VARIATION IN pH, VOLATILE FATTY ACID CONCENTRATION AND PROPORTIONS OF THE INDIVIDUAL ACIDS WITHIN THE RUMEN OF THE DAIRY COW.

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF AGRICULTURAL SCIENCE IN THE MASSEY UNIVERSITY OF MANAWATU.

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by

A.W.F.DAVEY.

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PART I

THE EFFECT OF LEVEL OF INTAKE OF PASTURE AND VARIATION WITH TIME AFTER FEEDING.

INTRODUCTION

An increased interest in rumen physiology and metabolism has resulted in the accumulation of a mass of data on the subject over the last two decades but information, particularly on quantitative aspects, is far from complete. It is now well established that the main non-nitrogenous end-products of rumen fermentation, the volatile fatty acids (VFA) acetic, propionic and butyric play a major part in the energy metabolism of the ruminant. They provide the major energy source for the animal and the amounts and proportions of the acids absorbed influence the efficiency with which the diet is used for fattening and can affect the milk composition of the lactating cow. Thus a knowledge of the type of fermentation produced is necessary, as a contribution to the assessment of the nutritive value of feedstuffs and the efficiency with which they are converted to animal products.

The results obtained by different investigators are seldom strictly comparable as the pattern of rumen fermentation and the concentration and proportions of the VFA s may be considerably modified by factors other than the composition of the diet. Such factors are the level of intake, the feeding régime and sampling techniques adopted.

The present experiment followed the observations of Bryant (1961) and Davey, Robinson and Campbell (1962), that differences occurred between days in rumen pH and VFA concentration and proportions of the individual acids in cows grazing pasture. It was thought that some of these differences could be accounted for by variations in the level of intake between days.

In much of the work on rumen fermentation carried out overseas, mainly hay and concentrates have been used and there is limited information on rumen fermentation in relation to changes in the composition of pasture.

The main experiment reported herewas designed as a 3 x 3 latin square, primarily to obtain information on the effects of different levels of intake of pasture fed indoors on the pH and on the concentration, proportions and total weight of VFA s produced in the rumen of three dairy cows, and as an aid to the design and interpretation of future experiments. It was also possible to collect additional information on the changes that occurred in pH and VFA concentration proportions with increased time after feeding. This information aided the interpretation of the results of the experiment dealing with intake.

The 3 x 3 latin square used, was repeated three times over a period of three months to assess possible variations in rumen fermentation caused by changes in pasture composition. From time to time the cows were grazed on pasture similar to, or different from that used in the indoor feeding experiment and rumen pH, VFA concentration and proportions of the acids were measured.

Although the experiment was not designed specifically to this end, the yield and composition of the milk of the cows was recorded in all experiments.

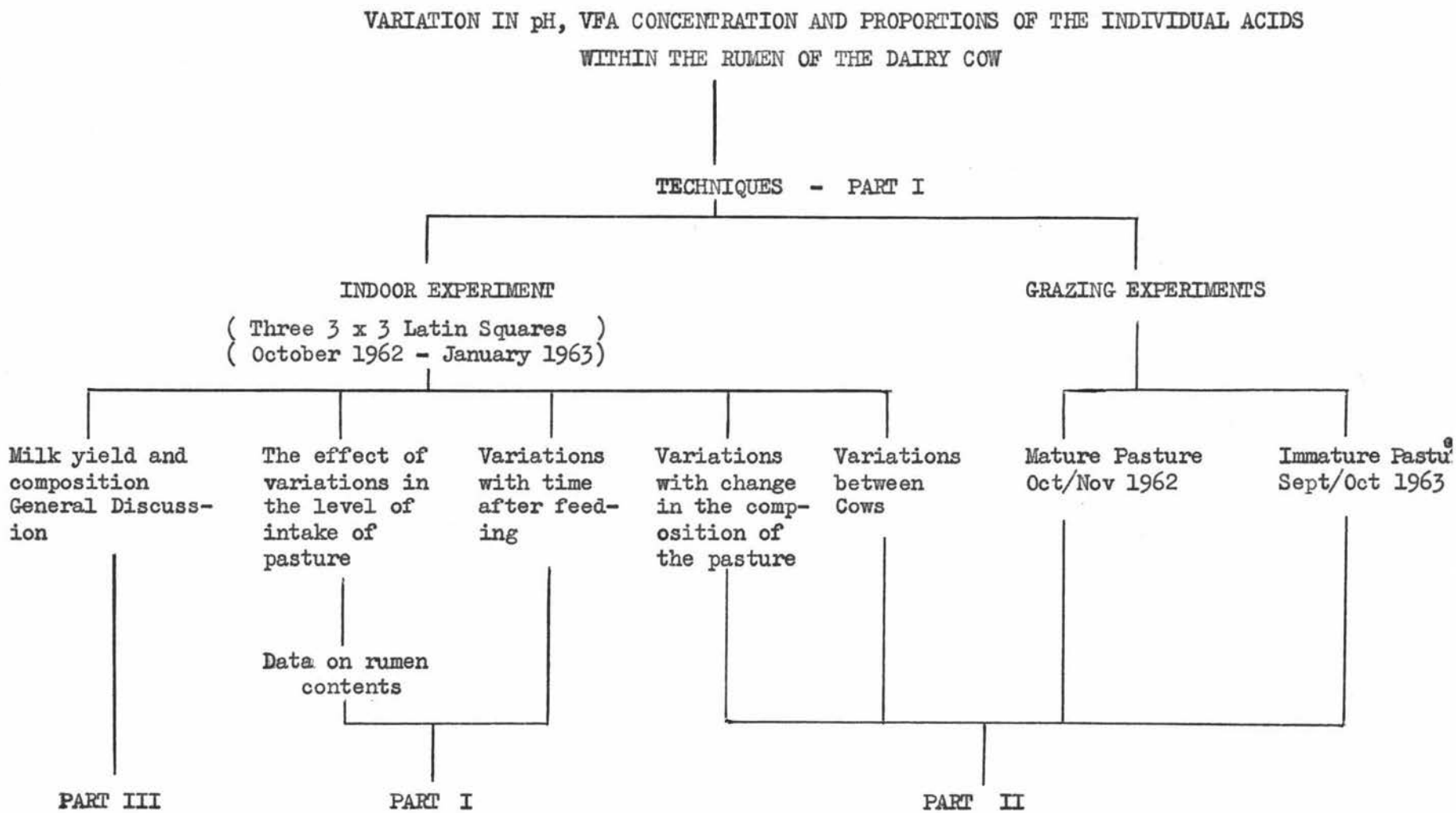
Part I of this report presents the review of literature and results and discussion on the effects of level of intake of pasture and time after feeding on rumen fermentation.

Part II contains the review of literature, results and discussion on variations in rumen fermentation over the season with grazing cows and with cows fed indoors and the chemical and botanical composition of the pasture.

Part III contains a brief discussion of the results obtained on milk yield and composition in relation to the proportions of the individual acids in the rumen liquor. The general discussion and conclusions are also included in this part.

For ease of reference the outline of the experiments and organisation of the thesis are given in Figure A. (facing Page 4.).

FIGURE A: OUTLINE OF THE EXPERIMENTS AND ORGANISATION OF THE THESIS



CHAPTER I

REVIEW OF LITERATURE

VARIATION IN RUMEN pH, VFA CONCENTRATION AND PROPORTIONS OF THE INDIVIDUAL ACIDS

Variation in rumen pH, VFA concentration and proportions of the individual acids as influenced by level of intake and time after feeding is reviewed. Information on rumen-fluid volume and the amounts of dry matter (d.m.) per cent of the ingesta as influenced by the diet and the level of intake is also reviewed.

1.1 The Relationship Between pH and VFA Concentration of the Rumen Liquor

The pH of the rumen liquor varies inversely with the concentration of the VFAs (Phillipson, 1942; Balch and Rowland, 1957; Briggs, Hogan and Reid, 1957; Bryant, 1961). Kay and Hobson (1963) stated that a clear correlation between pH and VFA was seldom seen as the VFA concentration and buffering power of the rumen ingesta vary independently. They further stated that the closest agreement between rumen pH and VFA concentration occurred after a meal.

A poor relationship between rumen pH and VFA concentration was observed by Williams and Christian (1956). They observed a lowering of VFA concentration of the rumen liquor of sheep with decreased intakes of dried grass but there was no equivalent increase in pH values. However their method of sampling by stomach tube may have introduced errors through contamination of the sample with saliva and through the difficulty of placing the end of the tube in a known position. An

inverse relationship between rumen pH and VFA concentration was demonstrated by Phillipson (1942) but he showed that when sheep drank water, there was a large drop in VFA concentration but no corresponding change occurred in the pH of the rumen liquor. Briggs et al.(1957), in studies with sheep on different diets, noted that the pH - VFA relationship was modified by high ammonia nitrogen which was attributed partly to the effects of varying stimuli on salivary secretion. They also found that rumen pH values were considerably higher in relation to the VFA concentration on all roughage diets, compared with those containing concentrates, because of a postulated greater secretion of saliva with the roughage diet.

1.2 The Effect of Varying Levels of Intake

(1) Rumen pH and VFA concentration

A limited number of experiments have been carried out, specifically to examine the effects of variations in the level of food intake on the pH and VFA concentration of rumen liquor.

Williams and Christian (1956) working with sheep, obtained a positive linear relationship between the intake of dried grass and the VFA concentration of the rumen liquor (0.44 mM. VFA 100 ml rumen liquor per 100 g feed). Mean values for rumen VFA concentration for intakes of 400g and 1000g of dried grass were 7.1 $\pm$ 0.8 and 9.7 $\pm$ 0.8 mM per 100 ml rumen liquor respectively. There was no change in the pH value of the rumen liquor with the varying levels of intake.

Since the present experiment was completed, Bath and Rook (1963) have reported the effects of the level of intake of hay alone and a mixed hay and concentrate ration on the ruminal concentration of VFAs, using two dry dairy cows. Increased levels of d.m. were associated with a fall in pH and an

increase in the concentrations of the VFA s. Portions of Bath and Rook's (1963) results are presented in Table I.

TABLE I. The Effect of Level of Intake on Rumen pH and VFA Concentration in the Rumen Liquor from Two Cows Fed Hay or a Hay and Concentrate Ration. (Adapted from Bath and Rook 1963).

		HAY		HAY AND CONCENTRATES (1 : 1)	
Fed lb/day	Cow	VFA mM/100 ml.	pH	VFA mM/100 ml.	pH
10	C	7.8	6.61	9.3	6.46
	D	7.1	6.70	9.3	6.37
15	C	10.1	6.41	10.4	6.51
	D	10.6	6.43	10.8	6.41
20	C	11.9	6.07	12.1	5.99
	D	9.2	6.51	11.4	6.01
30	C			12.6	5.98
	D			12.3	6.01

With the hay and concentrate ration, decreases in rumen pH and increases in VFA concentration were less marked between the two highest levels of intake. Terry and Tilley (1963), in a brief report, noted that rumen VFA fermentation could be influenced by the level at which a given feed was fed to sheep. Thus, as the amount of feed given was reduced, rumen pH increased and VFA concentration decreased, the extent of these changes being dependent on the

feed. No data were given for VFA concentration and only limited data were given on pH values. Variations in the pH of the rumen liquor of one cow fed lucerne hay at three levels of intake were reported by Hale, Duncan and Huffman (1940). Rumen pH values were highest at the lowest level of intake but differences were small six hours after feeding, when pH values were at their lowest.

Indications that level of intake may be responsible for some of the variations in rumen pH and VFA concentration were noted in the work of Davey et al. (1962) using three dairy cows grazing a mixed pasture. Highly significant differences ($P<0.01$) in rumen pH and VFA concentration, occurring between days and cows, were attributed possibly to differences in intake. Variations in rumen pH and VFA concentration between animals and days have been noted by Johns (1955 b) and between days by Bryant (1961) who also attributed differences partly, to variations in the level of intake.

The effects of starvation on rumen pH and VFA concentration were studied by Coop (1949) using one sheep. Starvation periods were up to four days in duration and in one trial, rumen pH rose as high as 8.11 and in another, 7.92 at the end of the starvation period. At the same time, rumen VFA concentration fell to as low as 3.8 mM per 100 ml but on return to feeding recovery was rapid with normal pre-treatment levels of VFAs being reached in from 12 to 24 hours. The author found no correlation between rumen pH and VFA concentration, nor was there any consistent effect on fermentation of variations in food and water intake, over the feeding period of the experiment. On exposure to air the rumen sample loses CO_2 and the pH rises. (Turner and Hodgetts, 1955). The delay in making the pH determination after removal of the liquor from the rumen and the method of sampling by stomach tube may have contributed to the unusual results obtained by Coop (Loc.cit.). Phillipson (1942)

using four sheep, reported an extreme pH value of 7.48 after a period of 48 hours starvation, with VFA levels of 3.27 mM per 100 ml rumen liquor. Masson and Phillipson (1951) stated that, in theory, the highest possible pH value in the rumen in almost the complete absence of VFAs was 7.4. Values of 7.6 for pH and 3.1 mM per 100 ml for VFA concentration were obtained by Davey and Wilson (1960) with two cows, after a fasting period of approximately 12 hours. Return to normal values for pH and VFA concentration was rapid and complete recovery to pre-fasting levels had taken place in 12 hours.

(2) Proportions of the individual VFA

High intakes of up to 1000 g dried grass per day fed to sheep gave a lower acetic to propionic acid ratio than did low intakes of 400 g (Williams and Christian, 1956). No changes occurred in butyric acid proportions between treatments. Considering the size of what are presumably the standard deviations (Table 2), differences between treatments in the proportions of acetic and propionic acid appear to be unimportant.

TABLE 2. The Effect of Varying Levels of Intake of Dried Grass on Rumen VFA proportions in Sheep (Adapted from Williams and Christian, 1956)

Intake g	PROPORTIONS OF VFAS (%)		
	Acetic	Propionic	Butyric
400	68.3 ± 1.7	21.2 ± 1.0	10.5 ± 1.2
1000	66.0 ± 1.8	23.4 ± 1.1	10.6 ± 1.0

Since the present experiment was completed, the effects of level of intake on rumen VFA proportions have been briefly reported by Terry and

Tilley (1963). They showed that, as the amount of hay given to sheep decreased, the proportion of the acetic acid in the rumen liquor increased. They stated that the extent of the effect appeared to be dependent on the feed, being most marked on feeds giving a low proportion of acetic acid. Their results given in Table 3 do not support this statement as no errors of estimate or details of the experiment were given to assess whether or not differences in rumen VFA proportions for Hay 3, were meaningful.

TABLE 3. Effect of Level of Feeding of Hay on Rumen VFA Proportions in Sheep. (Adapted from Terry and Tilley, 1963.)

Sample	Level of Feeding	PROPORTIONS OF VFAS (%)		
		Acetic	Propionic	Butyric
HAY 1	ad. lib.	72	18	10
	$\frac{2}{3}$ ad. lib.	71	19	10
HAY 3	ad. lib.	59	30	11
	$\frac{2}{3}$ ad. lib.	63	25	12

In addition, some degree of selection might have been expected with the animals fed to appetite, compared with the restricted animals, which could affect rumen fermentation.

