balch and Kelly, 1950). A mixture of fermentation gases is normally present above the ingesta (Dukes, 1955).

The collection of the fibrous ingesta in the dorsal regions has been attributed to the rapid movements of the reticulum and dorsal and ventral sacs of the rumen (Balch 1950, 1961). The density of the food particles and the buoyant effect of fermentation gases may be important (Nichols, 1954).

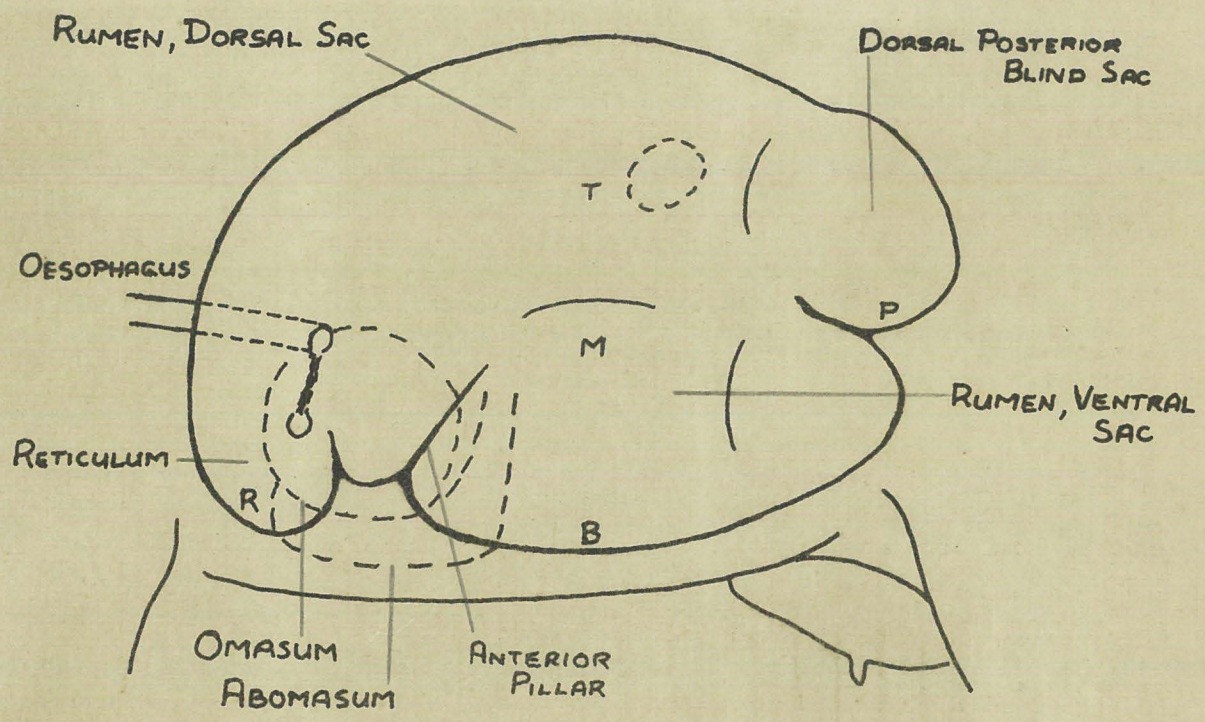


FIG. 1 DIAGRAM SHOWING THE APPROXIMATE POSITION OF THE RETICULO-RUMEN AGAINST THE LEFT SIDE OF THE COW (AFTER BALCH, 1959).

Factors that determine the movement of food particles from the dorsal rumen to the liquid ingesta in the ventral regions are not known with certainty (Balch, 1961).

(2) Fermentation Products

Smith et al (1956) sampled the dorsal and ventral rumens of three fistulated steers at hourly intervals over a 12 hour period which included two feeds. Analyses for crude fibre, ash, ether extract, total nitrogen, ammonia, non-protein nitrogen, water and alcohol soluble sugars, volatile fatty acids and digestion of cellulose in vitro, showed that in all instances except alcohol soluble sugars, a statistically significant difference ($P < 0.01$) existed between the two positions. Concentrations of all factors measured except ether extract and cellulose digestion in vitro were higher in the samples from the dorsal rumen. Data from samples obtained by rumenotomy established that the variation was not an artifact induced by the presence of the fistulae.

That cellulose digestion is more rapid in the ventral than in the dorsal rumen has been observed by others (Balch and Johnson, 1950; Miles, 1951).

Differences in the volatile fatty acid concentration between the dorsal and ventral regions of up to 50% in favour of the dorsal region were observed by Lampila (1955). In a later report (Lampila, 1960), a similar but less distinct difference was found in the NH_3 concentration during the first half of the period between feeds.

In contrast to the above observations, samples from six inches below the surface of the ingesta have been considered representative in VFA concentration as variation throughout the rumen was non-existent (Balch, Balch, Bartlett, Bartrum, Johnson, Rowland and Turner, 1955) or of no

consequence (Balch and Rowland, 1957).

(3) pH

Monroe and Perkins (1939) observed that pH varied throughout the rumen. The validity of their results has been questioned (see P. 20) and cannot be taken as unequivocal. However, the findings of Smith et al (1956) and the determinations in vivo of Smith (1941) and Lampila (1955) have established that the pH of the bovine rumen contents varies according to location within the rumen, the dorsal regions being consistently more acid than the ventral regions.

(4) Microbial Population

Differences in total bacterial count between the dorsal and ventral regions of the bovine rumen have been observed by Gall, Stark and Loosli (1947) and Fulghum, King and Moore (1958) but opposite conclusions were drawn as to which region had the highest concentration. Hungate (1947) found no difference between the two regions, an observation supported by Annison and Lewis (1959).

When interpreting these results it should be remembered that the three main counting techniques (total, total viable and differential counts) are all subject to serious limitations (Annison and Lewis, 1959; Hobson, 1961) and information obtained by each method must be regarded as complementary. Some earlier results have been obtained by crude culture techniques that are inherently more variable than the variability one might expect in the actual samples (Bryant, 1959; Huhtanen, Rodgers and Gall, 1952). Some more recent methods are considered to be of greater accuracy (Boyne, Hadic and Raitt, 1959). Furthermore, there is some doubt as to whether the total bacterial

population remains constant even under apparently constant conditions (Wilson and Briggs, 1955). Under such conditions the viable population was found to vary within a relatively wide range which the heterogeneity of the ingesta affected little or not at all. Samples from different depths within the rumen varied indiscriminately in total bacterial count, variations of the same magnitude were obtained with samples from the same depth.

These workers suggested that failure to establish the normal variation in a single animal has led to smaller overall changes being regarded as indicative of significant quantitative changes in the flora.

The little that is known about the distribution of the microbial population within the rumen is based on quantitative counts. Even though changes in total concentration may not be evident, qualitative differences may exist.

The existence of three quite distinct layers within the rumen contents has been unequivocally established. Reports on variation in fermentation end products and pH are not extensive but it appears that under some conditions at least, considerable stratification occurs. It is suggested that no major stratification exists in the concentration of the microbial population but reports are not extensive.

B. Factors affecting Stratification

Many factors are known to affect the composition of ingesta within the reticulo-rumen but only those factors known to affect its heterogeneous nature will be reviewed here.

(1) Type of ration

As mentioned above, most of the newly ingested food material is found in the dorsal rumen. This also applies to concentrates and succulents such

such as mangolds and young legumes (Balch, 1961) but there is a definite tendency for these feeds to gravitate to the ventral regions more rapidly than the fibrous material. Nichols, 1954; Smith et al, 1956; Louisiana A.E.S., 1956).

On a low roughage diet, or one in which the roughage is finely divided, there is little tendency for layering to occur (Jacobson, 1955) and the dry matter content of the ingesta in the ventral rumen approaches that of the ingesta in the dorsal rumen (Balch, 1950).

The only observation which has indicated that the type of ration affects stratification of fermentation end products is that of Lampila (1960). It was found that the extent by which the ammonia level in the dorsal rumen exceeded that in the ventral rumen was dependent on the type of processed silage that was added to the basal ration.

(2) Time after Feeding

As the time since last feeding increases, the volume of the rumen contents decrease (Ritzman and Benedict, 1938; Coop, 1949), the ingesta becomes more liquid (Quin, Oyaert and Clarke, 1951; Dukes, 1955) and the layering of food particles is not evident (Balch, 1961). There is no direct evidence of a concomitant alteration in the stratification of fermentation products. However, Lampila (1955) found that the pH difference between the dorsal and ventral regions of the rumen increased upon feeding simultaneously with the pH of the ingesta. Monroe and Perkins (1939) made a similar observation. In a more recent study (Lampila, 1960) a difference in the ammonia concentration between the dorsal and ventral regions of the rumen was found only in the first half of the period between two feeds.

Although these observations suggest maximum heterogeneity during

the period of maximum fermentation, the absence of significant Time x Position interactions (except in the instance of total sugars) in the results of Smith et al (1956) fail to support this. However, the statistical interpretations of this study should be viewed with caution as they are based on a small number of observations on variables which, in some cases, are not additive.

C. Sampling rumen contents.

One of the consequences of rumen ingesta heterogeneity is the difficulty experienced in obtaining representative and reproducible samples of the ingesta (Pearson and Smith, 1943).

Sampling procedures based on slaughterhouse material, cud (Davey and Briggs, 1959), and rumen puncture (Turner and Hodgetts, 1955) have useful but obviously very limited applications. The main methods are those of fistulation (Dougherty, 1955) and stomach tube (Watson and Jarrett, 1945; Pounden, 1954). The advantages and disadvantages of these two methods have been discussed by Doetsch and Robinson (1953), Hungate et al (1955), and Hobson (1961). It is generally agreed that fistulation is the most satisfactory method. Some degree of abnormality may be induced in the rumen environment (see P. 21) but no major abnormalities are evident (Smith et al 1956).

With fistulated animals, the problem of sample withdrawal remains. Thorough mixing of the whole of the rumen contents, with or without removal from the rumen (Agrawala, Duncan and Huffman, 1953) can be practised only for infrequent samples because of aeration of the ingesta. Temporary destruction of ingesta stratification is also involved but whether this is of importance is unknown.

With studies involving serial sampling it is common to sample from a fixed location in the rumen in an endeavour to reduce sampling errors (Bryant,

1959). Collection of large samples (Johns, 1955; Hungate et al, 1954) or composite samples from different regions within the rumen (Briggs et al, 1957a) serve a similar purpose.

Many devices have been used for the withdrawal of samples. Sampling devices such as described by Nichols (1955) and McClyment (1950) help prevent admixture with material from adjacent areas but because of rumen pillar movement, cannot attain the accurate placement of a hand borne container.

After removal from the rumen, the sample is usually expressed through muslin to remove the larger food particles (McDonald, 1952), the resultant liquor being used for analysis. What proportion of soluble components is retained with the solid material is not known. Loss of organisms is known to occur and possibly qualitative changes as well (Hobson, 1961).

Unless chemical analysis is performed without delay, further fermentation must be prevented. For this purpose, use has been made of $HgCl_2$ (Davis et al, 1957), H_2SO_4 (Emery et al, 1956), and alcohol (Agrawala et al, 1953).

Sampling for pH determination requires special attention. It has been established that on exposure to air, the rumen sample loses CO_2 and the pH rises (Turner and Hodgetts, 1955). Some earlier workers (Monroe and Perkins, 1939; Olson, 1941) have failed to take this effect into account. The pH levels which they recorded are subject to a variable error particularly where the samples were subjected to long periods of standing before the determination was made (Smith, 1941; Hyburgh and Quin, 1943; Clarke and Lombard, 1951; Turner and Hodgetts, 1955). Measurement of pH on samples obtained by stomach tube results in high values because of salivary contamination (Hyburgh and Quin, 1943; Clarke and Lombard, 1951) or loss of CO_2 during aspiration (Turner and Hodgetts, 1955). In vivo measurements have been made by Smith (1941) and Lempila (1955) by means of a specially constructed electrode assembly.

It has been established that loss of CO_2 with consequent rise in pH occurs immediately the fistula is opened (Smith, 1941; Turner and Hodgetts, 1955; Matscher, Borghi and Beghelli, 1957). Matscher et al also found that a leaking fistula caused high pH values but Turner and Hodgetts (1955) have stated that the pH of only the surface ingesta is affected.

Provided that the determination of pH immediately followed the removal of a sample, Briggs et al (1957a) found that the pH so obtained was identical to that determined with the sample in situ. Thus, in absence of suitable apparatus for in vivo determinations a procedure similar to the one used by these authors should produce satisfactory results, provided that the surface ingesta is not sampled.

D. Diurnal variations in the concentration of fermentation end products.

Determination of the fate and fermentation paths of dietary constituents by experiments in vivo has, in general, necessitated a controlled feeding regime. The ingestion of food is restricted to short periods that are often repeated throughout the experiment. Such studies have resulted in a comprehensive understanding of the changes that occur with time in the composition of the ingesta (Annison and Lewis, 1959; Boyne et al, 1957; Balch and Rowland, 1957). Similar information concerning free grazing conditions is very limited.

Balch and Rowland (1957) found that compared with a variety of diets fed at 12 hour intervals, the lowest sustained pH values were observed when the cows were grazing pasture. Although it was stated that pH varied inversely with the concentration of total volatile fatty acids, the form of data presentation allows for no further comment.

No distinct diurnal variations in the pH of the rumen contents of a grazing cow were observed by Lampila (1955). A similar lack of variability within and between days was found for various ingesta components by Williams and Christian (1956a) using pastured sheep sampled by stomach tube.

No other reports concerning diurnal variation of ingesta components have been found in the literature.

Reports of seasonal variations are also noticeably absent from the literature. Those that have been published (Johns, 1955; Barnett and Reid, 1957) are considered irrelevant to this review.

It is apparent that further research is required before the normal concentration of fermentation end products can be defined for the grazing ruminant.

II. EXPERIMENT I

THE EFFECT OF A LIMITED FEEDING PERIOD

A. Materials and Methods

(1) Animal

A 2½ year old pregnant Jersey cow was fitted with a large rumen fistula (Balch and Johnson, 1948) on 12/11/60. An initial period of handling was necessary for the animal to become accustomed to grazing alone and to the sampling procedure.

(2) Experimental design

The dates of sampling were 21/3/61, 24/3/61 and 27/3/61. On each of the three days there were four sampling times at three hour intervals commencing at 8.30 a.m.. Feed was withheld during the twelve hours preceeding the 8.30 a.m. sampling by confining the animal to a concrete yard. Water was available at all times. The feeding period consisted of one hour of grazing following the 8.30 a.m. sampling which was denoted as 0 hrs. after feeding. The same area (2½ acs.) was grazed on each occasion.

At each sampling time, samples were removed from the following five positions within the reticulo-rumen (Fig. 1).

Top (T)	Three inches below the centre of the surface ingesta.
Middle (M)	Intermediate to the dorsal and ventral positions.

Bottom (B) The floor of the ventral rumen.

Reticulum (R) The floor of the reticulum

Posterior (P) The floor of the dorsal posterior blind sac.

The positions were sampled in order as listed, each position being entered twice to give two samples (Sample A and Sample B) per position.

(3) Sampling

Samples (approx. 200 gm.) from the Top, Middle and Posterior positions were removed by hand. Samples from the remaining positions were removed using a 50 ml wide-necked bottle. The thumb was held over the neck of the bottle when moving to and from the sampling position.

The samples were expressed through muslin and the pH values determined to the nearest 0.05 pH units within one minute of removing the sample from the rumen. The pH meter (Radiometer, pH Meter 23) was standardized against a standard buffer solution (pH7) between samples.

The rumen liquor (25 ml) was transferred to a 50 ml glass bottle containing 5 ml of 10 N H_2SO_4 and stored at 5°C. The complete sampling procedure required approximately 25 mins.

(4) Analytical

Total volatile fatty acid (VFA) concentration was determined in duplicate for each sample using Procedure D as

described in Part I. All samples were analysed prior to the next sampling day. The order of analysis was randomised by use of the random numbers given by Fisher and Yates (1957).

Following titration and addition of excess alkali, two distillates, one from each of the two samples taken from the same position at the same time were mixed. Half this volume was taken and reduced in vacuo to approximately 5 ml over a water bath at 60°C using a rotary evaporator. After transference to a small test tube, the soaps were evaporated to dryness in an oven at 100°C and stored in a desiccator to await chromatographic analysis.

For determination of the relative proportions of acetic, propionic and butyric acids, the gas-liquid chromatogram of James and Martin (1952) was employed using the column packing described by Hawke (1957). Columns were run at 137°C with a N_2 flow rate of 12-15 ml/min.

In order to transfer the VFAs to the chromatographic column, the soaps were dissolved in a small volume of water and taken up in a hypodermic syringe. Approximately 0.05 ml (1-2 drops) of the aqueous solution was added to a platinum boat containing equal parts of Celite and $KHSO_4$ as a dry mixture. The boat was quickly pushed into the column with the aid of an attached copper wire and the gas supply connected. Although purely qualitative, this method of applying the samples was rapid for sample preparation was minimized by the use of an aqueous solution of soaps. In addition, the column

remained at operating temperature throughout the transfer of the soaps to the column.

The reliability of this method was checked using a standard solution containing a mixture of acetic, propionic and butyric acids in the proportions of 6 : 3 : 1. Good agreement was obtained between the calculated proportions and those found by chromatographic analysis, the proportions as determined chromatographically not differing by more than 2% from the calculated proportions.

TABLE I

ANALYSIS AND COMPONENTS OF VARIANCE FOR VFA LEVELS ON ALL DAYS

Analysis of variance

Source	df	SS	M.S.	F	
Days (D)	2	87.69	43.8	12.7	**
Times (T)	3	617.39	205.8	54	**
Positions (P)	4	150.73	37.7	9.9	**
Samples (S)	1	0.07	0.07	<1	NS
D x T	6	20.77	3.46	8.05	**
D x P	8	11.46	1.43	3.32	**
D x S	2	1.07	0.54	1.37	NS
T x P	12	45.60	3.80	8.85	**
T x S	3	1.65	0.55	1.28	NS
P x S	4	1.35	0.34	<1	NS
2nd and 3rd order interactions	74	32.03	0.43		
Duplicates within samples	120	0.20	0.002		
Total	239	970.01			

Components of variance and their estimates

Source	Duplicates	"Error"	PxS	TxS	TxP	DxS	DxP	DxT	S	P	T	D		
df	120	74	4	3	12	2	8	6	1	4	3	2	Component	Estimate
	1	1	1	1	1	1	1	1	1	1	1	1	σ^2	0.0016
	2	2	2	2	2	2	2	2	2	2	2	2	E^2	0.216
			24						24	24			PS^2	-0.004
				30					30		30		TS^2	0.004
					12					12	12		TP^2	0.281
						40			40			40	DS^2	0.003
							16			16		16	DP^2	0.063
									20		20	20	DT^2	0.151
									120				S^2	-0.004
										48			P^2	0.687
											60		T^2	3.314
												80	D^2	0.491

NS Not significant at the 5% level (P 0.05)

* Significant at the 5% level (P 0.05)

** Significant at the 1% level (P 0.01)

B. Results

(1) Total volatile fatty acids

The concentration of total VFA are presented in Appendix I. The statistical analysis of these data, together with estimates of the components of variance are presented in Table I.