In their recent experiment (Page 5), Bath and Rook (1963) obtained only minor changes in the proportions of the rumen VFAs with an increased intake of hay alone. Increasing the mixed hay and concentrate diet from 10 to 30 lb resulted in a progressive decrease in the percentage of acetic acid from an average value of 68.5 per cent with a corresponding increase in the

percentage of butyric acid. Little change occurred in the proportions of propionic and valeric acids. No errors of estimate were attached to these results to enable an assessment to be made of the real differences between treatments.

1.3 Variations With Time After Feeding

(1) pH and VFA concentration

Results obtained by many workers e.g. Phillipson (1942); Gray and Pilgrim (1951); Annison (1954); Briggs et al.(1957) and Balch and Rowland (1957), showed that rumen VFA concentration reached a peak from two to six hours after feeding and dropped to the lowest level prior to the next feed. The rate at which peak values were attained and their magnitude were discussed by Balch and Rowland (1957), who reported that the changes were dependent on the nature of the diet, with least variation occurring with cows receiving a roughage diet high in fibre and greatest variation occurring when concentrates were fed. Davey et al.(1962) obtained lowest rumen pH values and peak VFA concentrations between four to six hours after cows were put to pasture.

(2) Proportions of the VFA s

The results of Reid et al.(1957); Gray and Pilgrim (1951), and El-Shazly (1952), showed that as the concentration of the total VFAs increased after feeding, there was a decrease in the ratio of acetic to propionic acid. The ratio of acetic to propionic acid was highest before feeding.

The changes in the proportions of the individual VFA s of the rumen liquor with time after feeding were observed by Gray and Pilgrim (loc.cit.) using two sheep. The extent of these changes in one of their experiments is shown in Table 4. The proportion of acetic acid appeared to vary less and butyric acid more, with lucerne hay compared with wheaten hay. A further experiment confirmed these differences but no explanation was offered.

TABLE 4. VFA Proportions in the Rumen Liquor from Sheep Obtained Prior to and Four Hours After Feeding. (Adapted from Gray and Pilgrim 1951).

FEED	PROPORTIONS OF VFA S (%)		
	Acetic	Propionic	Butyric
Wheaten Hay	61.3-69.4	16.7-24.4	13.1-14.6
Lucerne Hay	68.1-71.8	14.8-20.2	9.8-15.6

The recent work of Bath and Rook (1963), where sheep were fed twice daily, showed that the fall in pH and rise in VFA concentration after feeding were associated with a decrease in the proportion of acetic acid and a complementary increase in the proportions of the other acids. They observed the extreme range in the proportions of acetic acid for two feeds given at 12 hour intervals to be 66.9 to 73.0 per cent and from 60.9 to 67.4 per cent.

No marked duirnal trends in the proportions of VFAs were noted by Bryant (1961) with one cow grazing pasture unrestrictedly, although the small differences noted were significant at the 10 per cent level. However, Bryant (loc.cit.) obtained highly significant differences ($P < 0.01$) in VFA proportions throughout the day, where the cow was restricted to a morning period of grazing. Balch and Rowland (1957) observed no consistent variation in the proportions of the VFA s with time after feeding. The cows used were fed varying proportions of hay and concentrates twice daily. Shaw (1961) attached considerable importance to this matter of changes in proportions of the acids with time after feeding. He studied the effect of different feed

combinations on rumen fermentation and found considerable uniformity in the proportions of the VFA s with time after feeding. Feedstuffs used ranged from lucerne hay, giving proportions of acetic acid ranging from 72.4 to 69.3 per cent over the day, to steamed maize with a range for acetic acid of 54.3 to 53.7 per cent. Total VFA concentration ranged widely over the various combinations of hay and concentrate fed. A similar uniformity in VFA proportions with time after feeding was obtained by Gray et al.(1960), with sheep fed wheaten hay. Ensor (1959), cited by Shaw (1961), suggested that the extent to which the rumen contents were a homogenous mixture was the "operating factor". Rations which gave a more homogenous mixture should maintain a more uniform microbial population and a greater uniformity of dissimilation.

The fact that contrasting results have been obtained on the extent of the changes in VFA proportions with time after feeding, appears to make serial sampling an essential routine technique in studies involving rumen fermentation.

1.4 Sampling Rumen Contents

Variations in rumen pH and VFA concentration arising through the method of sampling have been well reviewed by Bryant (1961). In general a dorso-ventral gradient occurred in the rumen ingesta, with the dorsal region being higher in VFA concentration and lower in pH than the ventral region. Bryant (loc.cit.) noted no evidence of the occurrence of anterior-posterior gradients in the rumen, for VFA concentration. In reviewing the results of other workers, he noted that a number of factors affected this gradient or stratification. The type of ration affected the degree of stratification with a tendency for less fibrous fractions to gravitate to ventral regions. Homogeneity of the contents increased with time after feeding and maximum heterogeneity was associated with the period of most rapid fermentation.

PHYSICAL CHANGES IN THE RUMEN

To interpret changes that occur in the concentration of the fermentation end-products in the rumen, account must be taken of the rates of absorption of the VFA s and volume changes in the rumen.

1.5 Absorption of VFAsFrom the Rumen

Absorption of VFA s from the rumen of sheep was first demonstrated by Phillipson and McAnally (1942). Since then considerable work has been carried out to determine rates of absorption. Various methods were adopted in these studies e.g. incubation *in vitro*, continuous rumen infusion of VFA s, arterio-venous differences in VFA s, isotope dilution, perfusion of the isolated rumen and a consideration of changes that occurred in the proportions of the VFA s after feeding. The conflicting results obtained probably reflect the various experimental techniques used.

Barcroft, McAnally and Phillipson (1944) analysed the blood draining from the rumen and claimed that the rate of absorption decreased as the molecular size of the VFA s increased. The pH in the rumen was not controlled. Danielli, Hitchcock, Marshall and Phillipson (1945) infused VFA s into the empty rumen at a pH of 5.8 and showed that the order of absorption was butyric > propionic > acetic, the reverse of the results obtained by Barcroft *et al.* (*loc.cit.*) and the indications were that pH may control the relative rates of absorption of the acids.

The changes in the proportions of the VFA s after feeding or incubation *in vitro* were used by Gray and Pilgrim (1951) to explain differences in absorption rates. Values for propionic acid were considerably higher *in vitro* than *in vivo* and supported the view that propionic acid was absorbed more rapidly than the other acids. Reid, Hogan and Briggs (1957) also stated

that the change in the proportions of the VFA s after feeding were partly explained by different rates of absorption between the acids. If this is so, they stated, the proportions of the acids in the rumen represent a complex equilibrium between their individual rates of production and their rates of absorption.

The production and absorption of VFA s in two isolated goat rumens were studied by McCarthy, Shaw, McCarthy, Lakshmanan and Holter (1958). The goats received a diet of hay and concentrates prior to isolation and perfusion of the rumens. The blood perfusions lasted for 80 min in one and 115 min in the other rumen and over these perfusion times, the concentration of the VFA s in the rumens increased by 24 per cent and 66 per cent respectively. The proportions of acetic, propionic and butyric acid in the rumens varied only slightly during the perfusions, despite the large change in VFA concentration.

Increases in the amounts of the individual acids in the blood were a reflection of their increases in the rumen. It was concluded that the relative rates of production of the VFA s were acetic > propionic > butyric and that the absorption of the acids appeared to be a reflection of their relative rates of production. VFA s accumulated in the blood perfusate. The possible effects of this accumulation on absorption were not commented upon, although the authors stated that any conclusions must be tempered by the artificial conditions of the experiment.

Shaw (1961) from his own results, which showed that little variation occurred in the proportions of the rumen VFA s with time after feeding and from the above results of McCarthy et al. (1958), suggested that there was a close agreement between the proportions of the acids produced, the proportions present in the rumen and the proportions absorbed. He also stated that the main forces causing absorption of the VFA s from the rumen were their respective concentration gradients. Blaxter (1962) also stated that, provided the rate of production of the VFA s was fairly constant over 24 hours, then measurements of

the proportions of the rumen VFA s would be a good index of the proportions both produced and absorbed. This statement appeared to be based on the limited evidence of Shaw (1961) and is in contrast to the statement by Reid et al. (1957) mentioned above.

Volatile fatty acids are absorbed more slowly at a high pH and Danielli et al. (1945) suggested that this was due to the rumen epithelium being more permeable to the ionized fatty acid radical. Dobson (1961) concluded that the rumen epithelium was relatively permeable to H+ and OH- , moderately permeable to HCO_3^- and readily permeable to CO_2 . At neutral pH, the fraction of VFA s neutralised directly was about half that which was absorbed. At low pH s the proportion of free acid absorbed was greater and the rate of absorption increased.

1.6 Volume Changes in the Rumen

In addition to the production and absorption of end-products; outflow of fluid from the rumen, water intake and salivary secretion need to be considered when interpreting changes that occur in the concentration of rumen end-products.

(1) Outflow from the Rumen

Limited information is available relating intake to the outflow of fluid from the rumen. The influence of fasting on flow rate and fluid volume was studied by Hydén (1961) using polyethyleneglycol as a reference marker in two experiments with sheep. The fluid volume of the rumen was not influenced significantly, but flow rate out of the rumen decreased continuously. A basal flow rate from the rumen of about two per cent per hour of the fluid volume of the rumen was reached after two to three days fasting. With sheep feeding, the flow rate was about six per cent of the fluid volume per hour.

In cattle (Balch, 1950 and Mäkelä, 1956), the most consistent influence on the rate of passage of feed was the level of feeding. This was also demonstrated in sheep by Blaxter et al. (1961). Other factors such as the particle size and specific gravity of the feed and the rate of digestion also affect rates of passage of feed and outflow from the rumen. (Balch, 1959).

The latter worker also found that passage to the omasum was most rapid during eating.

(2) Water intake

Phillipson (1942) withheld water from sheep and noted a steeper VFA concentration curve than usual, with a higher peak VFA concentration and drier rumen contents, compared with animals receiving water. When the animals drank, VFA concentration dropped but pH remained the same. Balch, Balch, Johnson and Turner (1953) restricted the water intake of a fistulated cow to 70 per cent of her normal intake. Feed intake dropped and after one week of adjustment, the weight of rumen contents fell to about the same extent as the water intake and the ratio of water to d.m. in the rumen was more or less maintained. Phillips (1961) also noted a fall in d.m. intake with a 50 per cent restriction in water intake.

(3) Salivary secretion

The volume of saliva secreted depends to a large extent on the quantity and nature of the food eaten. Estimates of the total amount secreted by cattle range from 98 - 190 l daily (Bailey,1961). This worker demonstrated that the amount of saliva added to the food also depended on the length of the consumption period rather than to the water content of the food. Wilson (1963) found that the amount of parotid saliva secreted when chaffed hay was fed at three levels of intake to sheep, was linearly related to the d.m. intake.

1.7 Rumen Fluid Volume, Amounts of Ingesta and Dry Matter Per Cent of the Ingesta

In their study of the effects of intake on rumen fermentation end-products of sheep, Williams and Christian (1956) stated that if a decreased level of intake of feed resulted in a reduction in the feed per unit volume of the rumen contents, then a fall in the concentration of the end-products would be expected. However feed per unit volume, as measured by the d.m. percentage of the

rumen ingesta of the sheep determined after slaughter in their experiment, was widely variable and did not appear to be related to intake. They were thus unable to explain their results in terms of a dilution effect.

Hale, Duncan and Huffman (1940) observed that the d.m. per cent, the weight of d.m. and the total weight of the rumen digesta were more or less constant when the rumen was emptied 14 hours after feeding, despite differences in intake of 10, 15 and 30 lb hay per day. One fistulated cow was used and the observations were restricted to only two determinations for each level of intake. With intakes ranging from three to thirteen Kg hay per day, Mäkelä (1956) observed that the total amount of rumen digesta varied to only a small extent. At high intakes, the time of retention of food was short and a positive relationship was established between d.m.intake and the d.m. percentage of the rumen contents. With a reduction in intake, the constant total weight of the ingesta was maintained by an increase in the proportion of water. Mäkelä (loc. cit.) also stated that the amount of d.m. in the rumen did not increase at the same rate as d.m. intake.

Extensive information of the effects of three levels of intake on the rumen contents of two fistulated steers was provided by Burroughs, Gerlaugh, Silver and Schalk (1946). The rumen was emptied every four hours over a 24-hour period. The ration was fed twice daily and consisted of lucerne hay, maize, with and without cobs and a protein supplement. Accelerated losses of d.m. from the rumen occurred during periods of eating. A close positive relationship was seen between d.m.intake and the total d.m. in the rumen. The amount of d.m. in the rumen preceeding feeding was approximately the same in amount as that consumed in one feeding period. The d.m. percentage of the rumen contents, calculated from the data of Burroughs et al. (loc. cit.) from the total water and weight of d.m. in the rumen, increased with a rise in d.m. intake. Values were 9.7, 11.7 and 13.8 per cent for intakes of 20, 39 and 48 Kg.

of feed respectively. These values applied to the period immediately before the morning feed, or 15.5 hr after the evening feed of the preceeding day. The mean d.m. per cent of the rumen contents for the 24-hour period were 10.3, 14.9 and 15.2 for the low, medium and high intakes respectively.

The effects of eating on the rumen ingesta of three cows and two steers were studied by Balch (1958). He found that the mean loss of d.m. from the rumen by absorption and passage to the omasum was 0.6 to 0.7 lb per 100 min between meals and rose to a loss of 1.4 to 2.1 lb per 100 min during eating. A positive relationship between the d.m.intake and the rate of loss of d.m. from the rumen between meals was also obtained. As the intake increased from 12 to 22 lb, the rate of loss of d.m. increased from 0.4 to 1.2 lb d.m./100 min.

1.8 Summary - Review of Literature

(1) The pH of the rumen liquor varies inversely with the VFA concentration. However limited results, in each case the observations from one experiment, showed that the relationship may be affected by the drinking of water, high rumen ammonia levels, time after feeding or the roughage content of the diet. In one experiment, the method of sampling by stomach tube may have affected the results obtained.

(2) In experiments with sheep and cows, a positive relationship between the level of intake and rumen VFA concentration was obtained. In one experiment, the extent of the change in VFA concentration was stated to be dependent on the nature of the diet, although no data were given. In three experiments with sheep and cows, a negative relationship between the level of intake and rumen pH was obtained. In one experiment with sheep no change in rumen pH occurred with a change in the level of intake. The method of sampling by stomach tube probably contributed to this negative result.

(3) Starvation in sheep and cows, in three experiments, was associated with high rumen pH values and low VFA concentrations. Recovery of pH values and VFA concentration to pre-starvation levels was rapid when the animals were fed.

(4) An increase in the level of intake of hay, fed to sheep and cows, was shown to have no influence on rumen VFA proportions in two experiments. In another experiment, a change in the level of intake of a hay producing low proportions of rumen acetic acid was associated with small changes in the proportions of acetic and propionic acid in the rumen liquor of sheep. One experiment with cows showed that increased intakes of a hay and concentrate diet were associated with decreased acetic and increased butyric acid proportions in the rumen liquor. The changes reported in all cases were comparatively small.

Moreover in most experiments no estimates of error were given, by which some assessment of the differences could have been made.

In an experiment with sheep, increased intakes of dried grass resulted in a decrease in the proportion of rumen acetic acid and an increase in propionic acid. The changes were small.

(5) Many workers have shown that the rumen VFA concentration reaches a peak from four to six hours after feeding. Some evidence showed that the greatest variation in VFA concentration occurred with concentrate diets, rather than with roughage diets high in fibre.