The four second order interactions were found non-significant when tested against the third order interaction. Hence, their sums of squares were pooled with that of the third order interaction to give a more accurate estimate of experimental error which was used for testing the first order interactions.

Approximate but conservative tests of the main effects were made using the largest mean square of the first order interactions involving the main effect being tested. All main effects except that of between samples proved significant ($P < 0.01$).

Examination of estimates of the components of variance showed that as would be expected under the feeding system used, most of the variation was due to differences between sampling times. This effect, together with those of Days and Positions, contributed approximately 85% of the total variation.

Laboratory error (Subsamples within Samples) contributed less than 1% to the experimental error.

The non-significance of the main effect, Samples and its

FIG. 4 DAILY AVERAGE VFA CONCENTRATION AT EACH POSITION (D x T INTERACTION).
MEAN OF 16 OBSERVATIONS
S.E. ± 0·16

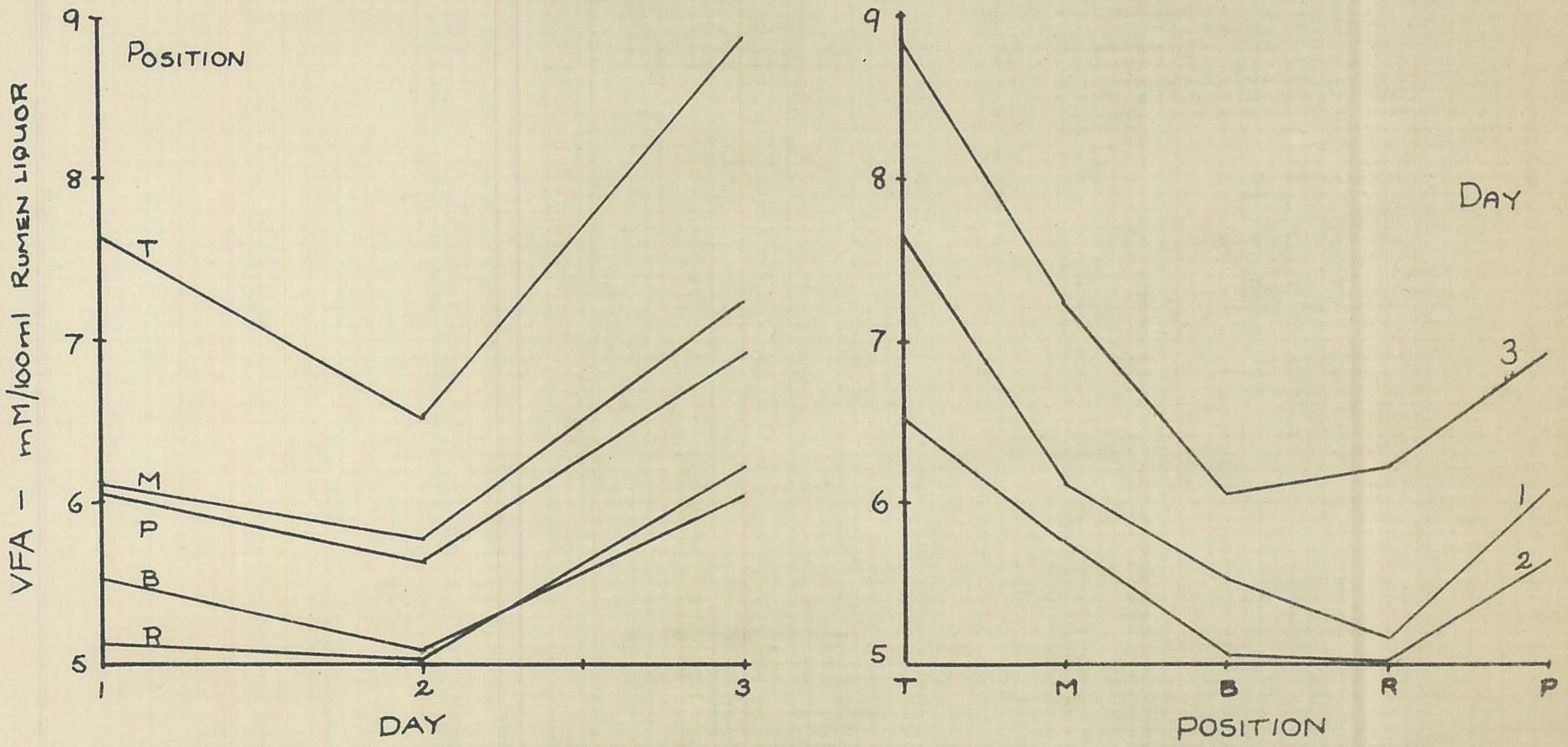
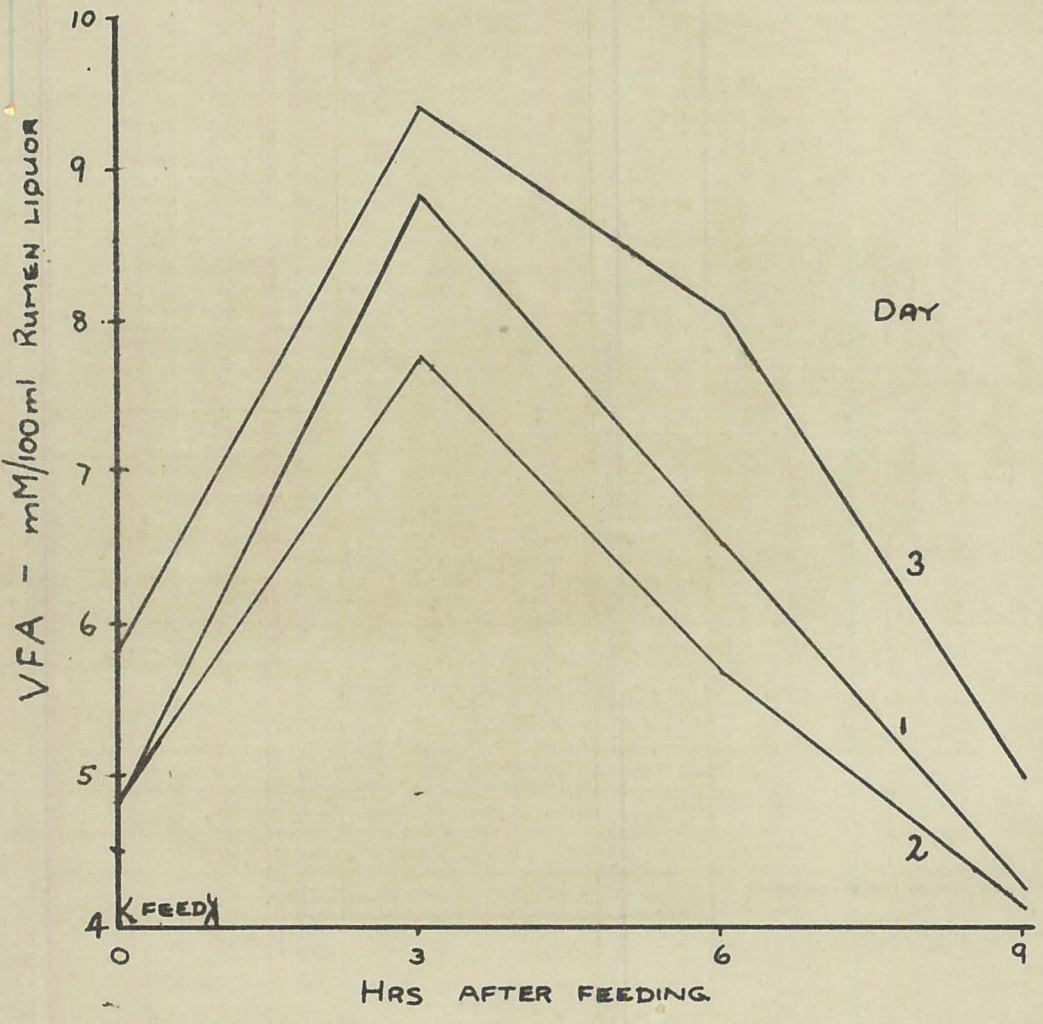


FIG. 3 DIURNAL TREND IN VFA CONCENTRATION FOR EACH DAY (D x T INTERACTION).
MEAN OF 20 OBSERVATIONS

S.E. ± 0·15



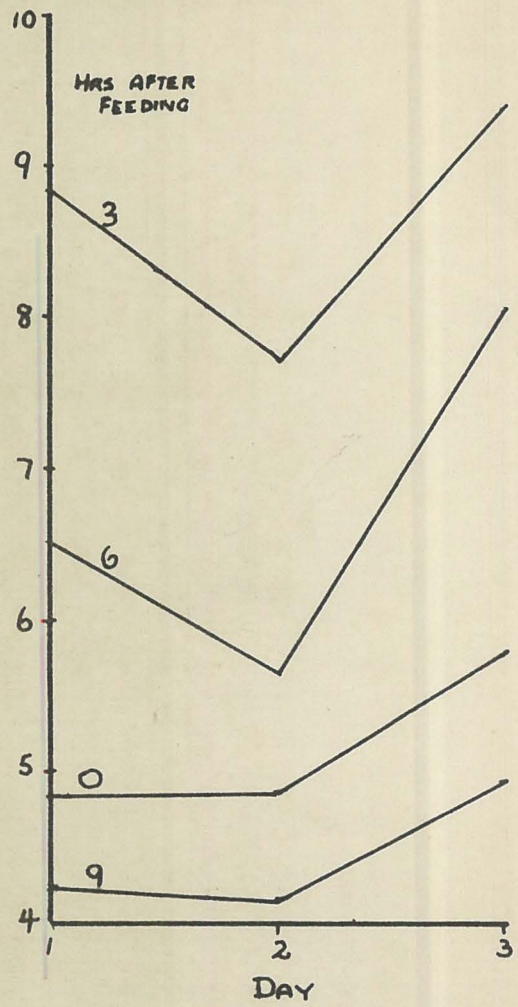
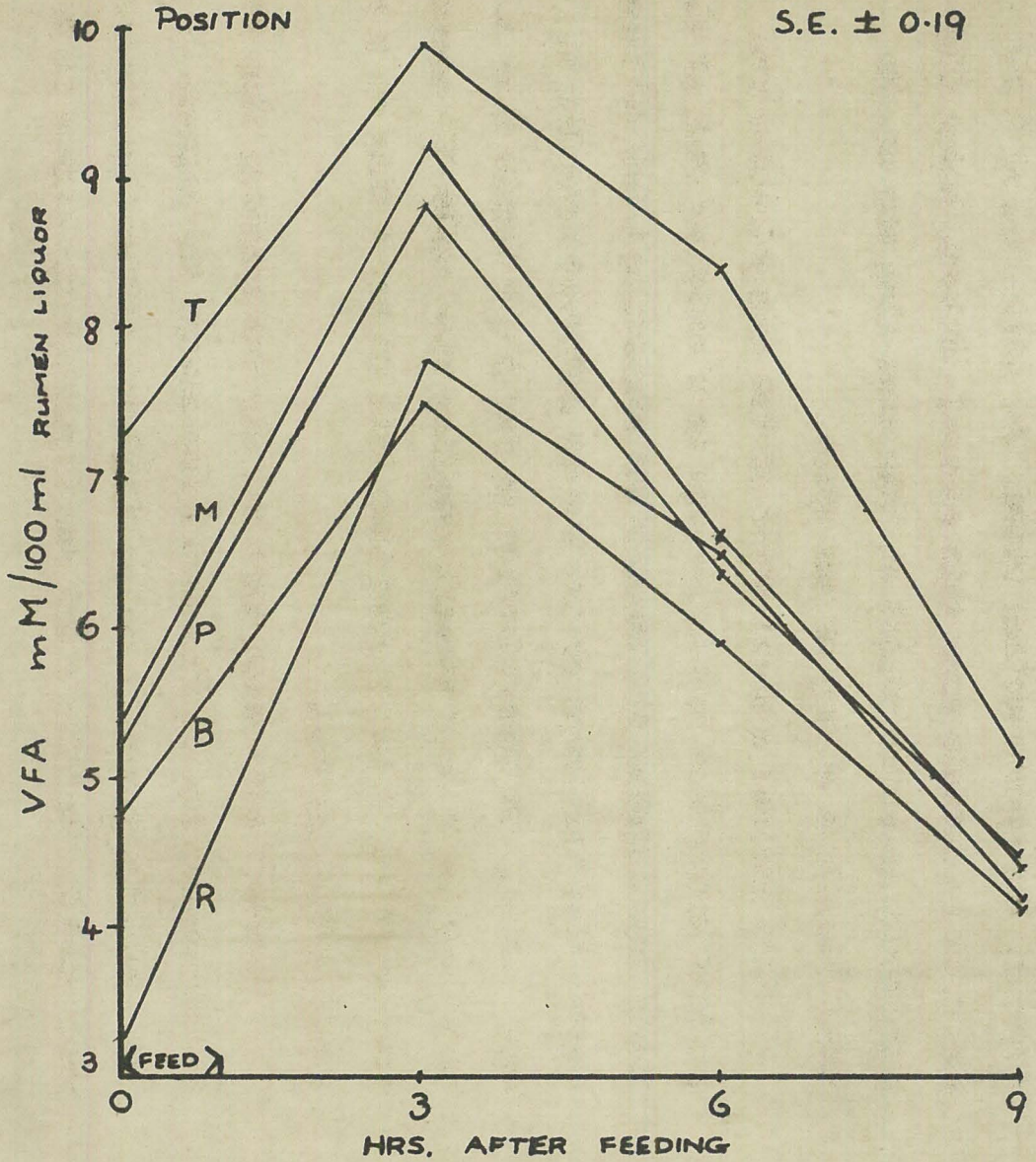
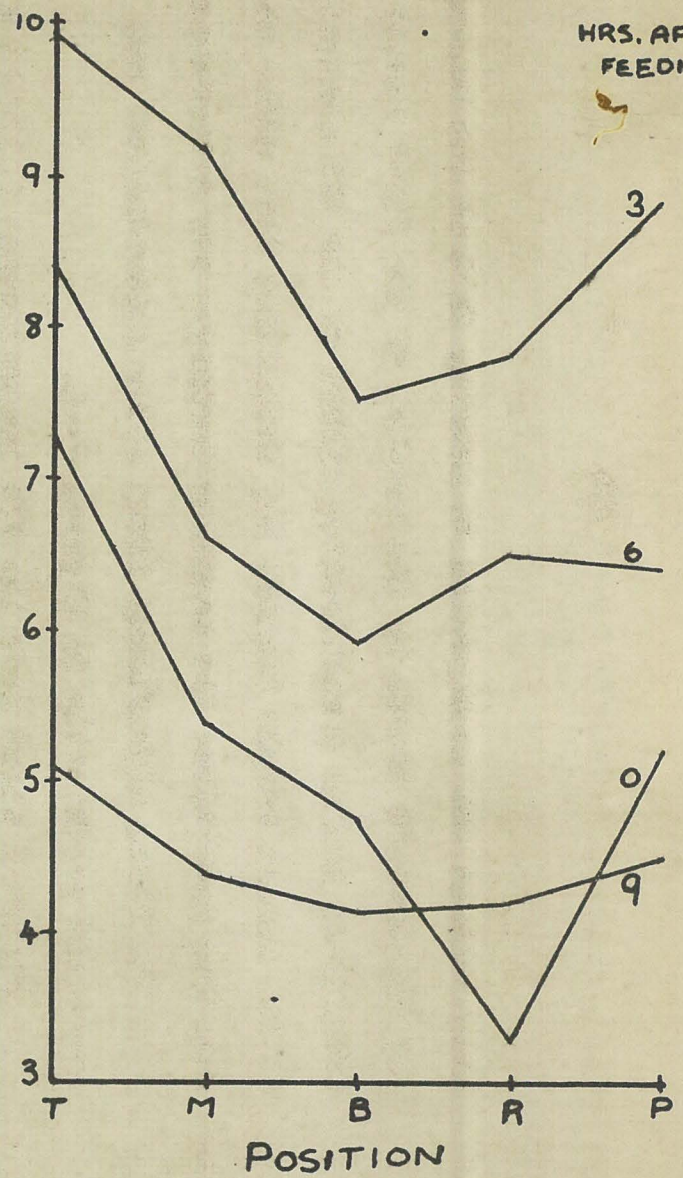


FIG. 2 DIURNAL TREND IN VFA CONCENTRATION FOR EACH POSITION (T x P INTERACTION). MEAN OF 12 VALUES

S.E. ± 0·19





interactions was interpreted as meaning that on the average, any disturbance caused by the removal of the first sample (Sample A) had no demonstrative effect on the VFA content of the second sample (Sample B), indicating that under the existing conditions the sampling technique was satisfactory.

The three significant first order intereactions are presented graphically in Figures 2 - 4.

Figure 2 shows that the VFA concentration followed a similar time trend for each position with observed maxima and minima at 3 and 9 hrs. after feeding respectively. However, the concentration within the reticulum at 0 hrs. appeared to deviate from this general pattern, being significantly lower ($P < 0.01$) at this time than any of the other positions at any of the times.

This general diurnal trend of VFA concentration was evident within the reticulo-rumen as a whole on each of the three days (Figure 3) although the significant interaction showed that the shape of the curves differed from day to day. Thus, VFA concentration on Day 3 was higher than that of either Day 1 or 2 at each sampling time while the concentration on Day 1 was higher than that on Day 2 only at 3 and 6 hrs. after feeding.

Figure 2 also shows that any ~~any~~ one sampling time the VFA concentration at Position T was always the highest ($P<0.01$). The concentration at Position M did not differ significantly from that in Position P but was significantly higher ($P<0.05$)

FIG. 8 DIURNAL TREND IN pH FOR EACH DAY
(D x T INTERACTION)
MEAN OF 10 OBSERVATIONS S.E ± 0·23

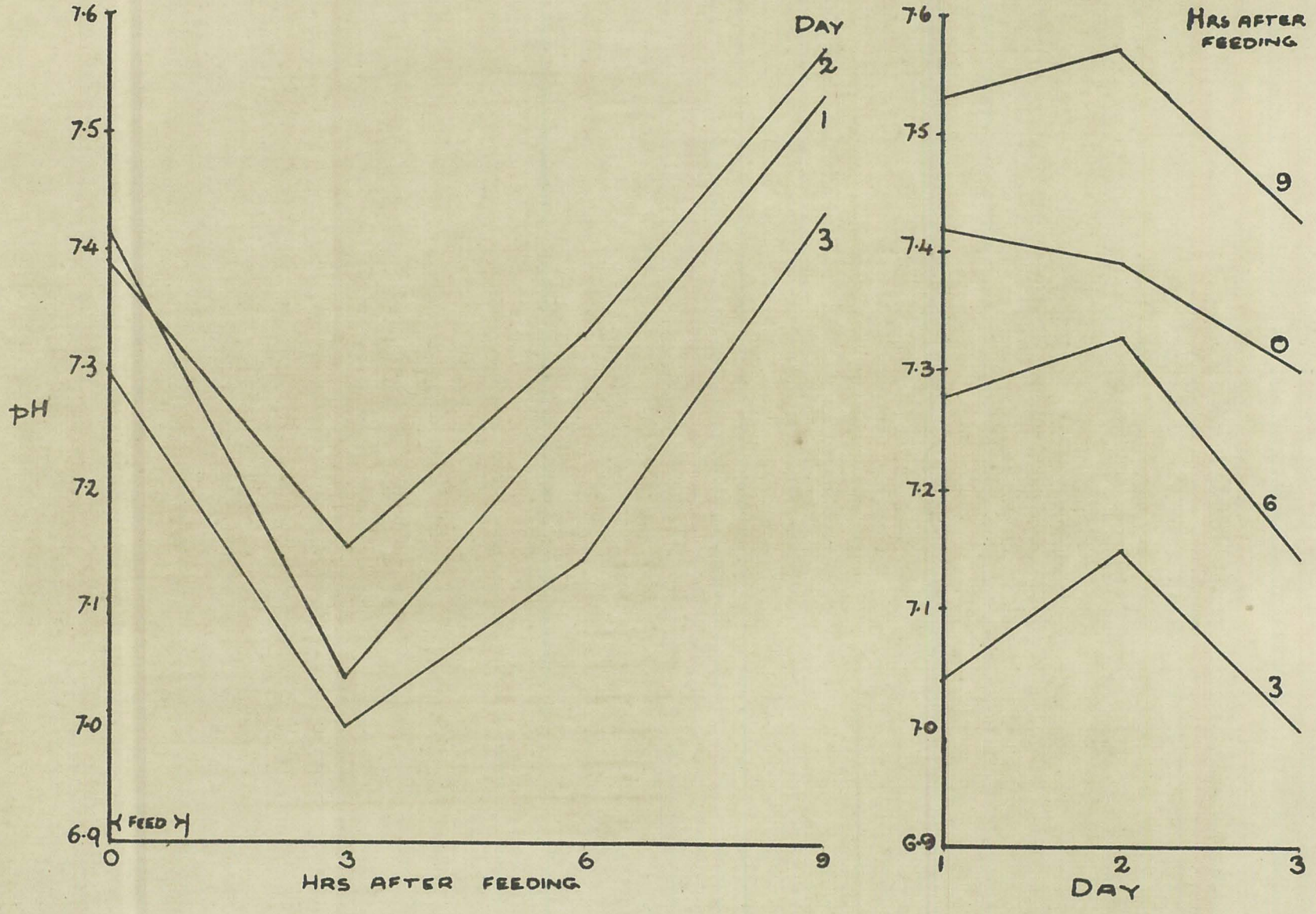
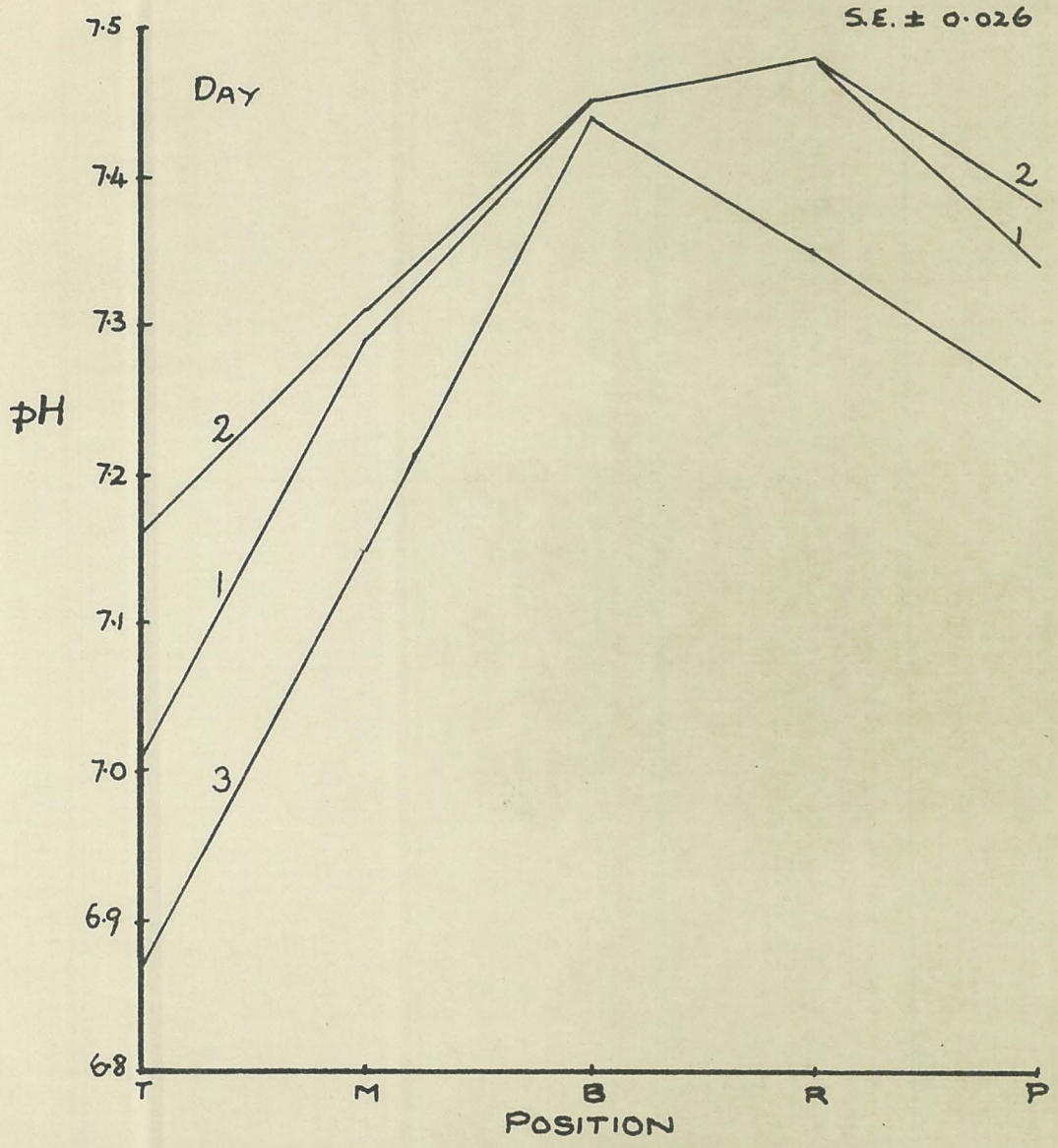


FIG. 7 DAILY AVERAGE pH AT EACH POSITION (D x P INTERACTION).
MEAN OF 8 OBSERVATIONS
S.E. ± 0·026



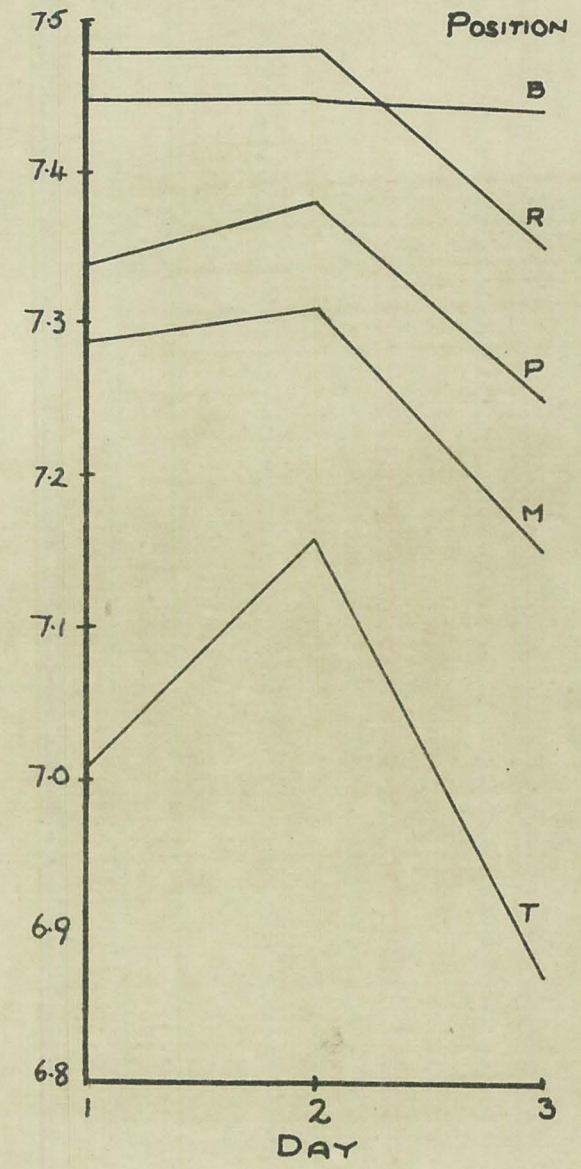


FIG.6 DIURNAL TREND IN pH AT EACH POSITION (T x P INTERACTION)
MEAN OF 6 OBSERVATIONS
S.E.± 0·03

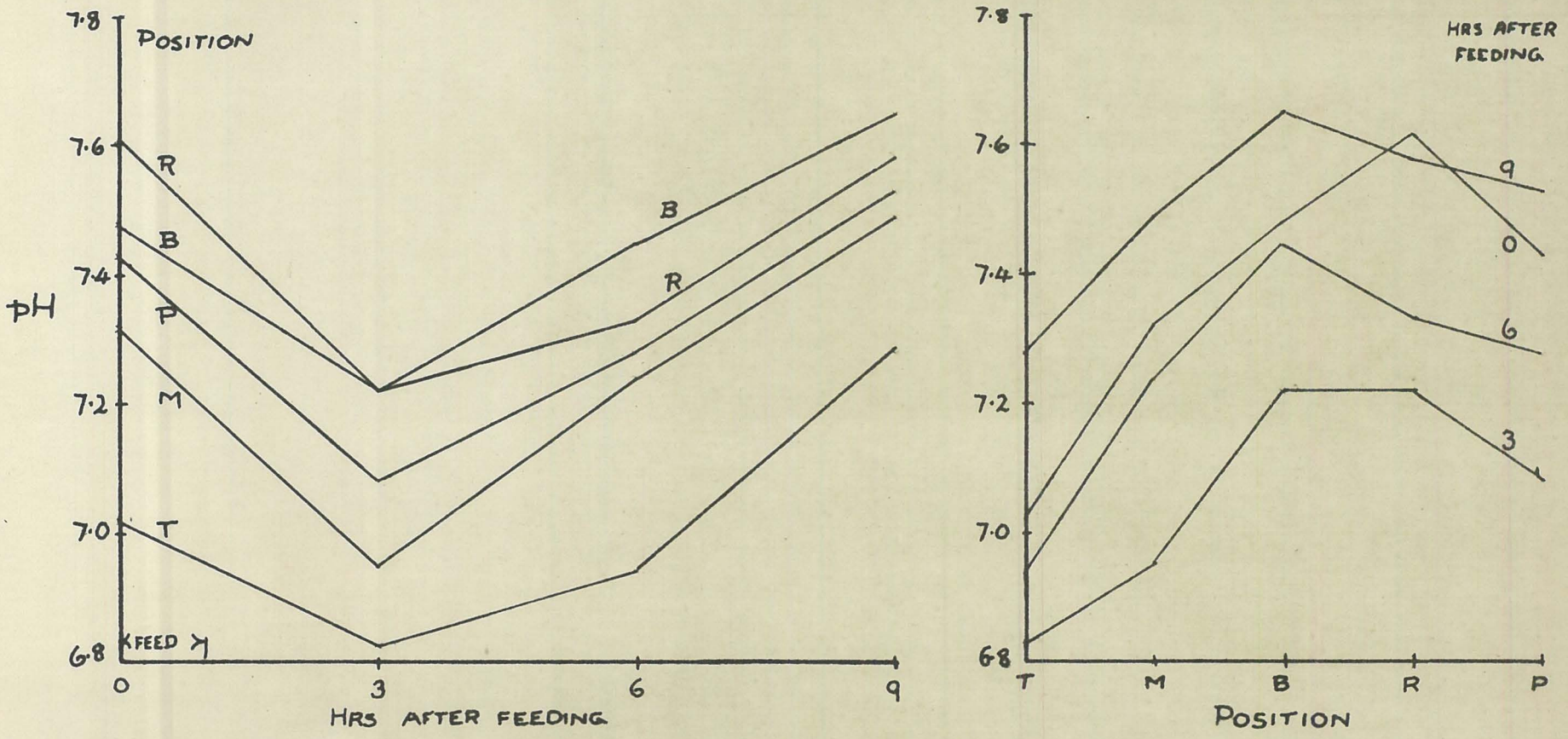


FIG. 5 DIFFERENCE IN pH BETWEEN SAMPLES FROM THE SAME POSITION (P x S INTERACTION), MEAN OF 12 OBSERVATIONS

S.E. ± 0·03

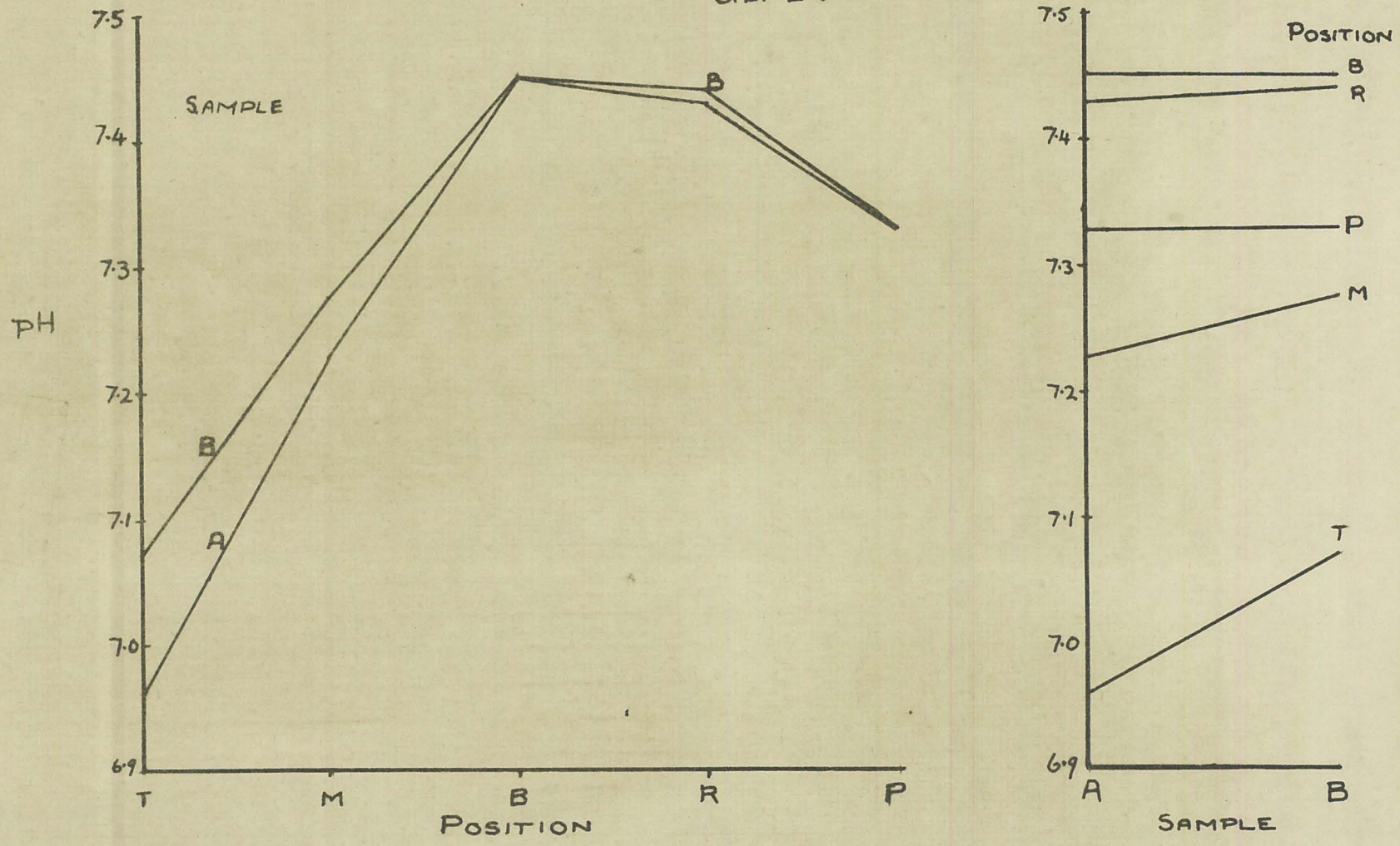


TABLE II

ANALYSIS AND COMPONENTS OF VARIANCE FOR pH LEVELS ON ALL DAYS

Analysis of variance

Source	df	SS	M.S.	F	
Days (D)	2	0.4383	0.2192	10.0	*
Times (T)	3	3.2274	1.0758	48.5	**
Positions (P)	4	2.9625	0.7406	33.5	**
Samples (S)	1	0.0301	0.0301	2.50	NS
D x T	6	0.0687	0.0115	2.22	*
D x P	8	0.1740	0.0218	4.18	**
D x S	2	0.0195	0.0098	1.87	NS
T x P	12	0.2647	0.0221	4.24	**
T x S	3	0.0244	0.0081	1.10	NS
P x S	4	0.0532	0.0133	2.56	*
2nd and 3rd order interactions	74	0.Ø3851	0.005204		
Total	119	7.6479			

Components of variance and their estimates

Source	"Error"	PxS	TxS	TxP	DxS	DxP	DxT	S	P	T	D		
df	74	4	3	12	2	8	6	1	4	3	2	Component	Estimate
	1	1	1	1	1	1	1	1	1	1	1	σ^2	0.005204
		12						12	12			PS^2	0.00068
			15					15		15		TS^2	0.00020
				6					6	6		TP^2	0.00281
					20			20			20	DS^2	0.00023
						8			8		8	DP^2	0.00207
							10			10	10	DT^2	0.00063
								60				S^2	0.00015
									24			P^2	0.02891
										30		T^2	0.03482
											40	D^2	0.00467

NS Not significant ($P > 0.05$)

* Significant at the 5% level ($P < 0.05$)

** Significant at the 1% level ($P < 0.01$)

than that at Position B at each sampling time except the last, 9 hrs. after feeding. At this sampling time, variation between positions was at a minimum.