(6) Evidence on the extent of the changes in the proportions of the rumen VFAs with time after feeding was conflicting. A number of workers have shown that the acetic acid proportion decreased and the propionic acid proportion increased with time after feeding. Generally these differences were greatest at peak fermentation. The variations can be quite extensive with the acetic acid proportion varying by as much as eight percentage units. However a number of workers have shown that variations in the proportions of the VFAs in the rumen with time after feeding were either inconsistent or were small. The extent of these changes in the relative proportions of the VFAs have an important bearing on the times at which the rumen is sampled in relation to feeding.

(7) The techniques of sampling from the rumen can influence the results obtained for pH and VFA concentration.

(8) Absorption of the VFAs from the rumen have been amply demonstrated. However results obtained on the relative rates of absorption of the acids varied greatly between workers. This was probably a reflection of the various experimental techniques used. The pH of the rumen liquor appeared to be an important factor governing rates of absorption. The conflicting evidence on

the extent of the changes in the VFA proportions with time after feeding and on the rates of absorption has given rise to two schools of thought on the significance of the mixture of VFAsin the rumen. One school states that the proportions of the VFA s in the rumen consist of a complex equilibrium between their individual rates of production and absorption, whereas the other group states that there is a close agreement between the proportions of the VFA s produced, the proportions present in the rumen and the proportions absorbed.

(9) The passage of fluid from the rumen to the omasum appeared to vary with the level of intake. Outflow was greatest during eating.

(10) Restriction of the water intake appeared to cause little change in the ratio of water to d.m. in the rumen, provided a period of adjustment was allowed.

(11) The volume of saliva secreted by the ruminant appeared to be largely dependent on the quantity and nature of the food eaten and the length of the consumption period.

(12) The most reliable information available showed that a close relationship occurred between d.m.intake and the total d.m. in the rumen and the d.m. per cent of the rumen ingesta. The total fluid volume of the rumen ingesta was dependent to a small extent on the level of intake. Results different from the abovementioned were obtained in one experiment, but the data were meagre.

(13) A knowledge of changes in the amount of ingesta in the rumen due to outflow, absorption, salivation and intake of feed and water was thought to aid the interpretation of changes in pH, VFA concentration and proportions of the individual acids.

CHAPTER 2

MATERIALS AND METHODS

2.1 Animals

Three lactating Jersey cows, each with a large rumen fistula, were used and a general description of them is given in Table 5. The live-weights given are the means of 10 weekly weighings for each animal, taken during the indoor feeding experiment.

TABLE 5. Characteristics of the Animals Used

Cow Number	Age (Yrs)	Mean live-weight (lb)	Calving (Date)	Production in 1961/62	
				Butterfat(lb)	Days in Milk
45	3	658	29/7/62	329	299
69	4	784	2/9/62	288	246
98	4	663	18/7/62	320	301

Cows 98 and 69 were both fistulated more than twelve months prior to the commencement of the experiment, but Cow 45 was fistulated six weeks before the experiment began. Healing was complete and no tenderness of the operation site was noted in this cow by the time the experiment commenced.

2.2 General Outline of Experiment

The experiment consisted of periods of indoor feeding of pasture at three levels of intake to the three fistulated dairy cows, each period comprising a 3 x 3 latin square. In addition, the cows were grazed on pasture from time to time. Rumen sampling was carried out to obtain data on the pH and the concentration and proportions of the VFA's in the rumen liquor under both sets of

conditions. The general outline of the experiment is given as follows:-

(a) 14 to 18 October, 1962 - Preliminary indoor feeding.

(b) 18 to 25 October - Cows grazing pasture similar to that fed indoors. (Part II).

(c) 26 October to 8 November - Preliminary indoor feeding.

(d) 9 to 21 November - Experimental indoor feeding.(Latin Square I).

(e) 21 to 28 November - Cows grazing pasture similar to that fed indoors.(Part II).

(f) 29 November to 2 December - Ad libitum feeding indoors.

(g) 3 to 17 December - Experimental indoor feeding. (Latin Square II).

(h) 18 December to 1 January,1963 - Cows grazing pasture, no treatment or sampling from rumen.

(i) 2 to 6 January - Ad libitum indoor feeding.

(j) 7 to 21 January - Experimental-indoor feeding (Latin Square III).

(k) (a) 23 to 29 September, 1963. } Grazing two cows on pasture (Part II).
(b) 29 September to 5 October. }

2.3 Experimental Design

(1) Preliminary Period

The main objects of the preliminary period were to accustom the animals to indoor feeding and to establish a satisfactory feeding routine. Success in achieving these objects was guaged by the behaviour of the animals and from their intakes of d.m. with ad libitum feeding.

In addition, a short period of feeding at the levels of intake intended for the main experiment (see below), together with pH determinations of the rumen liquor were carried out in order to arrive at a satisfactory routine. Because of the inverse relationship between rumen pH and VFA concentration, a measure of pH alone provides an approximation of the degree of fermentation in the rumen and its variation over the day although the fact that the relationship can be affected (Section 1.1) must be kept in mind. A short period of feeding,

at the levels of intake intended for the main experiment (see below) together with pH determinations of the rumen liquor, was carried out.

Various alternative feeding régimes were tried as follows, the pasture being fed at three levels of intake:-

(a) 30/10/62 - Equal amounts of pasture fed twice daily at 9 a.m. and 3 p.m.

(b) 31/10/62 - Proportionate amounts, according to the level of intake, fed every hour from 8.30 a.m. to 7 p.m.

(c) 1/11/62 - Equal amounts fed twice daily at 8.30 a.m. and 4.30 p.m. with a 'topping up' at 12.30 p.m.

Times of sampling are shown in Figures 1(a), 1(b) and 1(c). The results and discussion on which the design of the main indoor experiment was based are given in Section 4.2 (2) and 3·3(2).

(2) Experimental Period

Pasture was fed at three levels, the treatments being as follows:-

(a) Pasture fed to just below appetite, so that refusals were small.

(b) Pasture fed at approximately 80 per cent of A

(c) Pasture fed at approximately 60 per cent of A

The levels for treatments B and C were calculated from A on a green weight basis. The cows and treatments were arranged as 3 x 3 latin squares, replicated three times, to give sufficient error degrees of freedom for significance testing and to assess the effects of changes in pasture over the season, on rumen fermentation. The calendar date for each latin square is given in Section 2.2. Randomisation of the treatments by rows and columns was carried out, using the tables of Fisher and Yates (1957) and cows were allocated at random to columns.

The layout of the three squares was as follows:-

I			II			III		
98	69	45	98	69	45	69	98	45
B	A	C	B	C	A	A	C	B
C	B	A	A	B	C	B	A	C
A	C	B	C	A	B	C	B	A

From the results of Davey and Wilson (1962) and Coop (1949), (Page 7 & 8) it was considered that a period of four days on each treatment with sampling from the rumen on the fourth day, would avoid carryover effects. Where the rumen was emptied on the morning of the fifth day (section 2.6), ad libitum feeding was carried out for one day before changing to the new treatment.

The experiment was not designed to investigate variations in milk production and composition due to treatments, as the periods within squares were too short to allow carryover effects of milk production to disappear. However, the yield and the butterfat and solids-not-fat contents of the milk of the three cows were recorded as described in Part III .

2.4 Feeds and Feeding

At 9 a.m. each day sufficient pasture was cut by mowing to supply the following p.m. and a.m. feeds. The pasture was obtained from a single area kept free from grazing stock to avoid contamination from dung. From time to time the area was topped and efforts were made to keep the material as uniform as possible for any one treatment period of four days. The method of collection used and the area available meant that it was not always possible to use pasture at an optimum grazing height of 4 to 8 in. and at times the pasture used was stalky and up to 12 in. in height. The cut pasture, not required immediately

for feeding, was packed into bins and covered with polythene sheeting to prevent wilting over the day and in an endeavour to keep the d.m. content as even as practicable throughout the mass.

Feeding was carried out at approximately 8 a.m. and 5 p.m. each day. The feed was given in approximately equal amounts night and morning with the exception that some adjustments were made from time to time in one or other of the feeds, according to the amount of refusals remaining from the previous feed. Each 24-hour feeding period commenced at a p.m. feeding and terminated just prior to the a.m. feed on the following day. Samples for d.m.determination were taken from the pasture fed to each cow and from both the a.m. and p.m. refusals if they exceeded four lb green weight. The d.m. percentage of the feed was determined by drying sub-samples of approximately 200 g in a forced draught oven for 24 hours at a temperature of 70°C.

The dried a.m. and p.m. sub-samples of the feed for each cow were composited for each of the rumen sampling days. The bulked samples for each day were ground in a "Wiley" mill and stored in bottles at -10°C until required for chemical analysis (See Part II).

2.5 General Management

Water was available to the animals at all times, with the exception of each rumen sampling day, when water was offered for half an hour at 11 a.m., 2.30 p.m. and 8 p.m. and throughout the night. Grooming was carried out regularly and a close watch and record kept on the general condition and health of each animal. The animals were weighed each Tuesday prior to feeding. Records were kept of the green weight of pasture offered and refused and the green weight and d.m. intake of each cow for each 24-hour period.

2.6 Sampling of the Rumen Contents

Large samples of rumen liquor (Johns, 1955b) were obtained from the

middle of the ingesta (Bryant, 1961) on the fourth day of each treatment (review of literature Page12and discussion Section 4.1 (1)). The material obtained was strained through several layers of cheese cloth and approximately 150-200 ml of rumen liquor obtained. The rumen liquor was then bottled, tightly corked and held at 0°C in melting ice until required for VFA determinations.

It was decided to obtain rumen samples every three hours from 8 a.m. to 11 p.m. on the sampling day. To gain additional information on the efficacy of sampling from the middle of the rumen, a number of observations were made on the pH and VFA concentrations obtained from material sampled from the middle of the rumen and that sampled from the contents after they had been removed and thoroughly mixed. At approximately 9 a.m., following the fourth treatment day and prior to the a.m. feed, the rumen contents of each cow were removed. This was carried out throughout the trial with the exception of the first row in square one and for square three. The rumen contents were weighed, the volume measured and samples taken for d.m. determinations after thorough mixing of the ingesta. Approximately 500 g of the wet material was taken and after freezing at -10°C was dried in a freeze-drier for 32 hours. After removal from the freeze-drier the material was weighed immediately.

2.7 Chemical Methods

(1) pH readings

The pH of the rumen liquor was determined in duplicate using a glass and a KCl electrode mounted at a distance from a "Radiometer" pH meter. This enabled the electrodes to be placed in the large beaker of rumen liquor immediately it was collected giving a delay between sampling and reading of less than one min. Repeat determinations were made three to five min. later as a check on the loss of CO_2 from the sample (Review of literature Page7).

(2) VFA Concentration

All rumen samples were stored at 0°C until required for analysis, which was completed on the day following sampling. The concentration of the VFA s was determined by steam distillation in a Markham (1942) still. A five ml aliquot of rumen liquor was placed in the still with one ml of 10 N-H_2SO_4, saturated with $MgSO_4$ (McAnally 1944). One 60 ml portion of distillate was collected and titrated with approximately 0.05 N-NaOH. Prior to titration, carbon dioxide-free air was passed through the distillate for at least three min and this was continued during titration. In addition carbon dioxide-free water was used for washing. A second distillate was collected at least three times for each lot of 20 determinations and used as a blank correction for organic acids which are slightly steam volatile. The NaOH was standardised against potassium hydrogen phthallate. Phenolphthalein was used as the indicator of the titration end point. Determinations were repeated where the difference between duplicates were greater than one per cent. The duplicates were designated A and B, to correspond with the two stills used. To determine the recovery of the VFA s, known amounts of a standard solution containing 6.005 mM per 100 ml of acetic, propionic and butyric acids in the proportions 7 : 2 : 1 were added to rumen liquor and the total VFA s determined.

(3) Separation of the individual VFA s by gas-liquid chromotography

Following titration, approximately four ml of the NaOH used in the above-mentioned titration were added to each of the two distillates. These were mixed and approximately 80 ml evaporated to dryness in an oven at 90°C. The soaps were transferred to small test tubes and stored at -10°C.

For determinations 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). The columns were run at

137°C with a nitrogen flow rate of approximately 15 ml per min.

The soaps were transferred to the chromatograph column by dissolving them in one ml of water and taking them up in a hypodermic syringe. Approximately 0.03 ml of this aqueous solution was added to a platinum boat containing equal parts of celite and $NaHSO_4$ as a dry mixture. The boat was quickly pushed into the column and the nitrogen supply connected.

The reliability of the separations was checked by using a standard containing acetic, propionic and butyric acids in the molar proportions of 6 : 2 : 1. This solution was neutralised and the resulting soaps were evaporated to dryness. The standard soaps were transferred to the column as already described. Separations produced by the standard soaps were used as criteria in comparing the results obtained from the rumen VFA samples. Any graphs showing evidence of drift or poor separation were discarded and a repeat determination carried out. Finally as a further check on the method, 10 samples were selected at random and repeat determinations carried out to obtain a measure of the errors involved.

2.8 Statistical Analysis

(1) VFA concentration in the latin squares

Analysis of variance for the latin square as outlined by Snedecor (1961) and by Wilm (1945) for replications in time, was used to test differences between treatments, cows, days and times of sampling for rumen VFA concentration obtained in the three 3 x 3 latin squares. The classification of the experimental observations together with the coefficients of the variance components are given in Table 6.

The VFA concentration of the rumen liquor was calculated, using the missing plot technique of Snedecor (1961), for cow 98 at the 11 p.m. sampling time on 17 November, 1962.

TABLE 6. Coefficients of the variance components in the expectations of the mean squares. (Rumen VFA concentration in three 3 x 3 latin squares).

	Duplicates	CP:STTi	TiT	Ti	CP:S	TS	T	P:S	C:S	S
S	1	2			12			36	36	108
C : S	1	2			12				36	
P : S	1	2			12			36		
T	1	2	18			36	108			
TS	1	2	18			36				
CP : S	1	2			12					
Ti	1	2	18	54						
TiT	1	2	18							
CP : STTi	1	2								
Duplicates	1									

Note: S = Squares
C = Cows
T = Treatments
Ti = Times of sampling (Times).
C:S = Cows within squares
P:S = Days within squares
TS = Treatment x squares interaction (Cows x Days)
TiT = Times of sampling x Treatment interaction (Times x Treatment)

Cows and days were assumed to be random variables, with treatments fixed.

Testing was carried out as follows:-

(a) Treatments - The interaction TS did not contribute a significant amount to the treatment mean square and thus was used to test for differences between treatment means.

(b) Differences between cows within squares and days within squares were tested by using the CP : S mean squares.

(c) Differences between times of sampling were tested by using the TiT interaction.

(d) Squares - Differences between squares were tested by using the P : S mean square. The test was an approximate one and depended on the non-significance of C : S.

(e) The TS interaction was tested against the TiT mean squares and the TiT interaction was tested against the mean square for CP : ST Ti.

(2) Rumen pH values obtained in the latin squares

Rumen pH values were analysed in a similar manner to those described for VFA concentration, except that the duplicates were not included for pH, because of the time lapse in their determination (See Section 2.7 (1)).