This dorsal to ventral decrease in VFA concentration within the rumen held on each of the three days (Figure 4) but it appeared that the concentration within Positions B and R failed to rank consistently over the three days.

(2) pH

The analysis of variance of the pH data (Appendix II) is presented in Table II. Transformation of the data was deemed unnecessary (A. Glenday, pers. comm.) because of its narrow distribution about a mean of approximately pH 7 and lack of relationship between the variance and the mean.

The tests for significant effects were performed in a manner similar to that used in the analysis of the VFA data.

Estimation of the components of variance (Table II) showed that as with VFA concentration, most (85%) of the total variance could be attributed to the main effects, Days, Times and Positions.

In contrast to the results obtained with VFA concentration, a significant ($P<0.05$) interaction involving Samples was found (Fig. 5). A significant difference ($P<0.05$) existed between the pH values of the two samples from Position T but not at any of the other four positions although a difference that approached significance was apparent at Position M.

The interpretations of the significant Times x Positions and Days x Positions interactions are an approximate inverse of those for VFA concentration. Thus, a dorsal to ventral increase in pH was evident (Fig. 6) with a similar but not so evident variation in the value for the reticulum at 0 hrs. As was the case for VFA concentration, this dorsal to ventral trend was evident on each of the three days (Fig. 7) with a similar inconsistent ranking of Positions B and R with time. In contrast to the VFA concentration, the pH values at Position M and P were significantly different ($P<0.05$) at 0 and 3 hrs. after feeding (Fig. 6) and on the third day (Fig. 7).

A diurnal trend in pH levels was found at each position with a maximum and minimum occurring at 9 and 3 hrs. after feeding respectively (Fig. 6). This trend, as indicated by the mean of the values for the five positions, was repeated on each of the three days (Fig. 8) although the shape of the curves differed for each day. Thus within times, pH values on Day 1 did not differ from those on Day 2 except at 3 hrs. after feeding. Similarly, values on Day 3 were lower than those on Day 2 at all four sampling times and were also lower than those on Day 1 except at 3 hrs. after feeding.

(3) Regression of pH on VFA concentration

The regression of pH on VFA concentration was calculated using the average pH and VFA levels at each sampling position at each sampling time for the three days combined. The

regression coefficient was found to be -0.1131 ± 0.013 which proved to be highly significant when tested by analysis of variance (Appendix VIII).

(4) Relative proportions of individual VFAs

The percentages of acetic, propionic and butyric acids as determined by gas-liquid chromatography are presented in Appendix III.

In comparisons of the proportion of one acid with that of the other two acids combined, the distribution in each of the three cases was taken as binomial. Correction for anormality was applied using the inverse-sine transformation from tables given by Snedecor (1956).

The analyses of variance using transformed, coded data are presented in Appendix IV. In the absence of significant interactions, a pooled estimate of error variance was used for testing the main effects.

In all three cases there were no significant differences between Positions. Highly significant differences ($P<0.01$) were found between Times and between Days.

This indicated that the proportion of any one acid relative to the other two changed with time (average of three days), and with days (average of four times). Significant differences among the means were located using Duncan's (1955) multiple range test (Tables III and IV). Table III shows that the proportion of acetic acid was low at 3 and 6 hrs.

TABLE III

DUNCAN'S MULTIPLE RANGE TESTS FOR CHANGE IN PROPORTION OF INDIVIDUAL ACIDS WITH TIME

	Hours after feeding	Mean Transformed	S.E.	Results	Untransformed Means (%)
Acetic	0	63.69	±0.53	a A	80.4
	3	59.48		b B	74.2
	6	58.97		b B	73.4
	9	63.08		a A	79.5
Propionic	0	18.43	±0.36	a A	10.0
	3	21.41		b B	13.3
	6	20.63		b B	12.4
	9	18.93		a A	10.5
Butyric	0	17.73	±0.56	a A	9.3
	3	20.46		b BC	12.2
	6	22.05		c C	14.1
	9	18.45		a AB	10.0

Note:- Means which have a letter in common do not differ significantly whereas means which do not have a letter in common differ significantly. Small letters denote the 5% level and capital letters denote the 1% level.

Transformation is from percentages to angles where angle = $\text{Arcsin}\sqrt{\text{Percentage}}$

TABLE IV

DUNCAN'S MULTIPLE RANGE TESTS FOR CHANGE IN PROPORTION OF INDIVIDUAL ACIDS WITH DAYS

	Day	Mean Transformed	S.E.	Results	Untransformed Means (%)
Acetic Acid	1	63.21	±0.46	a A	79.7
	2	62.20		a A	78.2
	3	58.64		b B	72.9
Propionic Acid	1	19.35	±0.31	a A	11.0
	2	18.68		a A	10.3
	3	21.53		b B	13.5
Butyric Acid	1	17.57	±0.48	A	9.1
	2	19.75		B	11.4
	3	21.70		C	13.7

Note:- For significance of differences and transformation, see Note, Table III.

after feeding whereas the proportion of propionic acid was high during these times. The proportion of butyric acid was highest at 6 hrs. after feeding.

Figs. 3 and 8 indicate that 3 and 6 hrs. after feeding were times at which high VFA concentration and low pH levels occurred.

Table IV shows that the proportion of acetic acid did not differ for Days 1 and 2 but was low on Day 3. For propionic acid there was no difference between Days 1 and 2 but a higher proportion occurred on Day 3. For butyric acid, different proportions existed on each day, the lowest on Day 1 and the highest on Day 3.

C. Discussion

The feeding system used in this experiment did not prove entirely satisfactory. The highly significant positive linear correlation of rumen VFA concentration with intake observed by Williams and Christian (1956c) suggests that in this work the order of intake on the three days was Day 3 > Day 1 > Day 2 (Fig. 3: > denotes greater than). However, from Fig. 3 it is evident that the VFA concentration attained after feeding was also dependent on the VFA concentration present within the rumen before feeding commenced. Thus although it appears that the intake during the 1 hour's grazing was greater on Day 1 than on Day 2 and possibly even greater than on Day 3, because of the higher initial VFA concentration present within the rumen on Day 3, VFA levels were generally higher on this day.

The cause of the higher initial VFA concentration on Day 3 was not revealed by this investigation but it is suggested that it was due to differences in the extent of fermentation of food material ingested the previous day. In other words, there was more fermentable substrate present within the rumen at 0 hrs. on Day 3 than either of the other two days. It is also suggested that the differences in amounts of fermentable ingesta present within the rumen

accounted for the relatively higher VFA levels at 6 hrs. after feeding on Day 3 (Fig. 3).

Both these suggestions cannot be regarded as conclusive. Differences in VFA absorption rate and food composition between the days could account for the observed variation.

For a given diet, the fluctuations in pH are to a large extent a function of rumen VFA levels (Balch and Rowland, 1957; Briggs et al 1957a). As the regression of pH on VFA concentration was negative and highly significant ($P < 0.01$; Fig. 17), a similar relationship held in this investigation. Thus it is to be expected that the changes in VFA levels in Fig. 3 were reflected as inverse changes in pH (Fig. 8). The same should apply in all cases but some discrepancies were apparent and are discussed below. (p46)

The diurnal trends in both pH and VFA levels reported here are similar to those reported by many workers using different diets (e.g. Balch and Rowland, 1957). The rate at which peak values are attained and the magnitude of these values are largely a reflection of the diet (Balch and Rowland, 1957). The possible importance of such phenomena has been discussed elsewhere (Balch et al, 1955, 1957).

That the VFA concentration at 9 hrs. after feeding was lower than the concentration immediately prior to feeding (0 hrs.) is considered to have resulted from the presence of relatively greater amounts of unfermented ingesta within

the rumen at 0 hrs.

Variations in the levels of pH and VFA concentration at each position and the effect of Times and Days on these variations are discussed below (p56).

A change in the proportions of the individual acids with time after feeding has been reported by many workers (e.g. el Shazly, 1952; Briggs et al, 1957b) yet all reports do not confirm this (Shaw, 1959). Where changes have been observed, the general trend is similar to that reported here, namely, that after feeding the proportions of propionic and acetic acids rise and fall respectively reaching their maximum and minimum levels simultaneously with the attainment of maximum VFA concentration. Changes in the proportion of butyric acid are often erratic. Some variation in this pattern may occur with different diets (Briggs et al, 1957b; Gray and Pilgrim, 1951).

Briggs et al (loc. cit.) have suggested possible causes for these variations with time after feeding but the final solution awaits the results of further research.

The causes of the changes in the proportions of the individual acids with Days (Table IV) are also uncertain. The changes may be associated with the differences in pH and VFA levels between the three days because the lowest proportion of acetic acid and the highest proportion of propionic acid occurred on Day 3, the day of highest ($P < 0.01$)

average VFA concentration and lowest pH ($P<0.05$). Williams and Christian (1956c) found a similar relationship with sheep. High intakes (1000 gm dried grass/day) gave higher VFA concentrations and a lower ratio of acetic : proprionic acids than low intakes (400 gm/day). However, in contrast to the results reported here, no changes in pH or the proportion of butyric acid were observed by these workers.

III. EXPERIMENT II

THE EFFECT OF FREE GRAZING

A. Materials and Methods

(1) Experimental design

The same animal used in Expt. I was subjected to a further experiment in which pasture was available at all times during the experimental period.

Sampling times were every three hours during two 24 hr. periods commencing 11 a.m., 23/4/61, and 2 p.m. 25/4/61. For sampling, the animal was placed in a milking bail situated adjacent to the grazing area. Unforeseen circumstances prevented the first sampling (11 a.m.) on the second day.

Sampling procedure and pH determination were as for Expt. I. Grazing observations were made during each experimental period.

(2) Diet

From 14 days prior to the experimental periods, the grazing area had been grazed solely by the experimental animal. The sward was 3-12 inches in height. To obtain an indication of pasture quality, twenty, 1 x 1 ft. plots were cut and dissected into species. Composition of the sward on a dry weight basis is shown in Table V.

TABLE V

PERCENTAGE CONTRIBUTION BY WEIGHT OF SPECIES TO PASTURE YIELD (D.M. BASIS).

Species	% Contribution By Weight
Ryegrass (*Lolium* spp.)	33.7
White Clover (*Trifolium repens*)	25.0
Yorkshire Fog (*Holcus lanatus* L.)	28.3
Cocksfoot (*Dactylis glomerata* L.)	7.2
Other grasses	3.5
Weeds	2.3

(3) Analytical

Laboratory methods were similar to those used in Expt. I except that the high repeatability of VFA concentration determinations on the same sample observed during Expt. I, obviated the necessity for duplicate determinations. Furthermore, blank steam distillate titrations were dispensed with. Instead, blank titres were taken to be constant for each day. Each day, the value was determined and appropriate allowance made in the calculation of VFA concentration.

The relative proportions of acetic, propionic and butyric acids for the Top, Middle and Bottom positions were determined for only the first day.

FIG. 12 VFA CONCENTRATION OF SAMPLES FROM THE SAME POSITIONS ON DIFFERENT DAYS (D x P x S INTERACTION)
MEAN OF 7 OBSERVATIONS
S.E. ± 0.21

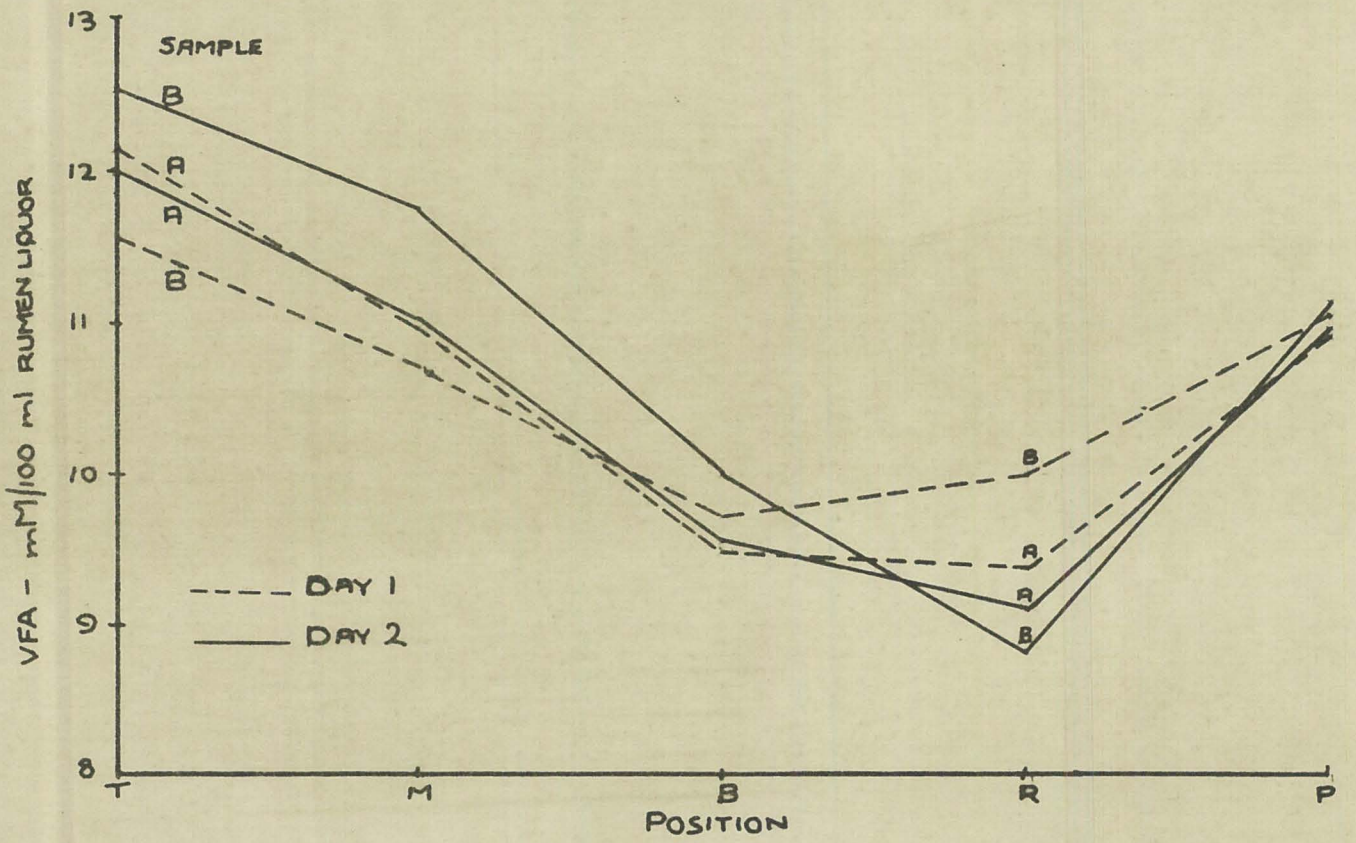


FIG. 11 DIURNAL TREND IN VFA CONCENTRATION FOR EACH POSITION (T x P INTERACTION).
MEAN OF 4 OBSERVATIONS
S.E. ± 0·28

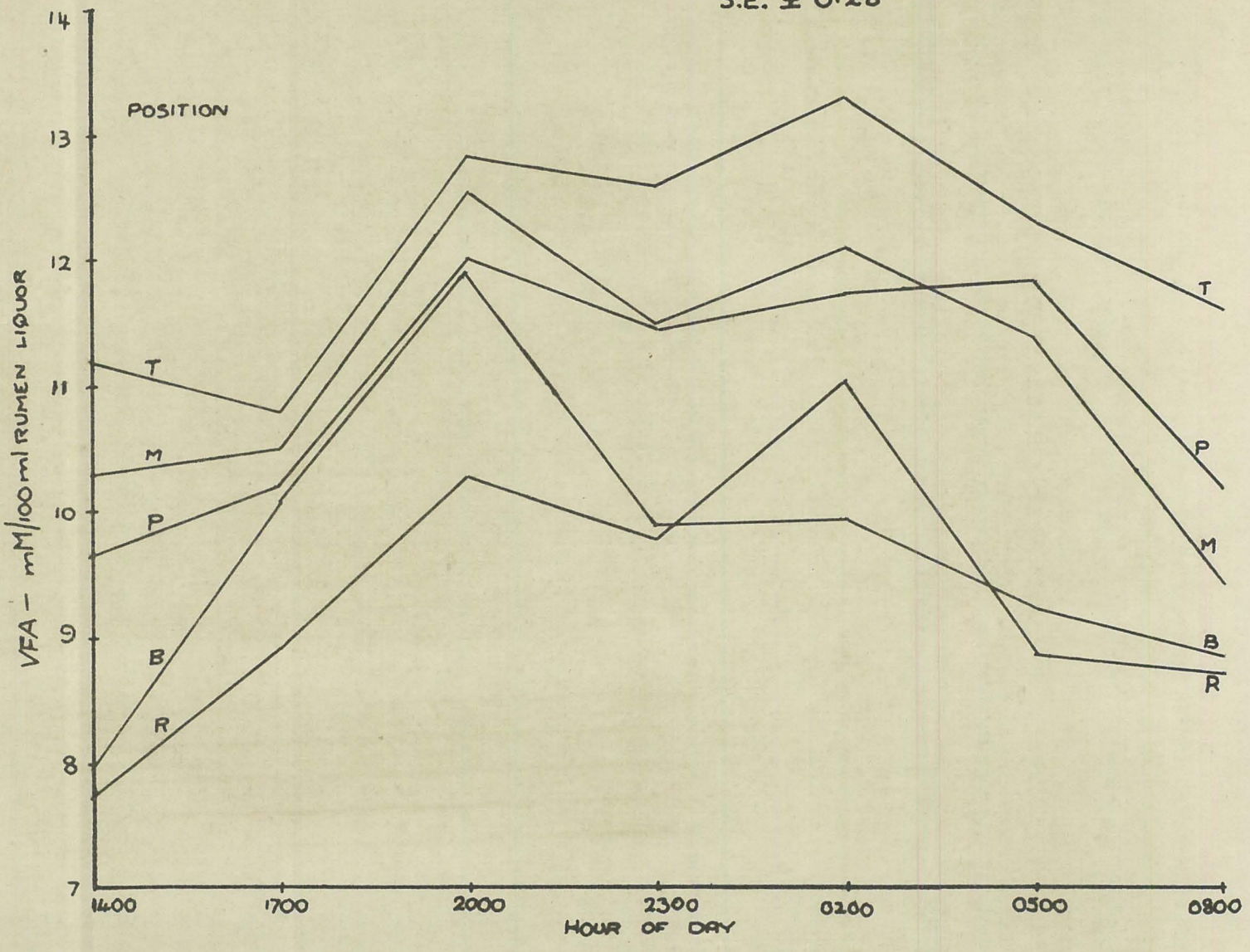


FIG. 10 DIURNAL TREND IN VFA CONCENTRATION FOR EACH DAY (D x T INTERACTION)
MEAN OF 10 OBSERVATIONS

S.E. ± 0·18

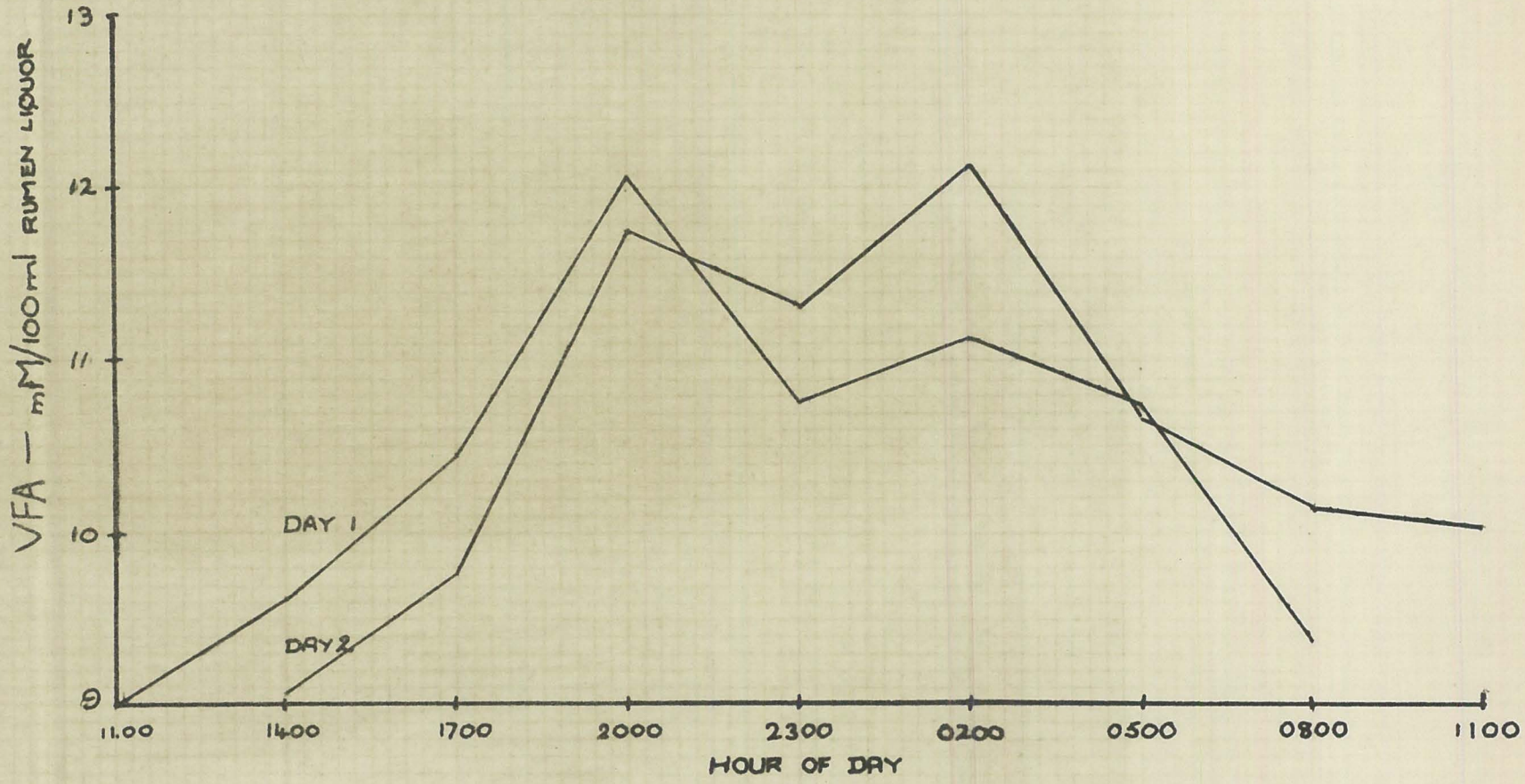


FIG. 9 DAILY AVERAGE VFA CONCENTRATION AT EACH POSITION (D x P INTERACTION)
MEAN OF 14 OBSERVATIONS
S.E. ± 0·14

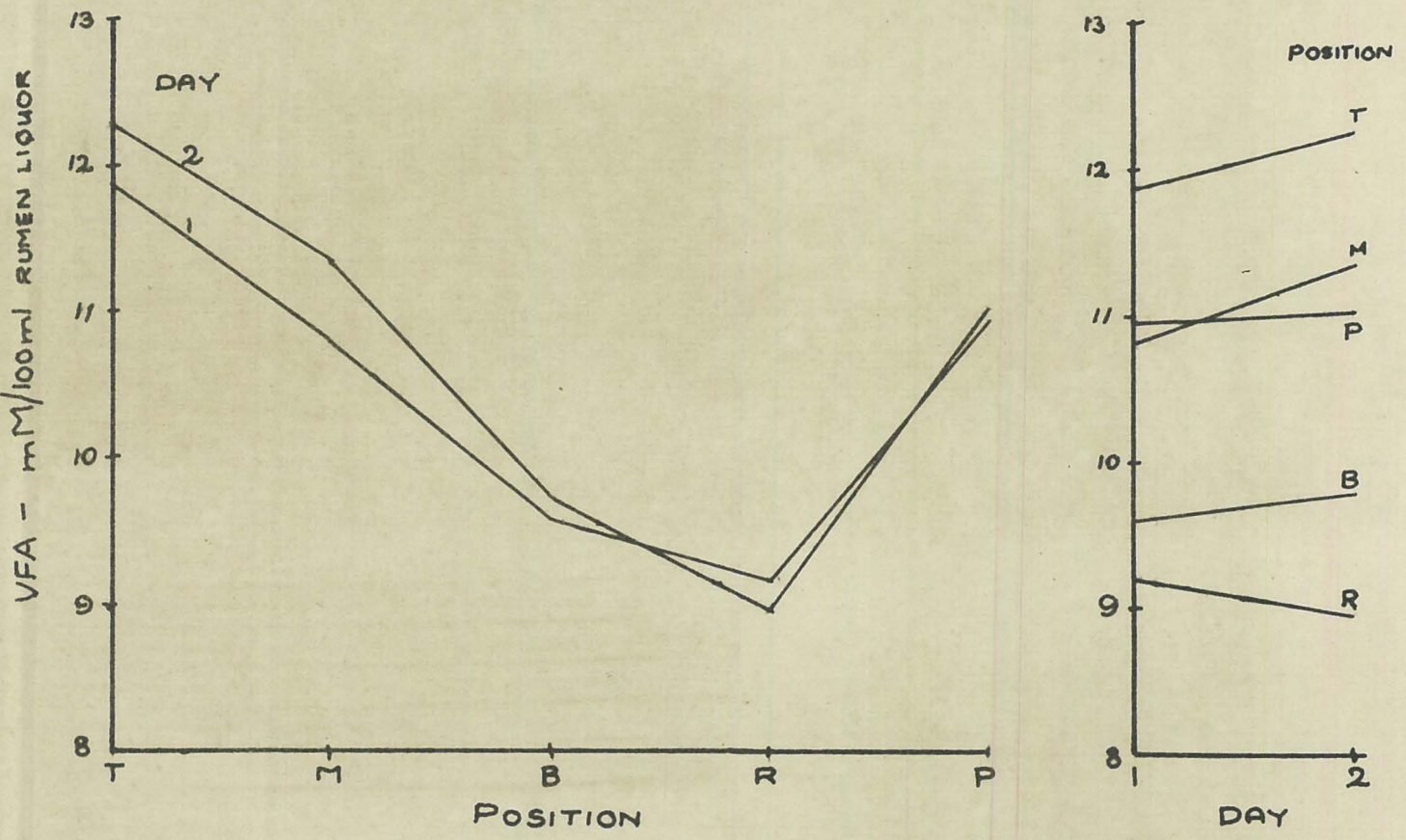


TABLE VI

ANALYSIS OF VARIANCE OF VFA LEVELS FOR DAY 1 AND 2.

(Expt. II)

Source	df	SS	M.S.	F	
Days (D)	1	0.360	0.360	< 1	NS
Times (T)	6	111.303	18.551	17.8	**
Positions (P)	4	138.987	34.747	31	**
Samples (S)	1	1.152	1.152	1.0	NS
D x T	6	13.530	2.255	7.18	**
D x P	4	6.537	1.634	5.20	**
D x S	1	0.787	0.787	2.50	NS
T x P	24	27.567	1.149	3.66	**
T x S	6	2.998	0.500	1.59	NS
P x S	4	0.509	0.127	< 1	NS
D x P x S	4	4.603	1.151	4.11	*
Error	78	24.491	0.314		
Total	139	332.824			

NS Not significant ($P > 0.05$)

* Significant at the 5% level ($P < 0.05$)

** Significant at 1% level ($P < 0.01$)

B. Results

The data for VFA concentration and pH levels are presented in Appendices V and VI respectively. Analyses of variance for each set of data were performed as for Expt. I. For this purpose, the data for the unpaired sampling times (first sampling on Day 1 and the last sampling on Day 2) were discarded.

(1) Total volatile fatty acids

The analysis of variance for the VFA concentration data is presented in Table VI.

The main effects, Between Times and Between Positions were significant although in both cases there were day to day variations as shown by the significant Days x Times and Days x Positions interactions (Figs. 9 and 10). Duncan's (1955) multiple range tests showed that for each day the order of acid concentration was $T > (M,P) > B > R$* but comparing between Days within Positions, the VFA concentrations on Day 2 at Positions T and M were higher than the concentrations at the respective positions on Day 1.

This dorsal to ventral decrease in VFA concentration held fairly constant for each sampling time during the days (Fig. 11) although a highly significant reduction in stratification was found at 5 p.m. and 8 p.m.. At some sampling

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* > denotes greater at the 5% level of significance
( ) denotes no difference at the 5% level of significance.

TABLE VII

ANALYSIS OF VARIANCE OF pH LEVELS FOR DAYS 1 AND 2

Source	df	SS	M.S.	F	
Days (D)	1	0.5531	0.5531	11.6	*
Times (T)	6	2.1957	0.3659	7.68	*
Positions (P)	4	5.6292	1.4073	46	**
Samples (S)	1	0.0778	0.0778	6.3	NS
D x T	6	0.2859	0.04765	9.88	**
D x P	4	0.0528	0.01320	2.72	*
D x S	1	0.0315	0.03150	6.53	*
T x P	24	0.7311	0.03046	6.32	**
T x S	6	0.0372	0.00620	1.30	NS
P x S	4	0.0495	0.01238	2.56	*
D x P x S	4	0.0544	0.01360	3.63	*
Error	78	0.3765	0.00483		
Total	139	10.0747			

NS Not significant ($P > 0.05$)

* Significant at the 5% level ($P < 0.05$)

** Significant at the 1% level ($P < 0.01$)

times, the difference in VFA concentration between Positions M and P approached 5% significance level and, as noted in Experiment I, there was a definite inconsistency in the ranking order of Positions B and R.

The significant interaction, Days x Times (Fig. 10) showed that the trend in fermentation, as judged by the mean of the values for the five positions, was different on each day. However, it is evident that peak values were attained between 5 p.m. and 5 a.m. (i.e. during the night) on each of the two days.

The diurnal trend in VFA concentration at each position is shown in Fig. 11 where it is apparent that a trend similar to that above was present in each case. The diurnal trend as noted in Fig. 10 was more evident than that exhibited by the VFA concentration at Positions T, M and P but even so, a highly significant increase of 2-3 in M/100 ml is observable at these three positions.

That some disturbance of the rumen contents occurred during sampling was apparent from the significant D x P x S interaction. The effect, shown in Fig. 12, was different on each of the two days.

(2) pH

The analysis of variance for the pH data is presented in Table VII.

In contrast to the results obtained from the VFA data, a

FIG. 16 pH OF SAMPLES FROM THE SAME POSITIONS ON DIFFERENT DAYS (D x P x S INTERACTION) MEAN OF 7 OBSERVATIONS S.E. ± 0·026