(3) Dry matter intakes for both the preliminary and experimental periods were analysed by analysis of variance. Rates of eating, the total weight of the rumen ingesta, the d.m. per cent of the rumen ingesta, the total water and the total amounts of VFA s in the rumen were also analysed by analysis of variance (Snedecor,1961). Expectations of the mean squares were not determined for these analyses.

(4) Proportions of the individual VFA s

The samples intended for chromatographic analysis for the third day in square two were inadvertently lost. Analysis of the two complete latin squares remaining for acetic, propionic and butyric acids showed that no significant

differences occurred between cows, in the proportions of the acids produced (Appendix 9). To gain complete use of the data, the proportions of the VFA s were therefore analysed as a randomised block design, ignoring cows (Glenday, A.C. pers. comm.). Each acid was analysed separately and arcsin transformation of the percentages was considered unnecessary as the percentages within each analysis did not vary greatly (Glenday, A.C. pers. comm.). In testing for differences between blocks, treatments and times of sampling, blocks and times were assumed to be random variables, with treatments fixed.

The classification of the experimental observations together with the coefficients of the variance components are given in Table 7.

TABLE 7. Coefficients of the variance components in the expectations of the mean squares. (VFA proportions in the randomised blocks).

	BTTi	BTi	TTi	Ti	TB	B	T
T	1				6		48
B	1	3				18	
TB	1				6		
Ti	1	3		24			
TTi	1		8				
BTi	1	3					

Note: B = Blocks
T = Treatments
Ti = Times of sampling
TTi = Treatments x times of sampling interaction
Bti = Blocks x times of sampling interaction

Cows and days were assumed to be random variables, with treatments fixed.

Testing was carried out as follows:-

(a) Differences between treatments and between blocks were tested by using the TB mean square and the BTi mean square respectively.

(b) The TTi and BTi interactions were tested by using the BT Ti mean square.

(5) Rumen contents and total quantities of the VFA s

As information on rumen contents and total quantities of the VFAs was collected for periods two and three in square one and for all of square two only, the data were analysed as a randomised block design, ignoring cow differences.

(6) Regression analyses

The relationships between rumen pH and VFA concentration, and between the d.m.intake and both rumen pH and VFA concentration were measured by analyses of regression (Snedecor,1961). As the regressions based on the pooled data were confounded by cows, days, squares and treatments, these effects were removed and a measure of the error regression obtained. Where variations in the relationships were suspected, regression analyses were made for separate treatments and times of sampling.

The relationship between the total amount of d.m. in the rumen and the d.m.intake was also measured by an analysis of regression, for the pooled data only.

(7) Significance of differences

Duncan's (1955) multiple range test was used to obtain information as to which differences between the means were significant.

TABLE 8. VFA Concentration of Rumen Liquor Sampled from the Middle of the Ingesta in the Rumen and from the Removed and Mixed Contents.

Sets	VFA CONCENTRATION (mM/100 ml. RUMEN LIQUOR)		
	Middle of rumen	Removed and mixed ingesta	Difference
1	7.75	7.70	0.05
2	6.39	6.55	- 0.16
3	8.84	8.96	- 0.12
4	7.84	7.66	0.18
5	6.29	6.09	0.20
6	7.66	7.26	0.40
7	5.86	5.49	0.37
8	6.76	6.91	- 0.15
9	9.52	8.75	0.77
10	6.52	5.40	1.12
11	9.02	9.40	- 0.38
12	6.88	6.84	0.04
Mean	7.44	7.25	0.33
S.E. of differences			± 0.12

TABLE 9. Recovery of the VFAs by Steam Distillation Using Rumen Liquor Plus a Standard Solution (6.005 mM/100 ml) of Acetic, Propionic and Butyric Acids in the Proportions of 7 : 2 : 1 (A and B duplicate determinations).

	Amount of standard solution added to 20 ml rumen liquor (ml).	VFA CONCENTRATION		Recovery (%)
		Determined (mM/100 ml)	Calculated (mM/100 ml)	
A.	0	15.53	-	-
	0.2	15.89	15.99	99.4
	0.4	16.54	16.42	100.7
	0.6	16.95	16.84	100.6
	0.8	17.31	17.26	100.3
	1.0	17.73	17.67	100.3
B.	0	15.50	-	-
	0.2	15.92	15.94	99.9
	0.4	16.69	16.37	101.9
	0.6	17.02	16.80	101.3
	0.8	17.33	17.21	100.7
	1.0	17.88	17.62	101.5
	Mean Recovery			100.7

CHAPTER 3

RESULTS

3.1 Techniques

(1) Sampling of the rumen contents

No general evaluation of the method of sampling to cover the full feeding cycle was made, but results are presented in Table 8 comparing *in vivo* sampling from the middle of the ingesta in the rumen with sampling from the removed and mixed rumen contents, prior to the morning feed. The results are from three cows fed pasture at three levels of intake.

The small difference between the means of the two methods of sampling was not significant. Between set differences were significant at the five per cent level (Appendix 1) and a between sample correlation of 0.94 was obtained.

(2) pH Readings

The means of 186 determinations taken over the whole of the indoor experiment were 6.66 $\pm$ S.E. 0.03, taken immediately on removal from the rumen and 6.68 $\pm$ S.E. 0.03 for a delay of up to five min after removal. There was no significant difference between the first and second readings.

(3) Recovery of the VFAs by steam distillation

The concentrations and the percentage recoveries of the VFAs for a sample of rumen liquor and from the sample with an added standard solution of VFAs are given in Table 9.

TABLE 10. Separation of the Individual VFA s by Gas-Liquid Chromatography.

(a) Results using a Standard Salt Solution of Acetate, Propionate and Butyrate (13 observations)

(b) Ten Random Duplicate Samples from the Indoor Experiment (A and B.).

	Acetic Acid	Propionic Acid	Butyric Acid
(a) Standard Solution (actual %)	70	20	10
Mean values obtained by gas-liquid chromatography	70.8	19.4	9.8
S.D.of the differences from the standard.	± 1.5	± 1.2	± 1.3
S.E.of the differences from the standard.	± 0.40	± 0.34	± 0.34
Range of values	69.1 - 73.3	17.5 - 21.3	8.4- 13.2
(b) Random Duplicates	A B	A B	A B
Mean VFA proportions	68.5 68.7	18.7 18.5	12.8 12.8
Means of differences between duplicates	1.4	0.95	1.8
S.D. of differences between duplicates	1.5	1.1	1.4
S.E. of differences between duplicates	0.46	0.33	0.44
Range of differences between duplicates	2.3 to -2.1	1.7 to -1.0	2.5 to -2.1
Correlation coefficient (A & B determinations)	** 0.92	** 0.88	** 0.76

Note. S.E. - Standard Error
S.D. - Standard deviation
** - $P < 0.01$.

In the indoor experiment, 162 determinations (324 duplicates) for VFA concentration were carried out. The mean VFA concentrations were 10.48 and 10.44 mM per 100 ml rumen liquor for duplicate A and B determinations respectively. The error between determinations was less than one per cent (Appendix 4).

(4) Separation of the individual VFA s by gas-liquid chromatography

The results using standard soap solutions of the VFA s are shown in Section (a) of Table 10 and those obtained with 10 random duplicate samples from the indoor experiment, are given in Section (b) of Table 10. There was no significant difference between the means of the random duplicates for each of the three VFA s.

3.2 Animals

(1) Health

The three animals became lame within three to four days of their introduction to the feeding barn. Lameness disappeared after the first week and no further foot troubles were experienced thereafter. Cow 98 scoured considerably over the preliminary period, but the condition had cleared up by the end of the first week in the experimental period, although the faeces were looser than those of the other two animals throughout the first latin square.

(2) Live-weights

The live-weights of the animals used in the indoor experiment are summarised in Table 11. The live-weights were obtained from 10 weekly weighings throughout the indoor experiment.

TABLE 11. Live-weights and Live-weight Changes of the Cows.

Cow Number	45	69	98
Means of first 3 weeks (lb)	637	780	671
Means of last 3 weeks (lb)	665	782	639
Difference (lb)	28	2	-32
Overall Means (lb)	658	784	663
S.E.'s of the overall means (lb)	± 10	± 8	± 7

Cow 69 was heavier ($P<0.01$) than either Cow 45 or 98. The results in Table 11, showing an apparent gain in weight for Cow 45 and a loss for Cow 98, cannot be regarded with confidence as weekly live-weights fluctuated widely. In addition there appeared to be no definite pattern with time in the distribution of weights exceeding or less than the overall means, even for Cow 98.

3.3 Preliminary Period

(1) Dry Matter Intake

On reintroduction to the barn (Section 2.2), the animals readily settled down and within two days were eating in excess of 20 lb of pasture d.m. per day. In Cow 98, oestrus was associated with a lowered intake, 5.4 lb of d.m. being recorded at the p.m. feed on 4 November, compared with a mean p.m. intake of 11.6 lb d.m. over the last week of the preliminary period. A missing plot value was calculated for this particular feed (Snedecor 1961). Ad libitum intakes of d.m. during the seven days prior to the experimental period are given in Table 12. Differences in intake between cows, days and

TABLE 12. Mean Dry Matter Intakes for the Final Week of *ad libitum* Feeding in the Preliminary Period.

Cow No.	a.m. (lb. d.m.)	p.m. (lb. d.m.)	24 hrs. (lb. d.m.)	S.E. of Mean (lb. d.m.)	V (%)
45	12.9	11.6	24.5		
69	13.4	11.9	25.3		
98	11.6	11.7	23.3		
Overall	12.6	11.8	24.4	± 0.16	10.2

TABLE 13. Mean Dry Matter Intake and pH of the Rumen Liquor from Cows Fed at Varying Levels of Intake and by Different Methods in the Preliminary Period.

DATE	Cow Number	45	69	98
30/10	Treatment	A	B	C
	Intake (lb. d.m.)	22.5	21.5	17.4
	Ratio of intakes	100	96	77
	pH	5.98	6.15	6.20
31/10	Treatment	C	B	A
	Intake (lb. d.m.)	13.0	18.7	21.0
	Ratio of intakes	62	89	100
	pH	6.56	6.65	6.52
1/11	Treatment	C	A	B
	Intake (lb. d.m.)	14.3	22.8	18.5
	Ratio of intakes	63	100	81
	pH	6.71	6.49	6.44

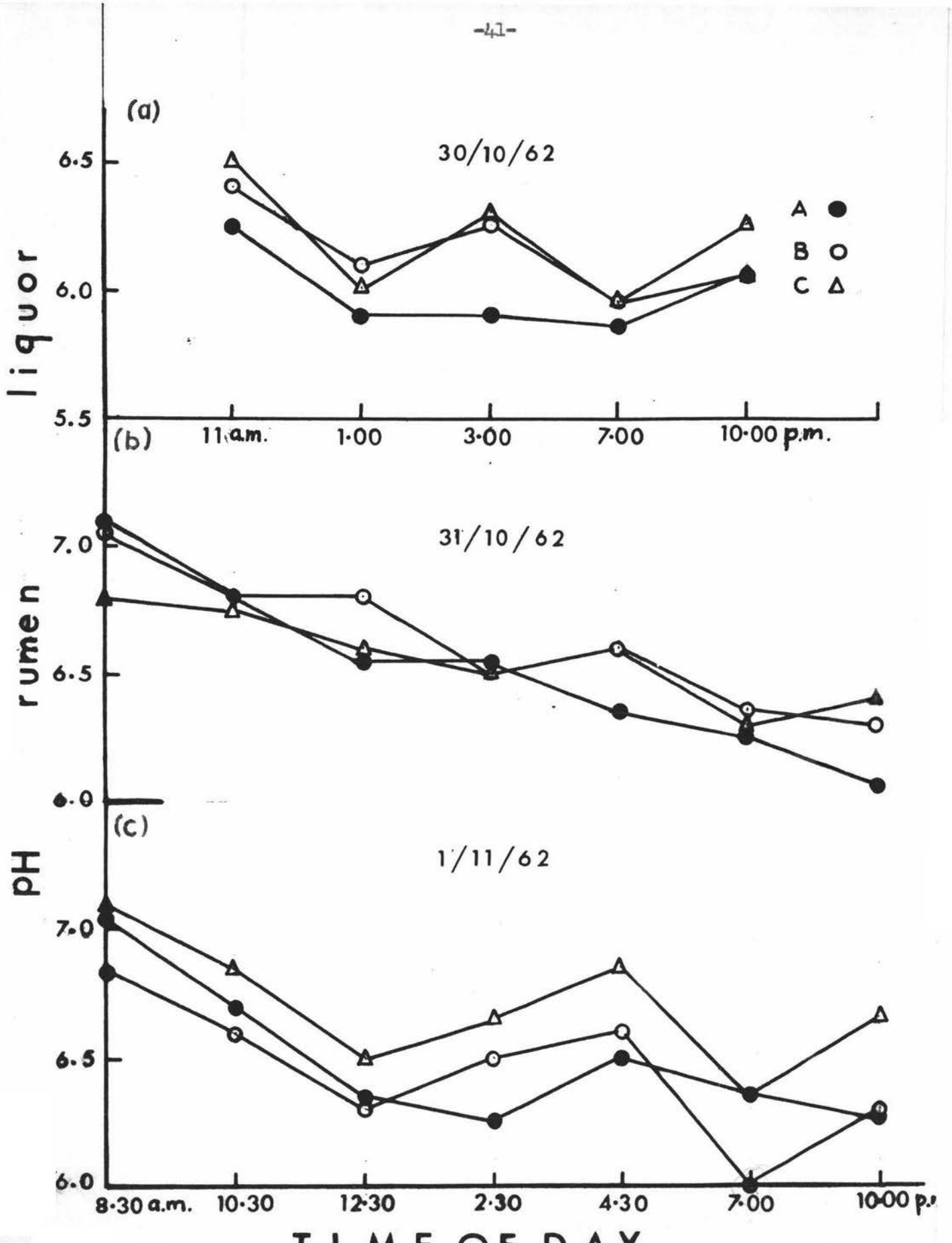


Fig. 1 (a) (b) (c) pH of the rumen liquor obtained during the preliminary period from three cows fed pasture at varying levels of intake.