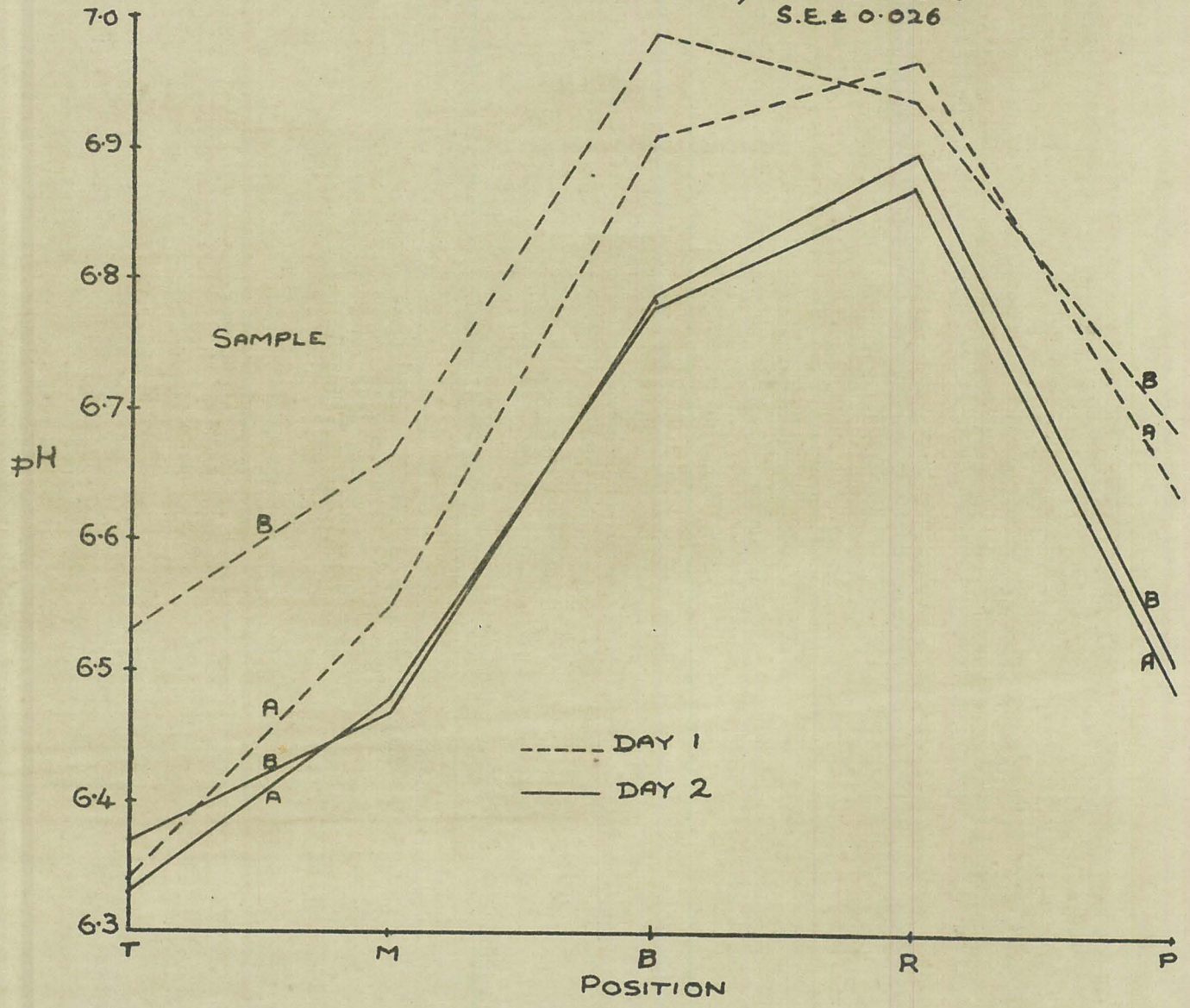


FIG. 15 DIURNAL TREND IN pH FOR EACH POSITION (T x P INTERACTION). MEAN OF 4 OBSERVATIONS S.E. ± 0·036

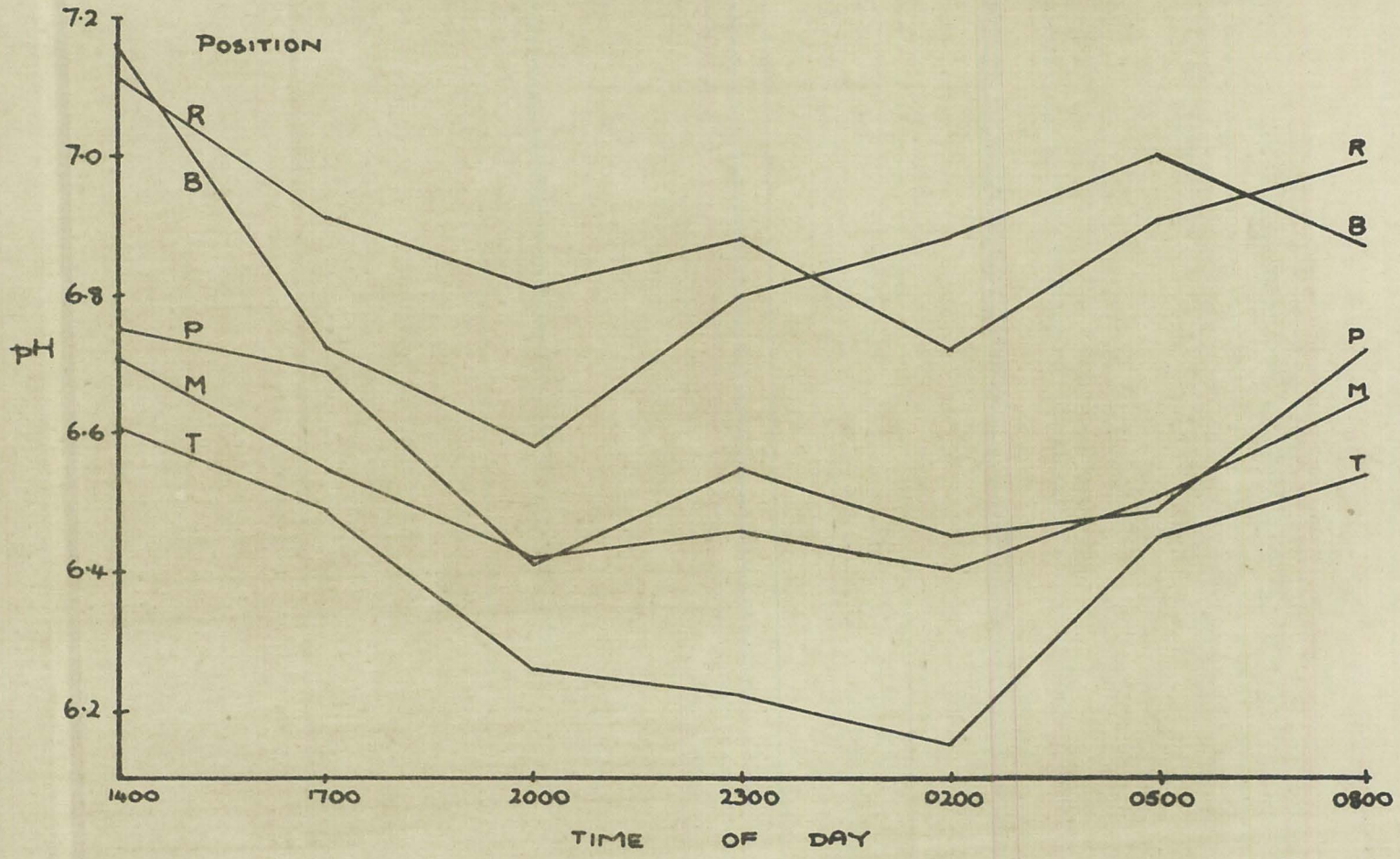


FIG. 14 DIURNAL TREND IN pH FOR EACH DAY (D×T INTERACTION). MEAN OF 10 OBSERVATIONS S.E. ± 0·02

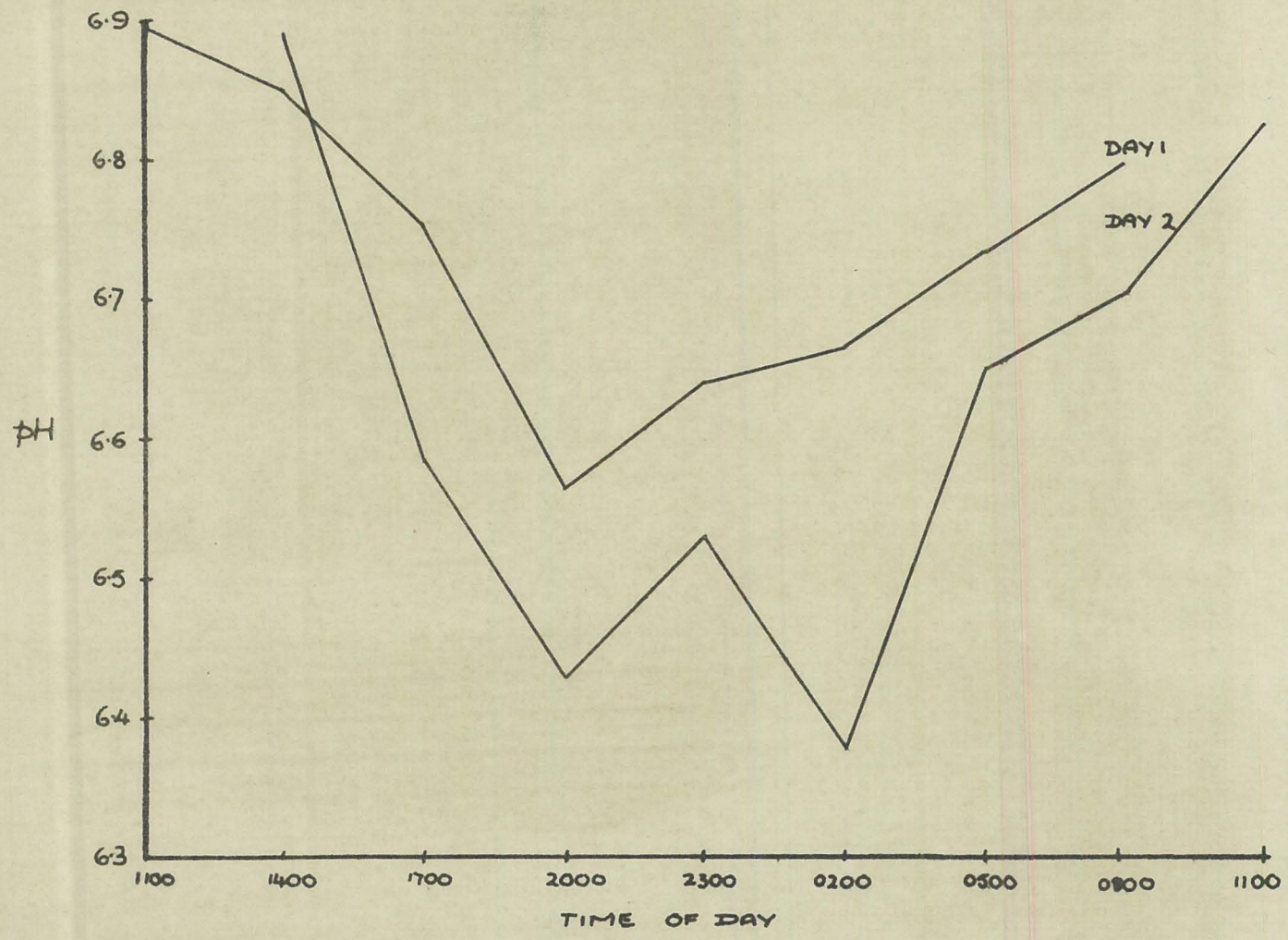
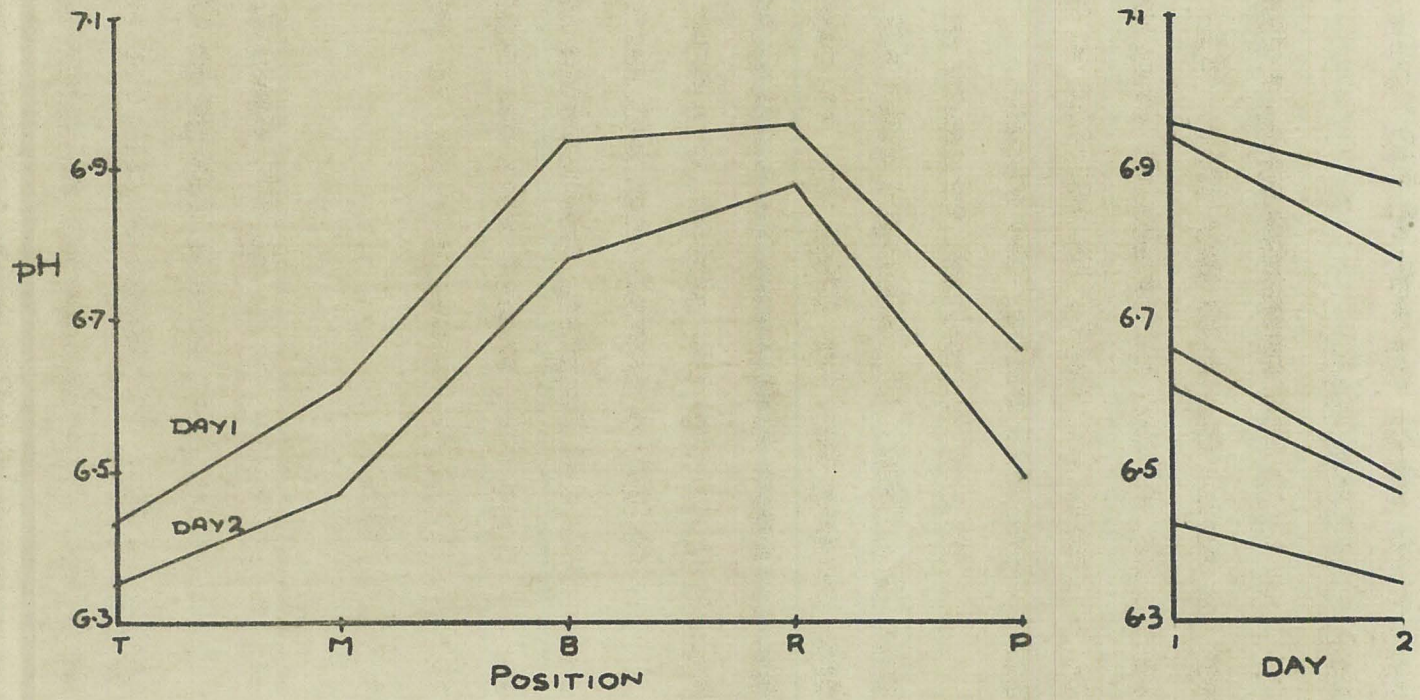


FIG. 13 DAILY AVERAGE pH AT EACH POSITION (D x P INTERACTION)
MEAN OF 14 OBSERVATIONS
S.E. ± 0·02



significant difference between Days was found. The pH levels on Day 2 were lower than those on Day 1 at all positions (Fig. 13) and at all times except the 2 p.m. sampling (Fig. 14).

As with VFA concentration, a diurnal trend in pH was apparent for each day (Fig. 14). This trend was exhibited by the pH levels at each position (Fig. 15), an increase ($P < 0.01$) in acidity of 0.3 - 0.4 pH units being observed during the night.

A dorsal to ventral increase in pH was found for each day (Fig. 13, averaged over all the times) and for each Time during the day (Fig. 15, average of the two days). The greatest difference in acidity between the positions occurred during the period of lowest pH.

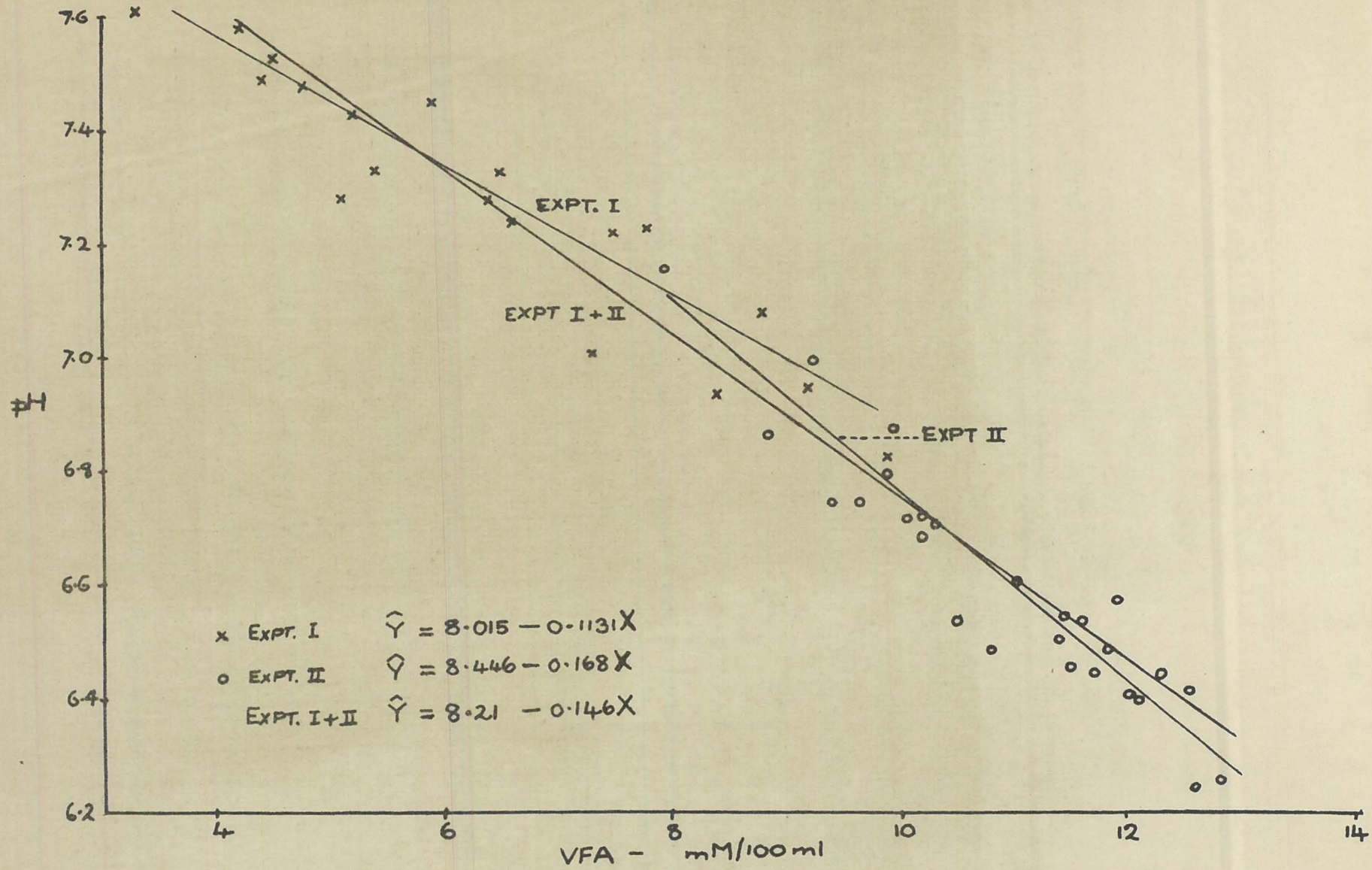
Although a significant interaction occurred between Positions and Samples, examination of the D x P x S interaction (Fig. 16) showed that the difference in pH between samples from the same position at the same time occurred only on Day 1.

(3) Regression of pH on VFA concentration

The regression line for pH on VFA concentration is shown in Fig. 17 together with the regression line for Expt. I. The regression analysis is shown in Appendix IX.

The data from Expt. I and II were pooled and a common regression coefficient of -0.1457 ± 0.005 was calculated

FIG. 17 GENERAL RELATIONSHIP OF pH AND VFA CONCENTRATION



(Appendix X). The deviations from the common and individual regression lines were not significantly different ($P = 0.20$) which indicated that the general relationship between pH and VFA concentration shown by the common regression line was valid. The relationship shows that for an increase in 1 m M/ 100 ml rumen liquor, the pH falls by 0.145 units (Fig. 17).

The close interdependence of the two variates, pH and VFA concentration, was shown by the correlation coefficient of -0.9704 (Appendix X).

(4) Relative proportions of individual volatile fatty acids

The relative proportions of the acids as found by gas-liquid chromatography are presented in Appendix VII.

Analyses of variance were performed in a manner similar to that described for Expt. I. The results of these analyses are presented in Tables VIII a, b, and c.

When the mean squares for Times and Positions were tested against the Error Mean Square, the results in all cases were not significant at the 5% level. When tested at the 10% level, the differences between the Time means were significant for acetic and butyric acids.

The means for each time are presented in Table IX.

TABLE VIII

ANALYSES OF VARIANCE OF THE PROPORTIONS OF INDIVIDUAL ACIDS OCCURRING ON DAY 1

VIII a Acetic acid

Source	d.f.	S S	M.S.	F	
Times	7	81.5175	11.6454	2.43	NS*
Positions	2	1.2019	0.6010	< 1	NS
Error	14	67.0282	4.7877		
Total	23	149.7476			

VIII b Propionic acid

Source	d.f.	S S	M.S.	F	
Times	7	21.0464	3.0066	1.74	NS
Positions	2	0.1469	0.0735	< 1	NS
Error	14	24.6205	1.7586		
Total	23	45.8138			

VIII c Butyric acid

Source	d.f.	S S	M.S.	F	
Times	7	63.9138	9.1305	2.72	NS
Positions	2	2.7429	1.3714	< 1	NS
Error	14	46.9158	3.3511		
Total	23	113.5725			

* N S denotes no significant difference at the 5% level.

TABLE IX

THE RELATIVE PROPORTIONS OF ACETIC, PROPIONIC AND BUTYRIC ACIDS WITHIN THE RUMEN AT EACH SAMPLING TIME ON DAY 1. (EXPT. II)

(Mean % of three positions)

Time	Individual acids (%)		
	Acetic	Propionic	Butyric
11.00	79.1*	13.4	7.5*
14.00	75.3	14.7	10.0
17.00	75.0	14.4	10.6
20.00	74.8	15.6	9.6
23.00	70.5	16.1	13.4
02.00	70.5	16.9	12.6
05.00	73.1	16.0	10.9
08.00	71.2	16.8	12.0
General Mean	73.7	15.5	10.8
5% Fiducial Limits	72.6-74.8	14.5-16.4	9.6-11.9

* denotes significant difference at 10% level using transformed data (inverse-sine transformation)

Table IX shows that there was some variation between the means at each sampling time. No differences between the means were significant at the 5% level. At the 10% level, the proportions of acetic and butyric acids were higher and lower respectively at 11 a.m. than at any of the other sampling times.

(5) Grazing habits

The grazing observations showed that each sampling time was followed by approximately 45 mins. of grazing except for the 2 p.m. and 5 a.m. samplings when grazing was for 2 and 1½ hrs. respectively. This pattern was quite constant for each of the two 24 hr. experimental periods.

C. Discussion

From the results of the grazing observations it is apparent that subjecting the animal to yarding, holding and sampling, caused considerable deviation from the normal grazing pattern (Hancock, 1954). Therefore this experiment failed in the sense that it gave no reliable indication of any diurnal trends which might exist in either the pH or the VFA concentration within the rumen of an animal following a normal grazing pattern.

The above is probably a partial explanation of why Williams and Christian (1956a) observed no change with time in ovine rumen pH or VFA levels but definite variations were found in this investigation. However, the results reported here indicate that the removal of rumen samples by stomach tube may lead to false conclusions (See p59).

The diurnal trends in pH and VFA levels which were found for the rumen as a whole (Figs. 10, 14) and for each position (Figs. 11, 15) were probably due to the relatively intensive grazing period that followed the 2 p.m. sampling time together with the shorter grazing periods which followed subsequent sampling times. Visual observation indicated that during this period, the rumen was tightly packed with ingested grass. Hancock (1954) and Gordon (1958) found that both sheep and cattle spend 7-8 hrs. ruminating, the greatest proportion of which occurs during the hours of darkness. Therefore, the continual exposure of fresh

substrate to fermentation consequent on rumination would be expected to facilitate the occurrence of the observed trends.

There were no diurnal trends evident in the proportions of the individual acids. However, as the differences between the means of the times were significant at the 10% level for acetic and butyric acids, it is apparent that the variation that did occur in these cases could not be wholly attributed to chance. Further investigation of this matter is required.

The significant D x T and D x P interactions (Figs. 9, 10, 13 and 14) were presumably the result of differences in the rate of food intake and total food consumption between the two days. Changes in the food composition may also have been a contributing factor. However, because of the short experimental period (36 hrs.), these changes may not have been of importance.

In view of the inverse relationship between VFA concentration and pH, it is perhaps surprising that a significant difference between Days should be obtained in the case of pH (Table VII) but not VFA concentration (Table VI). Similar discrepancies occurred not only in Expt. II but also in Expt. I indicating that the same linear pH - VFA relationship did not apply in all situations.

A similar discrepancy is apparent in the results of Williams and Christian (1956c). A possible explanation

is variation in the buffering properties of the rumen fluid as discussed on P50.

By calculation of either the regression or correlation coefficients, no inference is made as to the cause of the relationship between pH and VFA concentration even though VFA concentration was taken as the independent variable in the former calculation.

Although it has been stated that pH is largely a function of VFA concentration (Briggs et al, 1957a) suggestions that the effect is vice versa have been made (Lampila, 1955, 1959). Using steers fed a variety of hays, Cason, Ruby and Stallcup (1954) found significant positive correlations between the ash content of the rumen ingesta and rumen pH. No significant correlations were found between rumen pH and total fatty acids expressed as a percentage by weight of the weight of rumen contents. Cason et al concluded that strong chemical forces other than the fatty acids were present in the rumen. The chemical forces involved appeared to have been those shown by the buffering effects of the saliva and of the ingested materials.

The results of both experiments indicated that differences between sample A and Sample B were likely to occur. Any explanation as to why must be reconciled with the following observations.

(i) The differences were the differences between the means of either 7 or 12 observations.

(ii) Where a difference occurred it could have been the result of either a few relatively large observations or consistent smaller ones. The raw data indicates the latter.

(iii) Significant differences were more likely to occur when Position T and to a lesser extent Position M were sampled (Figs. 5, 12 and 16).

(iv) Where significant differences in pH occurred between samples from the same position, Sample A always had the lower pH (Figs. 5, 16).

(v) In Expt. II, differences in pH between samples from the same position occurred on one day and not the other (Fig. 16), but differences occurred on all three days in Expt. I (non significant D x P x S interaction).

(vi) In Expt. I no significant differences occurred in VFA levels of samples taken from the same position at the same time (Table I).

(vii) In Expt. II differences between the VFA levels of samples from the same position at the same time occurred on both days (Fig. 12) but the effects were opposite on each day.

(viii) The T x S interaction never approached significance, indicating the differences between samples were independent of changes with time of the amount or composition of ingesta within the rumen.

TABLE X

VALUES FOR TOTAL VFA, pH, AND INDIVIDUAL VFA EXPRESSED AS MOLAR PERCENTAGES OF THE TOTAL VFA FOR THE RUMEN LIQUOR OF GRASS FED CATTLE AND SHEEP.

Reference	Species	pH	Total VFA mm/100 ml	Individual acids: Acetic	Propionic	Butyric	Higher	Acetic / Propionic	Acetic / Butyric
Free Grazing									
Balch and Rowland (1957)	Cattle	5.07-5.92 5.48 *	12.2-16.5 14.3	63.4-68.2 66.3	17.4-20.2 18.5	10.2-12.3 11.5	3.0-4.5 3.7	3.6	5.8
Murdock and Roffler (1961)	Cattle	-	5.76±0.21	63.5±0.3	19.2±0.4	10.6±0.6	6.7±0.5	3.3	6.0
Annison et al (1959)	Sheep	-	14.1	57	26	16	2	2.2	3.6
Lampila (1955)	Cattle	6.05							
Beghelli et al (1958)	Sheep	5.26							
Reported Here	Cattle	5.95-7.25 6.65	7.6-15.2 10.6	73.7	15.5	10.8	-	4.7	6.8
Restricted Grass Diet (Levels before and after feeding respectively)									
el Shazly (1952)	Sheep	-	8.6 11.6	65.7 54.0	17.1 22.6	11.4 15.9	4.8 2.3	4.8 3.4	5.8 3.4
Johns (1955)	Sheep	-	2.6-6.6 10.1-18.7	50 - 62	21 - 30	12 - 17	3 - 10	-	-
Reported Here	Cattle	6.90-7.75 6.70-7.35	2.7-8.4 6.6-11.3	80.4 74.3	10.0 13.4	9.6 12.3	- -	8.0 5.5	8.5 6.0

* Mean

(ix) At Positions B and R the ingesta was of liquid consistency. At Position T and to a slightly lesser extent, Positions M and P, the ingesta was fibrous in nature and low in liquid content.

(x) Position T was the easiest to sample in that no forcing through a large mass of ingesta was required.

(xi) The differences between samples are considered to be a result of variable admixture with material from surrounding ingesta and/or heterogeneity already existing at that position.

Following consideration of these eleven observations, no adequate explanation for the differences can be suggested. However, it is suggested that the differences between samples from Position T were the result of heterogeneity already existing at the position rather than from varying admixture with surrounding material (See (x)). Similar reasoning may also apply to Position M although the possibility of varying admixture with material from surrounding ingesta is considered greater than for Position T.

Whether the differences would be eliminated by taking a larger sample is not revealed by these experiments.

On comparison of the general levels of pH, VFA concentration and the proportion of individual acids reported here with those reported in the literature (Table X), some differences are apparent. pH values are high even in the

presence of approximately normal concentrations of VFA, and also, the proportion of acetic acid is high.

In view of the established accuracy of the analytical methods used for the determination of pH, total and individual VFAs (Methods and Materials, Expt. I), it was unlikely that any of these methods were at fault. The validity of the sampling method used for pH determinations has been discussed elsewhere (p21).

The unusual pH - VFA relationship was probably the result of variation in the combination of factors that affect the buffering ability of the rumen fluid of the experimental animal.

The buffering properties of rumen fluid are affected by type of diet, time since feeding, consumption of drinking water and ammonia formation. (Turner and Hodgetts, 1955; Briggs et al, 1957a). Rumen fluid is poorly buffered against addition of alkali (Turner and Hodgetts, 1955). Ammonia is a weak base but can attain high concentrations in the rumen of an animal feeding on nitrogen-rich pasture (Johns, 1955; Mead, 1959) with consequent modification of the pH - VFA relationship (Briggs et al, 1957a).

Accumulation of lactic acid within the rumen can reduce rumen pH to low levels (Briggs et al, 1957a) but lactic acid may not accumulate in the rumen of a pasture fed animal (Balch and Rowland, 1957). Variation in salivary secretion rates can be considered important because of the buffering

properties of saliva (McDougall, 1948; Turner and Hodgetts, 1955). Factors affecting salivary secretion rates have been reviewed by Cole and Boda (1960). The nature of the diet, times spent eating and ruminating and psychic influences are important.

Ammerman and Thomas (1952) found that the rumen contents of different sheep were of different buffering capacity even when the feed was identical. Subsequent grazing trials employing the animal used in the investigation reported here revealed that its rumen contents were high in pH when compared with other animals receiving similar treatment, (Davey, A.W., pers. comm.). Variation in VFA concentration between animals did not appear to account for all the differences in pH.

Failure to make a determination of the proportion of valeric acid in a sample of VFAs results in higher percentages of acetic, propionic and butyric acids than in cases where such a determination is made. However, the ratio of one to another is not affected.

It has been suggested that the proportions of individual VFAs present within the rumen of a pastured animal are dependent on the seasonal changes occurring in the chemical composition of the pasture (Card and Schultz, 1953; Barnett and Reid, 1957). Johns (1955) failed to observe any obvious relationship. The plant species making up the pasture have been shown to be of importance (Tilley, Deriaz and Terry, 1960;

Butler and Johns, 1961).

Tilley et al (1960) found that the acetic : propionic acid ratio formed by in vitro fermentation of herbage by rumen liquor was dependent on the pH of the medium. Fermentation at a low pH (6.0) resulted in a low ratio. That pH is one of the factors influencing the acetic : propionic ratio in the fermentation of glucose by propionibacteria has been suggested by Johns (1951). High pH (6.5) results in a high acetic : propionic acid ratio. Thus the high pH values reported here may have affected acid production in favour of acetic acid.

The possibility of the high pH exerting a moderating effect on the relative rates of absorption of the acids cannot be overlooked (Briggs et al, 1957b; Barnett and Reid, 1961).

Knox and Ward (1961) found a significant increase ($P<0.05$) in total VFA concentration and a significant decrease ($P<0.05$) in the proportion of acetic acid when feeding frequency was increased from two to eight times a day. This suggests a possible explanation of why the proportion of acetic acid in Expt. I was higher than the proportion in Expt. II (Table X). The failure of Putnam, Gutierrez and Davis (1961) to observe such an effect emphasizes the tentative nature of this explanation.

IV. GENERAL DISCUSSION

The results of these experiments show that there was a stratification of pH and VFA levels within the reticulo-rumen of a cow following the consumption of pasture. Although only one cow was used in this investigation, in view of the confirmatory reports already existing in the literature it is tempting to state that the observed stratification was a natural physiological phenomenon of the bovine rumen. However, some other workers have failed to observe such a stratification in cattle under comparable conditions (Balch et al, 1955, 1957; Head, 1960).

In Expt. I where the feeding consisted of one hour's grazing, the ingesta in the dorsal region of the rumen (Position T) was on the average 38% higher in VFA concentration and 0.43 pH units higher in acidity than the ventral region (Position B). Similar dorso-ventral differences were observed under conditions of continuous grazing (Expt. II), the analagous differences being 29% and 0.47 pH units.

Smith et al (1956) reported differences of 45% in VFA concentration and 0.53 pH units for samples obtained by rumenotomy from a lactating dairy cow three hours after feeding 4 lb grain and hay ad. lib.. For pasture, differences of 0.2 - 1.1 pH units (Lampila, 1955) and 0.08 pH units (Smith, 1941) have been reported. In all cases the dorsal region of the rumen was more acid than the ventral

region.

The average differences in pH and VFA concentration as outlined above were found to vary in a definite manner. These variations will be discussed following consideration of the possible causes of stratification.

Smith et al (1956) based their explanation of the stratification of fermentation end products on the "hypothesis" that the physical pathways of the hay and grain particles through the rumen were different. They suggested that the movement of the hay to the dorsal regions and the grain to the ventral regions provided a sufficiently different environment to affect to a considerable degree the rate, and possibly the course, of fermentation.

> "Thus it would be anticipated that the fermentation intermediates and products associated with active fermentation of hay would tend to attain higher equilibrium concentrations in the top ingesta than in the bottom, when a low grain ration such as that fed here was consumed."

The ration fed was 4 lb grain and hay ad. lib. for 1 hr. The total nitrogen content of the strained rumen juice from the dorsal regions were taken as an indication that the

> "concentration of micro-organisms may also be greater in this nutritionally superior environment."

Although a conclusion such as the latter is tempting in view of the tendency for newly ingested food material to accumulate in the dorsal region (Review of Literature), reports in the literature, though not extensive, do not favour such a conclusion (Review of Literature).

Furthermore, reports that indicate cellulose digestion is more rapid in the ventral regions of the rumen (Balch and Johnson, 1950; Miles, 1951; Smith et al, 1956) are difficult to reconcile with this view.

Balch and Johnson (loc. cit.) observed a high correlation ($r = + 0.940$) between the time required to cause 50% reduction in the dry weight of the cellulose (cotton thread) and % dry matter content of the surrounding ingesta.

Lampila (1955) ascribed the cause of this to

> "the different degree of acidity of rumen contents (in the ventral region) which in turn is a result of the difference in water content."

However, the findings of Jacobson, Espe and Cannon (1942) suggest that the relatively small difference in acidity that exists between the two regions is insufficient to account for an effect so great as the two to threefold increase observed by Balch and Johnson.

Lower concentration of competing substrate as a causative factor (Smith et al, 1956) can be discounted on the evidence that the differences in cellulose digestion between the two regions still occur on diets of ground hay and concentrates (Balch and Johnson loc. cit.) when food material is approximately even in its distribution throughout the rumen (Balch, 1950).

In explanation of the stratification in pH observed in his work, Lampila (1955) made the suggestion that

> "...on account of the higher water content in the lower part the acids are washed away from the ingesta at a higher rate in this part of the rumen."

The results of the investigations reported here are in accordance with this hypothesis although effects other than this must be active for the different rates of cellulose digestion discussed above still remain unexplained.

Dilution of the liquid medium in the ventral rumen by saliva and drinking water probably accounts for some of the stratification. However, this does not appear to be reflected by differences in the distribution of micro-organisms and is also difficult to reconcile with the higher cellulose fermentation rate in the ventral rumen.

The pumping action of the reticulum flushes the mass of ingesta in the dorsal regions with liquid from the ventral rumen (Balch, 1959). It is suggested that this circulation of the fluid, aided by contractions of the rumen, results in the transport of the fermentation products to the site of absorption - the rumen wall. It is also suggested that factors which retard the fluid movement cause an accumulation of VFAs and other fermentation products in the dorsal region of the rumen.

If this hypothesis is correct, the observed increase in stratification to a maximum at 3 hrs. after feeding in Expt. I and during the hours of darkness in Expt. II, may be ascribed to not only increased production, but also to the accumulation of VFA in the dorsal mat of the ingesta formed from newly ingested food. The density and bulk of this mass of ingesta prevented the efficient removal of the

VFAs by the liquid medium. With gradual dispersal of the mass consequent upon rumination and microbial action, stratification decreased to an observed minimum at 9 hrs. after feeding in Expt. I (Figs. 2, 6). As this stage was approached, the increased liquid consistency and reduced volume of the ingesta may have allowed a more thorough mixing of the contents as has been suggested by Balch et al (1955). This, in its own right, would cause a reduction in the stratification of fermentation end products. Even so, homogeneity at 9 hrs. after feeding was not complete, for the concentration of VFA at Position T was still significantly higher than that at the other positions (Fig. 2). The pH was significantly lower than at other positions (Fig. 6).

The reduction in stratification at 5 and 8 p.m. in Expt. II (Figs. 11, 15) appears to be incompatible with the above hypothesis. However, it is suggested that the reduction was caused by the observed presence of such a large mass of ingesta within the rumen that the liquid, and consequently the VFAs, were more or less evenly distributed throughout.

If the hypothesis is correct, the differences in the extent of stratification between the three days in Expt. I (Figs. 4, 7) are explicable in terms of the different amounts of ingesta within the rumen on each day.

The inconsistent ranking of pH and VFA levels at Positions B and R are considered to be the result of rumination and

consequent entry of increased volumes of saliva into the rumen (Stewart and Dougherty, 1958). This effect was most marked during periods of greatest rumination, namely, the hours of darkness (Hancock, 1954) as shown in Figs. 11 and 15.

In Expt. I, the deviation in pH and VFA levels at Position R at 0 hrs. was probably the result of similar phenomena, entry of large volumes of alkaline (pH 8.0) saliva accounting for the rise in pH and a dilution effect accounting for the low VFA concentration.

The results of the experiments reported here indicate the possible errors involved in sampling the rumen contents for representative pH and VFA levels. Failure to obtain a representative sample may lead to erroneous conclusions regarding the general level of fermentation existing within the rumen at any particular time. However, it is to be remembered that although these results are in accordance with observations made by other workers, there are some reports discounting the existence of extensive stratification of pH and VFA levels (P.53). There is no analagous information concerning the ovine rumen. Furthermore, the results reported here are concerned only with pH and VFA concentration and have been obtained from only one animal. These comments should be borne in mind during the consideration of this discussion.

Before consideration of the merits and demerits of various positions within the rumen for sampling purposes,

it is obvious that the results reported here gave no reliable indication of the average pH or VFA concentration within the rumen as a whole. The Days x Times interaction presented in Figs. 3, 8, 10 and 14 are not a true indication for in using the average of the values for the five sampling positions, no allowance was made for the amount of ingesta each position represents. A true value could be obtained by removing the whole contents and thoroughly mixing the ingesta before sampling as has been done by some workers (Agrawala et al, 1953; Hale, Duncan and Huffman, 1947). Such a technique has obvious disadvantages in a study similar to the one reported here.

Because of the observed dorso-ventral stratification and absence of a definite anterio-posterior gradient (Smith, 1941; Lampila, 1955) it is suggested that a composite sample from Positions T, M and B or a single sample from Position M would serve as a satisfactory representative sample of the rumen contents. Either procedure would have the added advantage of counteracting the significant Days x Position interation that may occur consequent on differences in intake.

Sampling by means of a stomach tube is common in studies on sheep (e.g. Williams and Christian, 1956a, b) and has been applied to cattle (Pounden, 1954; Hungate et al, 1955). The uncertain placement of the end of the tube in the reticulo-rumen has been described by Watson and Jarret (1945). The possibility of salivary contamination can be reduced (Hungate

et al, loc. cit.) but it is suggested from the results reported here that the likelihood of obtaining samples from the reticulum and ventral regions would result in a false indication of pH and VFA levels existing throughout the rumen.

The same criticism is applicable to those methods which sample the ventral regions by other means (e.g. McClymont, 1950).

After a limited feeding period (Expt. I), because of reduction with time after feeding in the extent of stratification, the error involved would depend on the sampling time. With grazing, stratification appears more consistent (Figs. 11 15; Lampila, 1955) but there are some reservations to this general statement (P.56).

The results of this investigation also suggest that observations on changes with time of pH and VFA levels based on samples from either the reticulum or ventral regions of the rumen may give misleading results. The highly significant Times x Position interactions were considered to be mainly the result of (a) reduction in stratification with Time and (b) the irregular variations of pH and VFA levels at Positions B and R. On the assumption that the greatest absolute amounts of acids occur in that region of the rumen containing the more fibrous ingesta (Positions T, M and P), the consideration of (b) indicates that atypical trends may be noted when either the reticulum or the ventral regions are sampled.