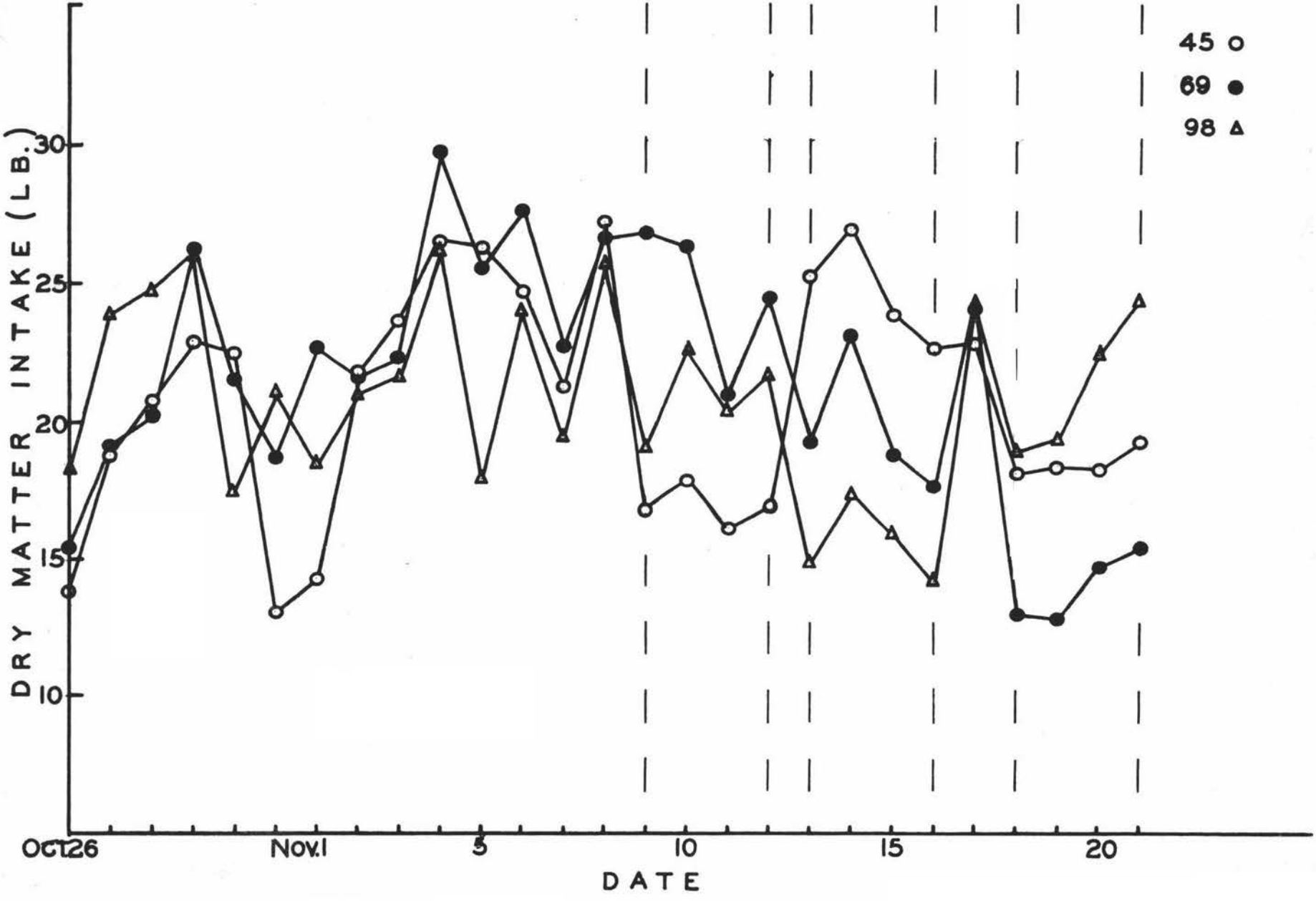


Fig.2: Dry matter intake of pasture for Square I.

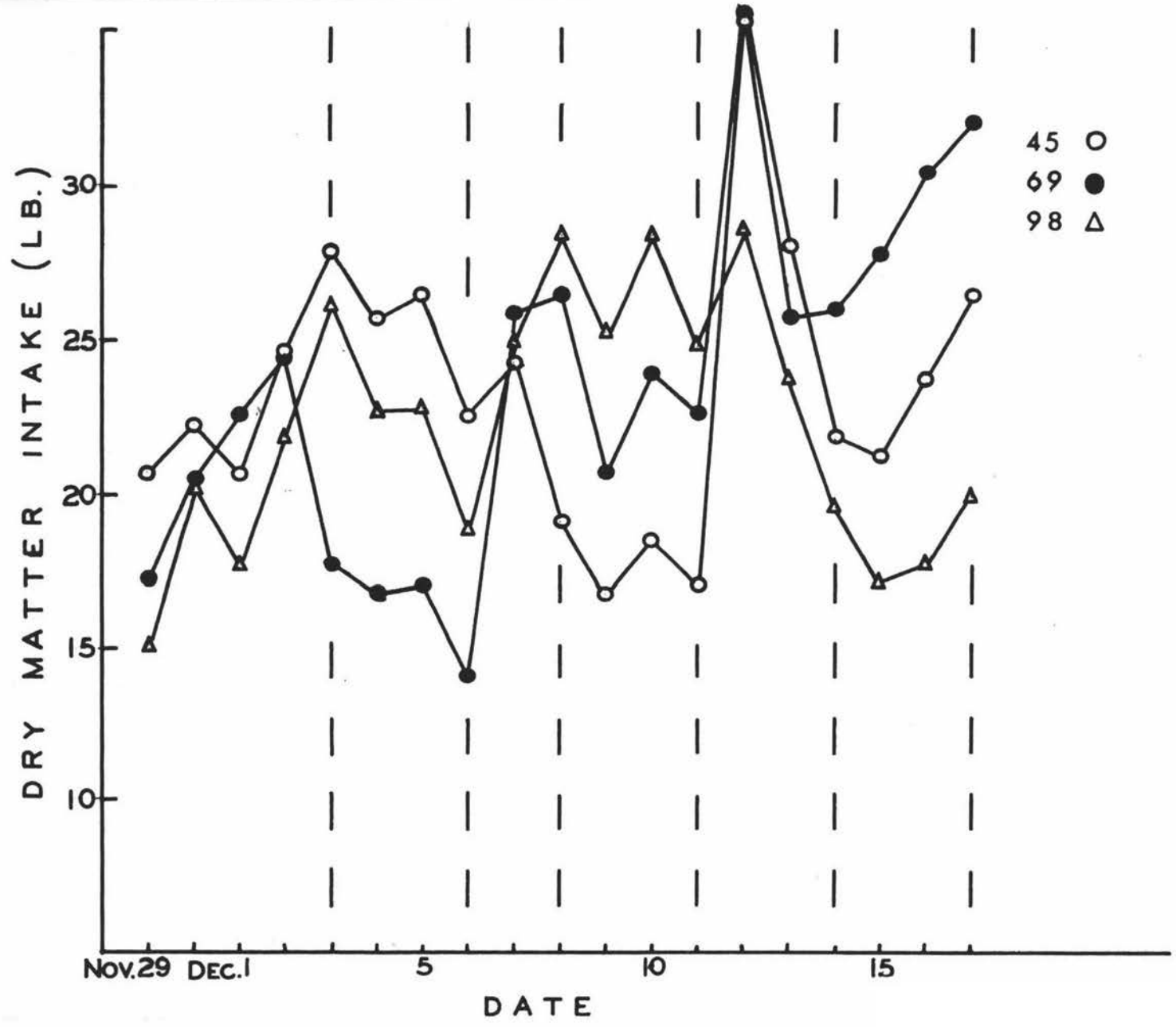


Fig. 3: Dry matter intake of pasture for Square II.

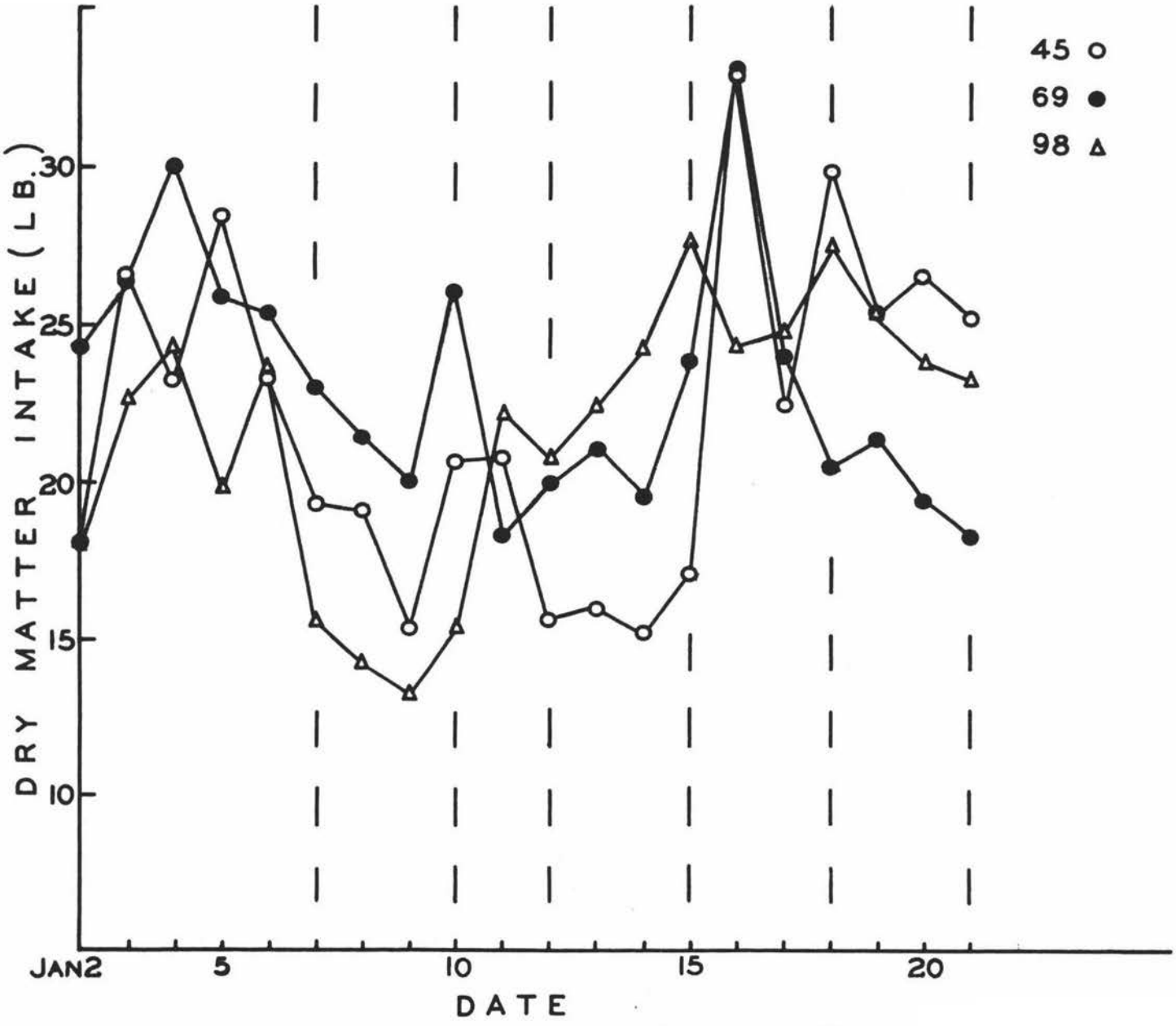


Fig.4: Dry matter intake of pasture for Square III.

TABLE 14. (a) Mean Four Day Intake (lb d.m./24 hr) and Ratios of Intakes Between Treatments.

(b) Mean Intake on Day Four (lb d.m./24 hr) and Ratios of Intakes Between Treatments.

Square		TREATMENTS A	B	C	S.E.
(a)					
1	Intake (lb d.m.)	23.6	19.8	15.5	
	Ratio	100	84	66	
2	Intake (lb d.m.)	26.9	23.2	17.6	
	Ratio	100	86	66	
3	Intake (lb d.m.)	24.9	21.5	16.7	
	Ratio	100	86	67	
Overall Mean (lb d.m.)		24.9	21.5	16.7	± 0.98
	Ratio	100	86	67	
(b)					
1	Intake (lb d.m.)	23.8	19.5	15.3	
	Ratio	100	82	65	
2	Intake (lb d.m.)	25.2	22.7	17.0	
	Ratio	100	90	68	
3	Intake (lb d.m.)	26.3	22.6	16.9	
	Ratio	100	86	64	
Overall Mean (lb d.m.)		25.1	21.6	16.5	± 0.69
	Ratio	100	86	66	

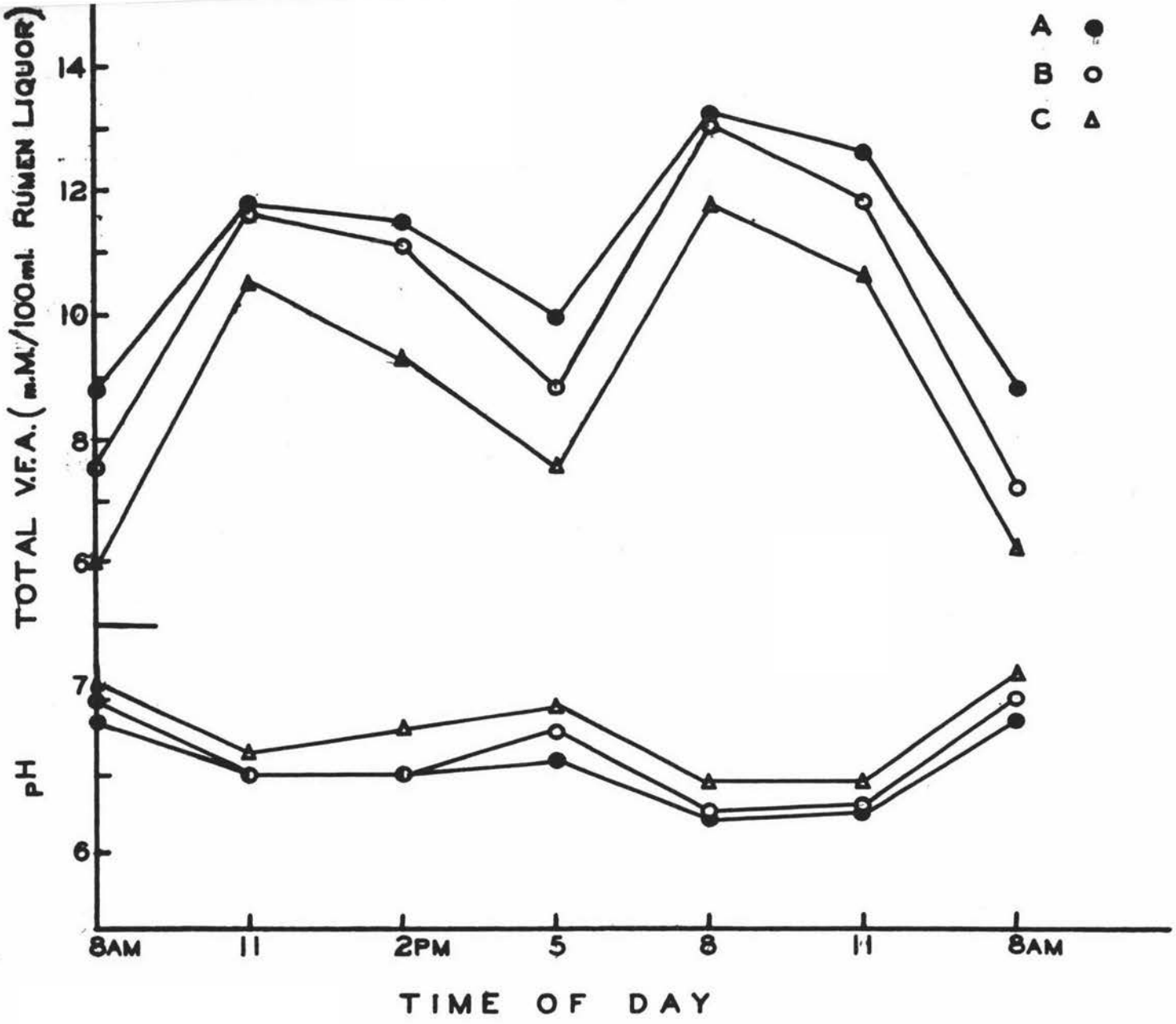


Fig.5: Mean pH and VFA concentration of the rumen liquor from three cows fed pastures at three levels of intake.