Avoidance of sampling the top few inches of ingesta is

indicated by the low repeatibility of sampling this site (P.49). However, the differences that occurred between samples from this position taken at the same time may not be considered of practical importance and may be reduced by removing larger samples of rumen ingesta.

The relatively high concentrations of VFA and low pH that exist at this position may facilitate studies on changes with time of pH and VFA levels but the absolute levels cannot be regarded as representative of the whole of the rumen ingesta. Furthermore, it should be remembered that the conditions may be aerobic in animals with badly sealed fistulae (Turner and Hodgetts, 1955; Matscher et al, 1957).

When changes with time are being studied the importance of sampling from a fixed position within the rumen is obvious.

Because of the nature of the disturbance to the normal grazing pattern caused by yarding, holding and sampling, it is apparent that in order to obtain reliable information on diurnal trends in rumen fermentation products, some modification to the experimental design would be necessary. If several animals were available, the principles of the design suggested by Williams and Christian (1956a) would be suitable. Interference to the animals' grazing habits and the number of analyses that can be handled in the laboratory limit the scope of this type of work.

V. SUMMARY

1. A study was made of the effect of two feeding systems on the variation of pH and VFA concentration within the reticulo-rumen of one cow. VFA concentration and pH were determined on samples of ingesta removed from five fixed positions within the reticulo-rumen.

2. With both feeding systems a dorso-ventral gradient in pH and VFA concentration was found.

3. The ingesta in the dorsal region of the rumen was higher in VFA concentration and lower in pH than the ingesta in the ventral regions of the rumen.

4. The differences in pH and VFA concentration between positions were dependent on the sampling time during the day and the day itself.

5. The proportions of individual acids did not vary with the sampling position.

6. Diurnal trends in pH and VFA concentration were observed for both feeding systems.

7. When feed intake was limited to one hour of grazing the proportions of individual acids were dependent on the sampling time.

8. No definite trends were found in the proportions of individual acids when the feeding system was one of free grazing. However, some variation was observed.

9. The relationship between pH and VFA concentration for both feeding systems was satisfactorily represented by a common regression line.

10. The possible causes of the dorso-ventral gradient in pH and VFA concentration were discussed.

11. The results were discussed in relation to sampling the rumen contents but it was emphasized that the results were obtained from only one cow.

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APPENDIX I

Data for duplicate VFA concentration determinations at four times after feeding (EXPT I).

Hrs after Feeding		DAY 1								DAY 2								DAY 3							
		0		3		6		9		0		3		6		9		0		3		6		9	
T	A	7.1	7.1	9.7	9.7	8.5	8.5	4.7	4.7	6.6	6.6	8.6	8.6	6.3	6.2	4.5	4.5	8.4	8.4	10.6	10.6	12.5	12.6	6.3	6.3
	B	7.2	7.1	11.3	11.3	7.5	7.4	5.1	5.1	6.4	6.4	8.8	8.8	6.5	6.5	4.4	4.4	8.1	8.1	10.5	10.5	9.1	9.0	5.7	5.6
N	A	4.9	4.9	9.8	9.8	5.9	5.8	4.1	4.1	5.2	5.2	8.4	8.4	5.5	5.5	4.0	4.0	5.8	5.8	10.2	10.3	8.2	8.2	5.4	5.4
	B	5.2	5.3	8.3	8.3	6.6	6.6	4.2	4.1	4.9	5.0	8.4	8.4	5.7	5.7	4.1	4.2	6.2	6.2	9.8	9.8	7.8	7.7	4.6	4.6
B	A	4.6	4.5	8.1	8.1	5.7	5.6	3.8	3.8	4.6	4.5	6.6	6.5	5.2	5.2	4.1	4.1	5.5	5.4	7.6	7.6	6.5	6.4	4.6	4.6
	B	4.5	4.5	8.3	8.2	5.6	5.6	4.0	4.1	4.3	4.4	6.6	6.6	5.3	5.3	3.8	4.0	5.0	5.0	8.1	8.1	6.9	6.9	4.3	4.4
R	A	2.7	2.6	7.6	7.7	6.2	6.2	3.8	3.8	3.3	3.4	7.1	7.1	5.6	5.6	3.9	3.9	3.9	4.0	8.1	8.1	8.0	8.0	4.6	4.6
	B	2.7	2.6	7.9	7.9	6.1	6.1	4.2	4.1	3.4	3.4	7.1	7.1	5.4	5.4	4.4	4.3	3.7	3.7	9.0	9.0	7.7	7.8	4.6	4.6
P	A	4.7	4.7	8.6	8.6	6.4	6.4	4.4	4.4	4.9	4.9	7.7	7.7	5.8	5.8	4.3	4.2	5.7	5.6	9.6	9.6	7.0	7.0	5.0	5.0
	B	5.0	4.9	8.6	8.6	6.8	6.8	4.2	4.3	5.0	5.0	7.8	7.8	5.5	5.6	4.1	4.2	5.9	5.9	10.5	10.4	7.0	7.0	4.7	4.8

NOTE: T, N, B, R, and P are the five sampling sites within the rumen.
A and B are the two samples taken from each position.
All concentrations expressed in ml/100 ml rumen liquor.

APPENDIX II

Data for pH levels at four times after feeding. (EXPT. I).

		DAY 1				DAY 2				DAY 3			
Hrs after feeding		0	3	6	9	0	3	6	9	0	3	6	9
T	A	7.00	6.75	6.85	7.25	7.00	6.95	7.20	7.35	6.90	6.70	6.55	7.05
	B	7.05	6.85	7.00	7.35	7.10	7.00	7.20	7.50	7.00	6.75	6.85	7.20
M	A	7.30	6.90	7.30	7.55	7.30	7.05	7.35	7.60	7.30	6.80	7.05	7.25
	B	7.45	7.05	7.25	7.55	7.35	7.00	7.35	7.50	7.25	6.90	7.15	7.50
B	A	7.50	7.20	7.50	7.65	7.50	7.30	7.30	7.65	7.40	7.25	7.45	7.65
	B	7.50	7.05	7.60	7.60	7.50	7.25	7.45	7.70	7.45	7.25	7.40	7.65
R	A	7.65	7.20	7.40	7.60	7.70	7.35	7.35	7.65	7.50	7.25	7.10	7.45
	B	7.75	7.20	7.45	7.60	7.60	7.20	7.35	7.60	7.45	7.15	7.35	7.55
P	A	7.50	7.05	7.30	7.55	7.40	7.25	7.30	7.60	7.35	7.00	7.25	7.40
	B	7.50	7.10	7.15	7.60	7.45	7.15	7.40	7.50	7.35	6.90	7.25	7.55

NOTE: T, M, B, R, and P are the five sampling sites within the rumen.
A and B are the two samples taken from each site.

APPENDIX III.

Data for proportions of acetic, propionic and butyric acids at five positions within the rumen on four times after feeding. (EXPT. I).

	DAY 1				DAY 2				DAY 3			
Hrs after feeding	0	3	6	9	0	3	6	9	0	3	6	9
	85.7	73.4	77.5	80.4	79.5	74.2	76.4	77.1	78.0	68.0	71.4	74.2
T	10.5	12.6	12.4	11.1	11.3	12.9	10.7	10.4	11.3	16.0	14.3	14.4
	3.8	14.0	10.1	8.5	9.2	12.9	12.9	12.3	10.7	16.0	14.3	11.4
	84.8	77.4	80.0	87.4	82.0	76.6	72.4	77.2	78.5	70.2	69.0	74.0
M	8.6	15.3	9.6	7.4	9.7	12.5	11.4	9.3	9.8	14.9	15.2	14.2
	6.6	7.3	10.4	5.2	8.3	10.9	16.2	13.0	11.7	14.9	15.8	11.8
	80.6	78.8	79.2	84.4	85.7	75.8	74.5	88.2	77.5	69.6	69.8	76.4
B	9.7	12.1	11.0	8.4	8.9	12.3	10.6	5.0	10.0	15.2	14.2	11.8
	9.7	9.1	9.8	7.2	5.4	11.9	14.9	6.8	12.5	15.2	16.0	11.8
	76.6	79.6	71.6	82.3	81.4	80.7	72.8	76.7	76.2	68.2	68.2	76.6
R	11.2	12.2	13.8	9.7	7.8	8.4	11.8	11.0	10.4	15.9	15.0	11.9
	12.2	8.2	14.6	8.0	10.8	10.9	15.4	12.3	13.4	15.9	16.8	11.5
	82.8	70.5	73.0	83.9	80.3	80.8	74.4	75.0	78.0	67.3	70.1	75.4
P	9.7	14.1	11.7	9.9	10.8	9.6	10.9	10.9	10.6	17.3	14.5	11.8
	7.5	15.4	15.3	6.2	8.9	9.6	14.7	14.1	11.4	15.4	15.4	12.8

NOTE: T, M, B, R, and P are the five sampling sites within the rumen.
The percentages are in the order acetic, propionic and butyric acids.

APPENDIX IV

Analyses of variance for the proportions of individual acids (Expt. I).

ACETIC ACID

Source	d.f.	S.S.	M.S.	F
Days (D)	2	230.5982	115.2991	27.0
Times (T)	3	277.2001	92.4000	21.6
Position (P)	4	28.8230	7.2060	1.69
D x T	6	36.9049		
D x P	8	35.1625	4.2700	
T x P	12	49.0183		
Error	24	92.4155		
Total	59	750.1225		

PROPIONIC ACID

Source	d.f.	S.S.	M.S.	F
Days	2	89.1812	44.59	22.8
Times	3	88.3978	29.46	15.0
Positions	4	11.2957	2.8239	1.44
D x T	6	23.2727		
D x P	8	10.1698	1.9585	
T x P	12	17.2892		
Error	24	47.1934		
Total	59	286.7998		

BUTYRIC ACID

Source	d.f.	S.S.	M.S.	F
Days	2	171.0609	85.53	18.4
Times	3	172.8244	57.61	12.4
Positions	4	26.0652	6.5163	1.40
D x T	6	34.0190		
D x P	8	29.6155	4.6576	
T x P	12	59.2191		
Error	24	80.4345		
Total	59	573.2386		

APPENDIX V

VFA concentrations at five positions within the rumen during free grazing. (EXPT. II)

		DAY 1								DAY 2							
Hr of the day.		11.00	14.00	17.00	20.00	23.00	02.00	05.00	08.00	14.00	17.00	20.00	23.00	02.00	05.00	08.00	11.00
T	A	10.4	11.8	10.9	12.9	12.0	12.7	12.5	12.3	10.8	9.9	12.4	13.5	13.7	12.2	11.5	11.7
	B	10.3	10.6	11.0	12.7	11.8	11.6	12.4	10.9	10.9	11.3	13.3	13.2	15.2	12.1	11.7	12.4
M	A	9.4	10.4	10.3	12.1	11.5	12.1	11.7	8.7	10.2	9.8	11.7	11.6	12.2	11.8	9.8	9.8
	B	9.2	9.4	11.0	13.0	10.9	11.3	10.6	8.7	11.2	10.9	13.4	11.9	12.8	11.5	10.5	9.6
B	A	7.[illegible]	7.9	10.0	12.1	9.5	8.8	9.3	9.0	7.8	9.9	10.5	9.9	11.5	9.1	8.2	7.9
	B	8.7	8.5	10.5	12.2	9.1	9.9	8.7	9.1	7.6	9.9	12.9	11.0	9.8	9.8	9.1	7.8
R	A	7.0	8.8	9.4	10.4	9.5	10.7	9.3	7.6	7.2	9.0	9.4	9.4	11.1	8.2	9.6	10.3
	B	7.5	8.7	9.9	11.3	10.6	11.6	9.8	8.1	6.2	7.4	10.1	9.8	10.8	8.1	9.6	9.2
P	A	9.7	10.0	10.9	11.8	11.4	10.6	11.5	9.7	9.3	9.9	11.9	11.4	11.8	11.8	10.5	10.6
	B	9.9	10.0	10.4	12.3	11.5	12.1	11.5	9.6	9.3	9.7	12.0	11.5	12.4	12.2	11.0	10.8

NOTE: T, M, B, R, and P are the five sampling sites within the rumen.
A and B are the two samples taken from each site.
All concentrations are expressed in ml/100 ml of rumen liquor.

APPENDIX VI

The pH at five positions within the rumen during free grazing. (EXPT. II).

		DAY 1								DAY 2							
Hr of the day.		11.00	14.00	17.00	20.00	23.00	02.00	05.00	08.00	14.00	17.00	20.00	23.00	02.00	05.00	08.00	11.00
T	A	6.55	6.50	6.40	6.20	6.20	6.20	6.45	6.40	6.55	6.45	6.25	6.15	6.05	6.35	6.50	6.55
	B	6.65	6.75	6.55	6.40	6.40	6.40	6.55	6.65	6.65	6.55	6.20	6.15	5.95	6.45	6.60	6.55
M	A	6.80	6.60	6.65	6.45	6.45	6.40	6.45	6.85	6.75	6.45	6.45	6.40	6.25	6.45	6.60	6.70
	B	6.80	6.75	6.65	6.50	6.65	6.60	6.65	6.90	6.75	6.40	6.30	6.35	6.35	6.50	6.65	6.85
B	A	7.10	7.15	6.85	6.60	6.80	7.10	7.00	6.90	7.15	6.60	6.60	6.70	6.60	6.95	6.85	7.05
	B	7.20	7.15	6.80	6.70	6.95	7.05	7.20	6.95	7.20	6.65	6.40	6.75	6.80	6.85	6.80	7.15
R	A	7.15	7.05	7.05	6.90	6.90	6.85	6.95	7.10	7.05	6.75	6.80	6.90	6.60	7.10	6.85	6.80
	B	7.15	7.10	7.05	6.80	6.80	6.80	6.95	7.10	7.25	6.80	6.75	6.90	6.65	7.05	6.90	6.90
P	A	6.75	6.70	6.75	6.60	6.55	6.60	6.55	6.75	6.75	6.55	6.25	6.50	6.30	6.40	6.65	6.85
	B	6.80	6.75	6.80	6.50	6.65	6.65	6.60	6.85	6.80	6.65	6.30	6.50	6.25	6.40	6.65	6.85

NOTE: T, M, B, R, and P are the five sampling sites within the rumen.
A and B are the two samples taken from each position.

APPENDIX VII

Proportions of individual acids within the rumen during free grazing (DAY 1, EXPT. II).

DAY I

Hr of the Day	11.00	14.00	17.00	20.00	23.00	02.00	05.00	08.00
T	80.0	75.0	77.7	76.2	66.7	73.3	66.2	69.9
	12.0	15.0	13.3	14.8	17.5	15.3	17.9	17.9
	8.0	10.0	9.0	9.0	15.8	11.4	15.9	12.2
M	79.3	74.0	75.0	73.8	74.0	67.3	73.7	74.2
	13.8	15.3	14.5	15.0	14.7	18.2	17.2	14.5
	6.9	10.7	10.5	11.2	11.3	14.5	9.1	11.3
B	77.9	77.0	72.3	74.3	70.8	70.6	78.7	69.3
	14.4	13.8	15.2	16.9	16.0	17.4	13.1	18.3
	7.7	9.2	12.5	8.8	13.2	12.0	8.2	12.4

NOTE: T, M, and B are the three sampling positions within the rumen.

The percentages are expressed in the order acetic, propionic and butyric acids.

APPENDIX VIII

ANALYSIS OF REGRESSION OF pH ON VFA CONCENTRATION BASED ON THE MEAN VALUES AT EACH SAMPLING POSITION AT EACH TIME OVER THE THREE DAYS COMBINED. (Expt. I)

Y = pH X = VFA concentration

Linear regression =

adjusted $Y = a + bX$

$\bar{Y} = 7.297$ $\bar{X} = 6.345$

$SSy = 1.0806$ $SSx = 69.0695$ $SPxy = -7.8143$

$b = \frac{SPxy}{SSx} = -0.1131$

SS due to regression of y on $x = bSPxy = 0.8838$

Test of significance of b

Source	d.f.	S S	M.S.	F	
Lin. Reg.	1	0.8838	0.8838	81.08	**
Error	18	0.1968	0.0109		
Total	19	1.0806			

$$S.E._b = \pm\sqrt{\frac{0.0109}{69.0695}}$$

$$= \pm 0.0126$$

$$Y = 8.015 - 0.1131X$$

APPENDIX IX

ANALYSIS OF REGRESSION OF pH ON VFA CONCENTRATION BASED ON THE MEAN VALUES AT EACH SAMPLING POSITION AT EACH TIME FOR TWO DAYS COMBINED (Expt.II)

Y = pH X = VFA concentration

Linear regression, adjusted $Y = a + bX$

$\bar{Y} = 6.657$ $\bar{X} = 10.649$

$SSy = 2.1415$ $SSx = 68.9568$ $SPxy = -11.5827$

$$b = \frac{SPxy}{SSx} = -0.1680$$

SS due to regression = $bSPxy = 1.9459$

Test for significance of b

Source	S S	d f	M.S.	F	
Lin. Reg.	1.9459	1	1.9459	329.8	***
Error	.1956	33	.0059		
Total	2.1415	34			

$$S.E._b = \pm\sqrt{\frac{.0059}{68.9568}}$$

$$= \pm 0.0092$$

$$Y = 8.446 - 0.168X$$

APPENDIX X

ANALYSIS OF REGRESSION FOR pH ON VFA CONCENTRATION OVER BOTH EXPT. I AND II.

Y = pH X = VFA concentration

Linear regression, adjusted $Y = a + bX$

$\bar{Y} = 6.89$ $\bar{X} = 9.084$

$SSy = 8.4216$ $SSx = 373.5736$ $SPxy = -54.430$

$$b = \frac{SPxy}{SSx} = -0.1457$$

Test of significant of b

Source	df	SS	M.S.	F	
Deviations from the common reg.	52	0.4962	.009542	1.24	NS
Deviations from the individual reg.	51	0.3924	.007694		

$$S.E._b = \pm \sqrt{\frac{0.4903}{19797}}$$

$$= \pm 0.0049$$

$$Y = 8.21 - 0.1457X$$

Correlation between pH and VFA concentration

$$r = \frac{SPxy}{\sqrt{SSx.SSy}}$$

$$= \frac{-54.430}{\sqrt{3145.0874}}$$

$$= -0.9704$$