TABLE 15. The Effect of Level of Intake on pH and VFA Concentration in the Rumen Liquor from Cows Fed Pasture Indoors.

	Treatments			SE	General Mean	V (%)	Significance of differences
	A	B	C				
Mean pH	6.48	6.57	6.74	± 0.02	6.60	2.4	**
Mean VFA (M/100 ml)	11.34	10.69	9.35	± 0.14	10.46	9.5	**

Note 1. The following conventions have been used throughout to describe the direction and extent of differences between means:-

(1) Any two means NOT underscored by the same line are significantly different.

(2) Any two means underscored by the same line are NOT significantly different.

(3) NO underscoring is used where two means only are compared.

(4) ** Differences significant at the 1 per cent level.

(5) * Differences significant at the 5 per cent level.

(6) + Differences significant at the 10 per cent level.

(7) N.S. Differences not significant

Note 2. (1) S.E. Standard Error of the means unless otherwise stated.

(2) V Coefficient of variation.

times of feeding were significant ($P<0.05$) and the interaction, Days x Time of feeding was highly significant ($P<0.01$) (Appendix 2).

(2) pH values and method of feeding

Mean pH values are illustrated in Figures 1(a), (b), and (c) and d.m. intakes and mean pH values are shown in Table 13, for the various methods of feeding tried.

3.4 Experimental Period

(1) Dry matter intake

Dry matter intakes throughout the experimental period are summarised in Table 14 and are shown in Figures 2,3,and 4. Variations in intake from day to day occurred, but in general, differences between treatments were well defined. The 100:80:60 intake ratio aimed at was not always obtained, mainly because the animal on Treatment A,at times, refused part of its ration (e.g. on November 11 and January 19). Differences in d.m. intake between days within squares and between treatments, based on both the total intake of four days per period or on the 24-hour intake on the fourth day of each period,were highly significant ($P<0.01$) (Appendix 2).

The results in Table 14(b) also illustrate the differences in intake between squares and treatments and show that success in attaining the 100:80:60 ratio was no greater on the sampling (or fourth day) than it was over the four days of the period, despite the time available for feed intake adjustments by the fourth day.

(2) Rumen pH and VFA concentration

Figure 5 and Table 15 summarise the effects of level of intake of pasture on rumen pH and VFA concentration. The major points of note are the highly significant differences ($P<0.01$)between treatments for both measures.

A highly significant($P<0.01$)Times x Treatment interaction was obtained for rumen pH values, in contrast to the non-significant interaction for rumen VFA concentration(Appendices 3 and 4).

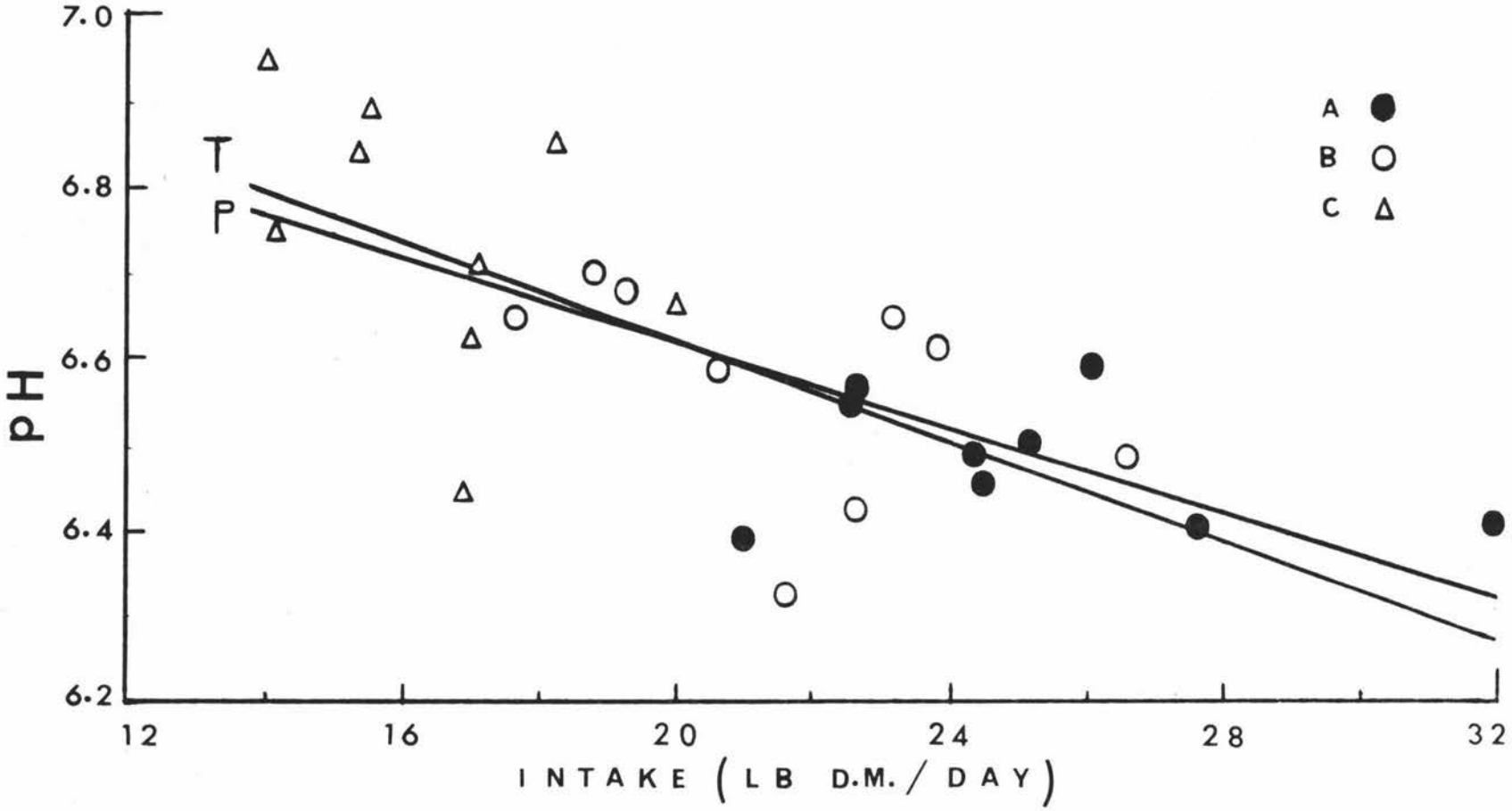


Fig.6: Relation between the dry matter intake of pasture and the pH of the rumen liquor.

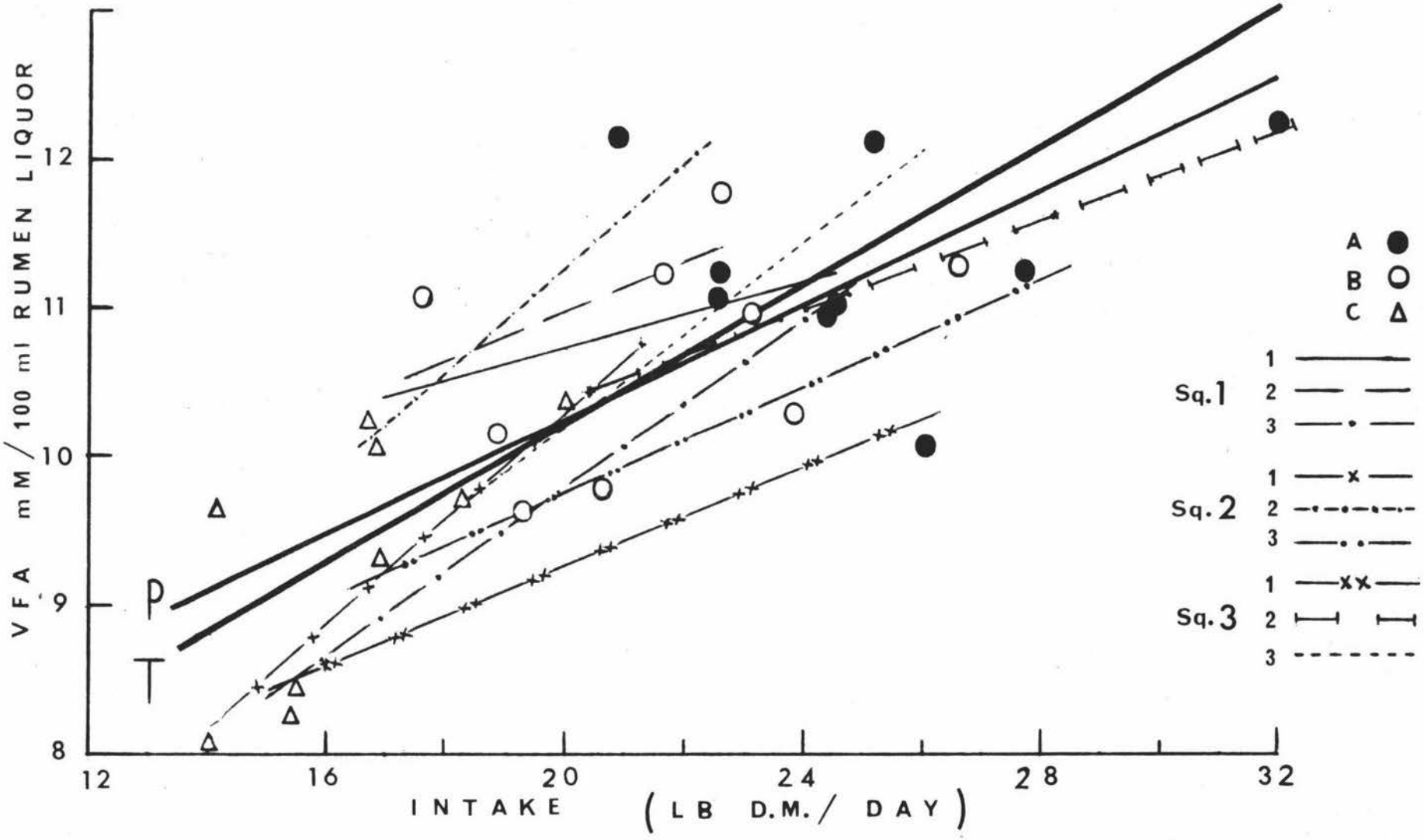


Fig. 7: Relation between the dry matter intake of pasture and the VFA concentration of the rumen liquor.

TABLE 16. Regression Coefficients for both pH and VFA Concentration of the Rumen Liquor on Dry Matter Intake

pH	Regression Coefficient	S.E. of regression coefficient	Significance of regression
(a) Total	-0.025	± 0.002	**
Treatments	-0.030	± 0.001	*
(b) Within Treatments			
A	-0.007	± 0.008	NS
B	-0.020	± 0.015	NS
C	-0.035	± 0.027	NS
(c) Within Times of Sampling			
8 a.m.	-0.012	± 0.008	*
11 a.m.	-0.015	± 0.005	**
8 p.m.	-0.034	± 0.008	**
VFA Concentration			
(d) Total	0.191	± 0.033	**
Treatments	0.233	± 0.021	
(e) Within Treatments			
A	0.037	± 0.080	NS
B	0.109	± 0.092	NS
C	0.316	± 0.120	*
(f) Within Times of Sampling			
8 a.m.	0.283	± 0.050	**
11 a.m.	0.177	± 0.055	**
8 p.m.	0.202	± 0.049	**

(3) The relationships between the d.m. intake of pasture and the pH and VFA concentration of the rumen liquor

Regression coefficients for the relationships between the d.m.intake of pasture and pH and VFA concentration respectively, from three cows, are given in Sections (a) and (d) of Table 16 and are illustrated in Figures 6 and 7. The regression slope P, based on the total data did not vary greatly from the line T for treatment where the effects of cows, days and squares were removed. (Appendix 5 and 7).

Figures 6 and 7 illustrate the poorer relationships between d.m. intake and both pH and VFA concentration within treatments. These poor relationships, particularly for treatments A and B, were confirmed by separate analyses of regression for each treatment. The results for pH and VFA concentration are summarised in Sections (b) and (e) respectively of Table 16.

Separate regression lines were fitted to the data for each day in each of the three latin squares for the relationship between d.m. intake and rumen VFA concentration. The regression lines are shown in Figure 7 and the regression coefficients in Appendix 7.

The relationships between d.m.intake and both rumen pH and VFA concentration were examined by separate analyses of regression for various sampling times. The d.m.intake for day three was used as the independant variable for the 8 a.m. sampling time, with d.m. intake for day four being used for the 11 a.m. and 8 p.m. sampling times. The results for both pH and VFA concentration are summarised in Sections (c) and (f) respectively of Table 16. Tests of heterogeneity showed that there were no significant differences between the times of sampling regressions for VFA concentration. Differences between the regressions for pH were highly significant ($P<0.01$), with the slope for 8 p.m. being steeper than either the 8 a.m. or 11 a.m. sampling times.

TABLE 17. The Effect of Level of Intake on the Proportions and Amounts of the Individual VFA s in the Rumen Liquor of Cows Fed Pasture Indoors

	Treatments A	B	C	S.E.	General Mean	V (%)	Significance of Differences
(a) Means & proportions for all sampling times							
Acetic Acid (%)	69.9	69.6	70.4	± 0.28	69.9	2.8	N.S.
Propionic Acid (%)	17.6	17.7	17.9	± 0.20	17.7	7.7	N.S.
Butyric Acid (%)	12.5	12.7	11.7	± 0.15	12.3	8.7	**
Significance 1%	———						
(b) Proportions for 8a.m. 11 a.m. & 2 p.m. sampling times							
Acetic Acid (%)	70.2	69.9	71.4	± 0.32	70.5	2.9	*
Significance 5%	———						
Propionic Acid (%)	17.2	17.6	17.0	± 0.21	17.3	6.0	N.S.
Butyric Acid (%)	12.5	12.4	11.6	± 0.20	12.2	8.0	*
Significance 5%	———						
(c) Molar quantities							
Acetic Acid(mM/100ml.)	7.82	7.36	6.46	± 0.10	7.21	9.5	**
Propionic Acid (mM/100 ml)	2.00	1.91	1.67	± 0.04	1.86	13.1	**
Significance 1%	———						
Butyric Acid (mM/100ml.)	1.41	1.46	1.09	± 0.06	1.32	33.1	**
Significance 1%	———						

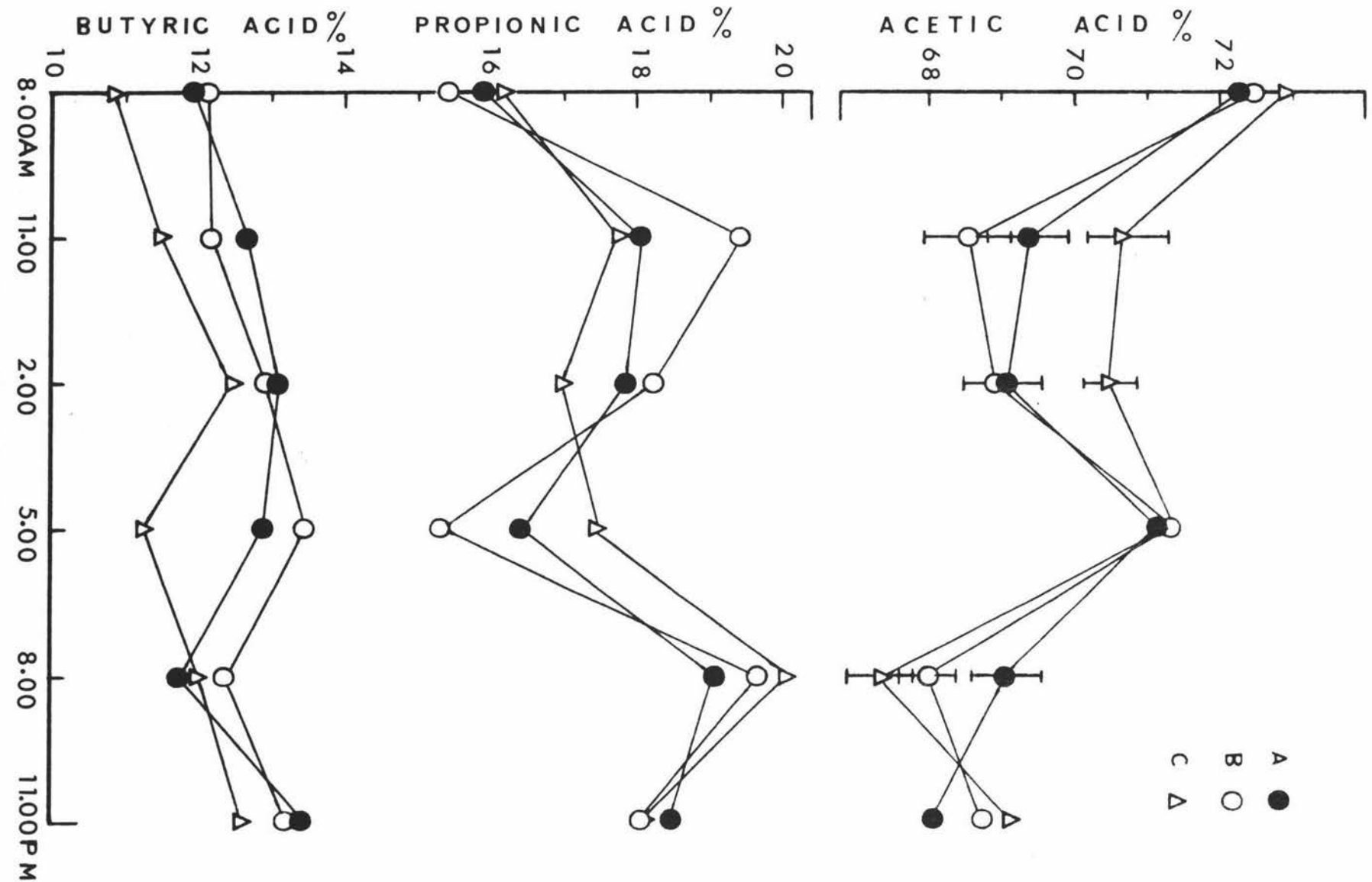


Fig.8: The Treatment x Times interaction for the proportions of acetic, propionic and butyric acids

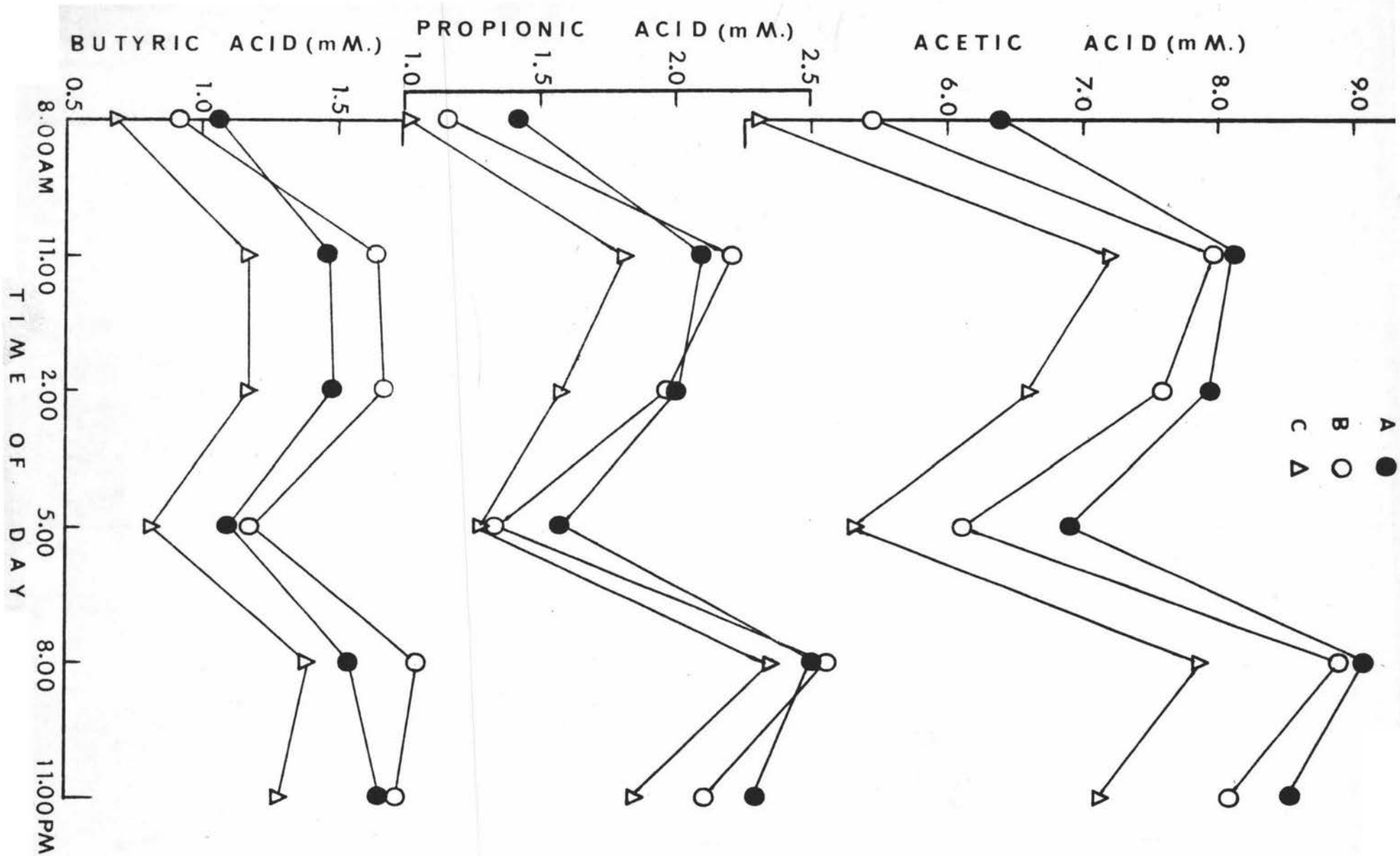


Fig. 9: The Treatment x Times interaction for the molar quantities of acetic, propionic and butyric acids in the rumen liquor.

(4) Proportions of the individual VFA s

The mean proportions of the individual VFA s for each treatment are given in Section (a) of Table 17. Variation in the level of intake of pasture had no significant effect on the proportions of acetic and propionic acid but had a highly significant effect ($P<0.01$) on the proportion of butyric acid, in the rumen liquor of three cows.

The Times x Treatment interaction was highly significant ($P<0.01$) for acetic acid (S.E. $\pm$ 0.4 per cent), significant ($P<0.05$) for propionic acid (S.E. $\pm$ 0.5 per cent) and non-significant for butyric acid . The Times x Treatment interactions for all three acids are shown in Figure 8. The S.E. s of the means for acetic acid for the 11 a.m., 2 p.m. and 8 p.m. sampling times are included in Figure 8.

Differences between treatments in the proportions of the VFA s where the data for the 8 a.m., 11 a.m. and 2 p.m. sampling times only were analysed, are summarised in Section (b) of Table 17.

(5) Molar quantities of the individual VFA s

The values for the individual VFA s expressed as mM per 100 ml rumen liquor are summarised in section (c) of Table 17. The Times x Treatment interaction for propionic acid was highly significant ($P<0.01$, S.E. $\pm$ 0.06 mM per 100 ml); for butyric acid the interaction was significant at the 10 per cent level (S.E. $\pm$ 0.05 m M per 100 ml) and for acetic acid it was non-significant. The Times x Treatment interactions for all three acids are illustrated in Figure 9.

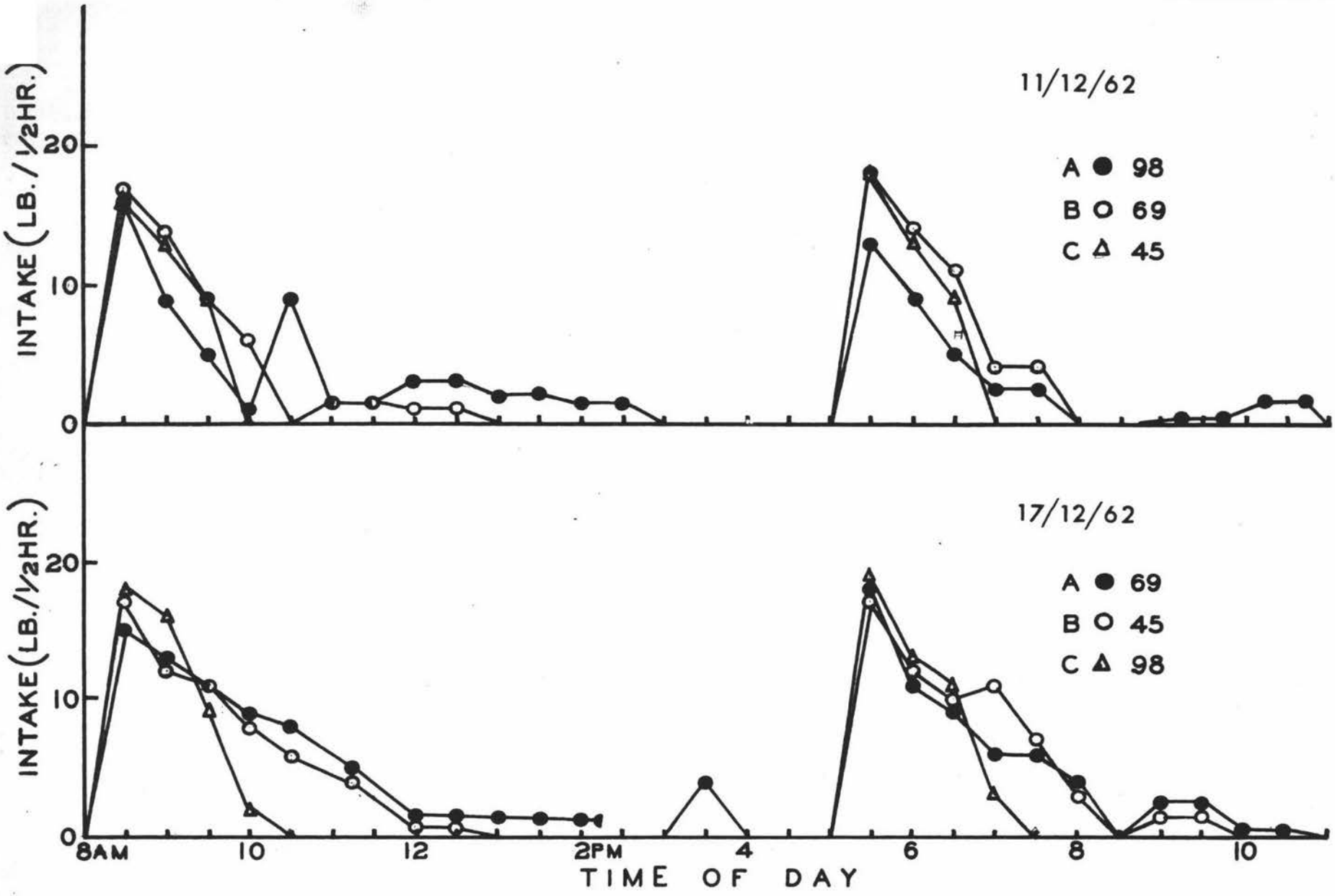


Fig.10: The pattern of feeding - Green weight of pasture eaten in 30 min periods.

TABLE 18. Variation in Rumen pH and VFA Concentration with Time after Feeding

							S.E.	Significance of Differences
Times	8p.m.	11p.m.	11a.m.	2p.m.	5p.m.	8a.m.		
Means pH	6.30	6.34	6.55	6.61	6.78	7.00	± 0.02	**
Significance 1%	———		———	———				
A.M. v P.M.		A.M.			P.M.			
Mean pH		6.72			6.47		± 0.02	**
Times	8a.m.	5p.m.	2p.m.	11a.m.	11p.m.	8p.m.		
Mean VFA values(mM/ 100 ml)	7.55	8.79	10.66	11.34	11.73	12.68	± 0.20	**
Significance 1%			———	———				
5%				———				
A.M. v P.M.		A.M.			P.M.			
Mean VFA values (mM/ 100 ml.)		9.85			11.07		± 0.14	**

(6) Rates of eating

Whilst rates of eating were measured throughout the day, to establish the pattern of feeding, it appeared that the first 30 min after the commencement of feeding was the only period when the intake of the animal on Treatment C was not restricted. Accordingly rates of eating for the first 30 min after both the morning and evening feed commenced were examined by analysis of variance. The mean rates of eating for Treatments A and B of 3.6 and 3.8 lb d.m. per 30 min respectively were significantly lower ($P < 0.05$, S.E. of means 0.1 lb) than the rate of 4.1 lb for Treatment C (Appendix 13).

(7) Pattern of feeding

The pattern of feeding in terms of lb of green matter eaten per 30 min for the three treatment groups on two days is given in Figure 10. Patterns of feeding were similar for other days also, with the Treatment A and to a lesser extent the Treatment B animal eating for longer periods than the Treatment C animal (see Table 21).

3.5 Variations with Time after Feeding

(1) Rumen pH and VFA concentration

Variations with time of sampling for rumen pH and VFA concentration are summarised in Table 18 and illustrated in Figure 5. Lowest pH values occurred at 8 p.m. and 11 p.m. with highest values at 8 a.m. and 5 p.m. prior to the commencement of the morning and evening feeds. Rumen pH values for the period 8 a.m. to 2 p.m. inclusive (AM) were consistently lower ($P < 0.01$) than values for the 5 p.m. to 11 p.m. period (PM). Estimates of the mean squares (Appendix 3) showed that most of the variance in pH values (58 per cent) was associated with time of sampling. Rumen VFA concentration showed a similar but inverse pattern to pH values over the day, although the values for 8 p.m. were significantly higher ($P < 0.01$) than for the 11 p.m. sampling time. As with pH, most of the variance in VFA concentration (57 per cent) was associated

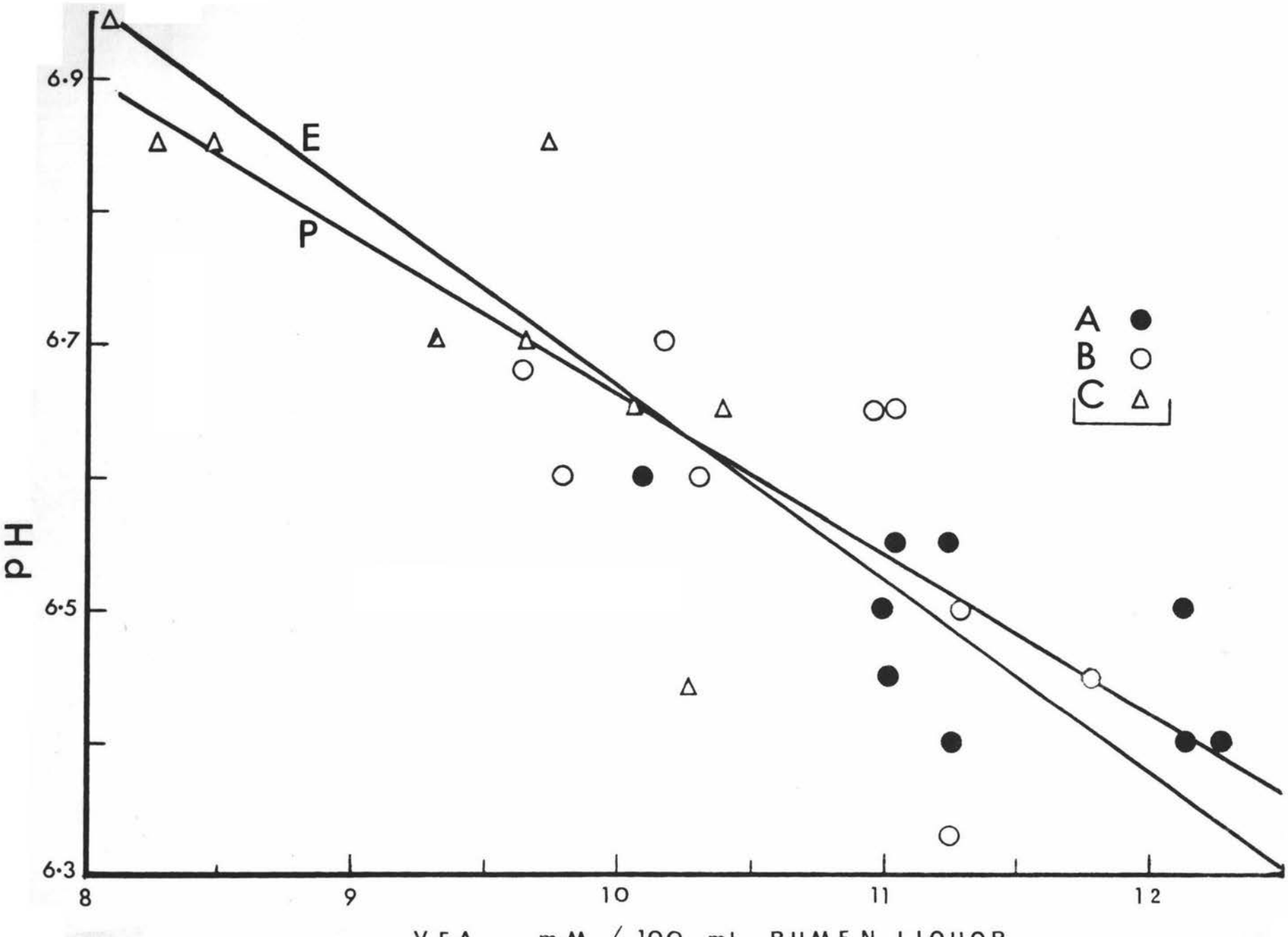


Fig.11: Relation between the pH and VFA concentration of the rumen liquor from cows fed pasture indoors.

TABLE 19. Regression Coefficients for pH on VFA Concentration of the Rumen Liquor

Regression	Regression Coefficient	S.E. of the regression	Significance of regressions
(a) Total	-0.122	± 0.005	* *
(b) Within group	-0.145	± 0.020	* *
(c) Treatments A	-0.115	± 0.010	* *
B	-0.123	± 0.009	* *
C	-0.123	± 0.009	* *
(d) Times 8a.m.	-0.096	± 0.010	* *
11a.m.	-0.058	± 0.015	* *
8p.m.	-0.122	± 0.023	* *

TABLE 20. Variation in the Proportions of VFAs According to Times of Samp

Times:	8p.m.	11p.m.	2p.m.	11a.m.	5p.m.	8a.m.	S.E.
Acetic Acid Means (%)	68.2	68.7	69.5	69.6	71.3	72.5	± 0.20
Significance 1%	———	———	———	———			
5%			———	———			
Times:	8a.m.	5p.m.	2p.m.	11p.m.	11a.m.	8p.m.	
Propionic Acid Means (%)	15.8	16.4	17.7	18.3	18.4	19.8	± 0.30
Significance 1%	———		———	———	———		
Times:	8a.m.	8p.m.	11a.m.	5p.m.	2p.m.	11p.m.	
Butyric Acid Means (%)	11.6	12.0	12.1	12.3	12.8	13.1	± 0.26
Significance 1%	———	———	———	———	———		
5%		———	———	———	———	———	

with time of sampling (Appendix 4).

(2) The relationship between pH and the VFA concentration of the rumen liquor

The inverse relationship, already mentioned, between pH and VFA concentration was examined in greater detail by regression analysis. To avoid the confounding effects of cows, times of sampling and treatments, regression coefficients were obtained separately for the various effects (Appendix 14). Figure 11 illustrates the close relationship between rumen pH and VFA concentration. The regression slope P based on the total data available, varied only to a small extent from the line E for the within group regression where the main effects had been removed. The regression coefficients are given in Table 19. The separate regressions for each treatment were all highly significant ($P < 0.01$) and tests of heterogeneity showed that there were no significant differences between them (Appendix 14.) Separate regression analyses were also made for the 8 a.m., 11 a.m. and 8 p.m. sampling times, the results being summarised in Table 19. Tests of heterogeneity showed that there was a significant difference ($P < 0.05$) between the regressions, with steeper regression slopes for the 8 a.m. and 8 p.m. compared with the 11 a.m. sampling time. The separate regressions for treatments and times of sampling were confounded by cows, days and treatments, and cows and days respectively.

(3) Proportions of the individual VFA's

The proportions of acetic, propionic and butyric acids varied with time after feeding ($P < 0.01$), with acetic decreasing, propionic increasing and butyric acid varying to a small extent. Highest proportions of acetic acid were obtained at 8 a.m. and 5 p.m. when the VFA concentration was at its lowest. Generally at 8 p.m., 11 p.m. and 2 p.m, VFA concentrations were highest and were associated with lowest acetic acid proportions (Figure 8 and Table 20). The extreme range for the proportion of acetic acid over the day was 61.5 to 72.6 per cent.

TABLE 21. Rumen Fill and Total Amounts of VFAs Determined 15 hours after Commencement of the p.m. Feed on Day Four. (Means of five determinations per treatment.)

	Treatments A	B	C	SE	Significance of Differences
Time since cessation of feeding (hr.)	11	approx 12	13		
Mean d.m. intake (lb)	24.5	21.0	16.2		
Ratio	100	86	66		
Total weight of ingesta (lb)	107	100	78	± 6	*
Significance 5%	———				
Ratio	100	93	73		
D.M. % of ingesta	11.2	9.6	8.9	± 0.4	*
Significance 5%		———			
Total d.m. in rumen (lb)	11.9	9.6	6.9	± 0.5	* *
Ratio	100	81	58		
Total water in rumen (lb)	95	90	71	± 6	*
Significance 5%	———				
Ratio	100	95	75		
Total VFA (moles)	3.82	2.96	2.11	± 0.15	* *
Ratio	100	77	55		
Acetic Acid (moles)	2.67	2.06	1.48		
Ratio	100	77	55		
Propionic Acid (moles)	0.67	0.52	0.38		
Ratio	100	78	56		
Butyric Acid (moles)	0.48	0.38	0.25		
Ratio	100	78	52		

3.6 The Amount of Digesta and Total Quantities of VFAs in the Rumen

The data relating to rumen fill and quantities of VFAs according to treatments are summarised in Table 21. The methods used in calculating the quantities of VFAs are described in Appendix 15.

The amount of d.m. in the rumen and the d.m. intake appeared to be related. Regression analysis measured this relationship. The regressions for total d.m. in the rumen on the p.m. intake for day four and on the 24-hour intake for the same day were 0.841 ± 0.02 and 0.472 ± 0.06 respectively. Both regressions were highly significant ($P<0.01$) (Appendix 16). The quantities of VFAs in the rumen and the d.m. per cent of the ingesta were also closely related to intake. However a poorer relationship was evident between intake and the total ingesta and total water in the rumen. Thus small non-significant differences between Treatments A and B were noted for these values. The total ingesta and total water in the rumen for treatment C, whilst significantly lower than A and B ($P<0.05$), were proportionately closer to Treatments A and B than were the corresponding d.m. intakes. (Appendices 15 and 16).

CHAPTER 4

DISCUSSION

4.1 Techniques

(1) Sampling of the rumen contents

Samples taken from the middle of the rumen, prior to the morning feed, were representative of the VFA concentration of the rumen as a whole for the conditions of feeding employed in this experiment. However, as already noted (Page 12), stratification of the ingesta was likely to be at a minimum at this stage in the feeding cycle with heterogeneity at a maximum during peak VFA concentration shortly after feeding (Bryant,1961). A further complication was the possibility of a different pattern of stratification occurring at the different intake levels. Whilst the results did not provide information on the efficacy of sampling from the middle of the rumen over the whole feeding cycle the VFA concentration varied appreciably between sets (Section 3.1 (1)), so that the results probably have a wider applicability than might be inferred from the fixed sampling time taken.

It is possible that a composite sample taken from various positions in the rumen may be a more efficient method of sampling. However it would be difficult to make allowance for the different amounts of ingesta represented by each position. Improved results could be obtained by carrying out separate determinations for each sampling position but the number of analyses that could be handled in the laboratory would limit such a study.

An important part of this experiment involved the study of changes in rumen pH and the concentration and proportions of the VFA s with time after feeding. Thus sampling from a fixed position within the rumen was thought

desirable, as samples taken between cows and at different times would probably be more comparable with each other. Nevertheless the data obtained in this experiment applied to material taken from a fixed point in the middle of the rumen ingesta, although the results of Bryant (1961) suggested that the values obtained from this position were approximately the mean in terms of pH and VFA values of a composite sample taken from the dorsal, middle and ventral positions. The difficulty lies in relating the results of the present experiment to the results of other workers. In a large proportion of the studies on rumen fermentation, little if any information is given on methods or positions of sampling from the rumen.

(2) pH readings

In the present experiment, the time between removal of the contents and recording the first pH reading ranged from 40 to 50 sec. However Smith (1941) and Turner and Hodgetts (1955) established that loss of CO_2 with a rise in pH occurred immediately the fistula was opened. It is also possible that a leaking fistula may result in high rumen pH values (Matscher, Borghi and Beghelli,1957). However Turner and Hodgetts (1955) stated that only the pH of the surface ingesta was affected.

It is perhaps surprising that the pH readings taken from three to five min after removal from the rumen, in the present experiment, were not higher than those taken immediately. The possibility exists that most of the CO_2 loss had occurred prior to the reading taken 40-50 sec after sampling. In the absence of equipment to enable direct readings to be taken in the rumen, pH readings were obtained in vitro as described under materials and methods.

(3) Recovery of the VFAs by steam distillation

The mean recovery of the VFAs by steam distillation of 100.7 per cent was considered satisfactory (See also Bryant,1961). The high percentage

recovery obtained may have been due to small negative errors in the estimation of the VFA concentration of rumen liquor before the addition of the standard solutions. As all recoveries were based on this initial value, errors at this stage would be reflected in all recoveries.

(4) Separation of the individual VFA s by gas-liquid chromatography

Considerable difficulty was experienced in obtaining satisfactory separations. Ideally all determinations should be duplicated. However in view of the number of samples involved and the difficulties experienced with the equipment, this was not possible.

The 10 random duplicates and the standard soap solutions provided a measure of the variability of the separations. The standard solutions also provided evidence of the accuracy of the separation and detection of the individual acids.

For individual separations the variation between determinations was shown to be comparatively large. Thus for butyric acid, the range of approximately four per cent represented a considerable variation around a mean of 10 or 12 per cent (Table 10). The results emphasised the need for adequate replication and estimates of laboratory error.

4.2 Preliminary Period

(1) Dry matter intake and liveweight

Although the data were limited, d.m.intake appeared to be associated with liveweight in that the intake of the heaviest animal, Cow 69, exceeded that of the other two animals. The d.m. intake in lb per 100 lb liveweight per day were, 3.8, 3.2, and 3.5 for Cows 45, 69 and 98 respectively. Thus Cow 69 ate rather less in relation to her liveweight than either Cow 45 or 98. The d.m.intake of Cow 98 does not appear to have been affected by the considerable scouring observed at this time, with this animal.

The above figures on d.m. intake, as a proportion of live-weight, are similar to those obtained by Hutton (1963) of 3.16 and 3.70 lb d.m. per 100 lb live-weight with lactating cows stall fed on pasture. The results of Hutton (loc.cit.) referred to periods from 1 tol2 weeks and 13 to 24 weeks after calving respectively. The period concerned in the present experiment was approximately 12 to 18 weeks after calving for Cow 45 and Cow 98 and 8 to 14 weeks for 69. The ad libitum intakes obtained during the preliminary period in this experiment were satisfactory and indicated that techniques of pasture collection and feeding were reasonable. It also indicated that the animals had become accustomed to stall conditions insofar as feeding was concerned.

(2) Methods of feeding

Feeding proportionate amounts several times daily should enable a closer control of intake at the various levels employed. Feeding patterns are also likely to be more similar between treatments than where the animals are fed twice daily. However the pattern of fermentation as shown by rumen pH was obscure (Figure 1 (b)) and no definite peak values were obtained. The limited advantages of the system, involving a great deal of work caused its abandonment.

A short period of starvation followed by feeding for a fixed period, has been adopted by a number of workers e.g.Johns (1955b) for testing the effects of different feeds on rumen fermentation. This avoids the complication of earlier feeding or variations in feeding patterns between animals. The method was considered, but was felt to be too divorced from normal feeding practice for lactating cows (See Part III).

When approximately equal amounts of pasture were fed twice daily, distinct peaks of fermentation occurred, (Fig. 1 (a) and (c)) and differences in rumen pH were evident between the levels of intake used. Despite a diminished control over intake at the different levels and the fact that feeding patterns

between treatments were likely to vary, this method of feeding was adopted in this experiment. The method provided a greater amount of data per period, in that rumen fermentation could be characterised twice in one day, following the morning and evening feeds. 'Topping up' the feed at mid-day (Figure 1 (c)) appeared to be an unnecessary complication to the pattern of feeding in the morning period and was not carried out in the main experiment.

4.3 Experimental Period

(1) Dry matter intake

The mean d.m. intake per day for Treatment A of 24.9 lb was similar to that for the preliminary period of ad libitum feeding of 24.4 lb. Whilst a strict 100 : 80 : 60 ratio of d.m. intake was not attained, treatments were sufficiently separated to achieve the objects of the experiment.

(2) Rumen pH, VFA concentration and d.m.intake

The results showed that a restriction in the d.m. intake of pasture, fed twice daily to three lactating Jersey cows, was associated with an increase in pH and a decrease in the VFA concentration of the rumen liquor. The results were similar to those of Bath and Rook (1963) who obtained similar increases in pH and decreases in VFA concentration for a comparable restriction in intake with two dairy cows (cf. Tables 1 and 15). The results were also similar to those of Williams and Christian (1956) for VFA concentration but not for pH, as these authors reported no change in the latter value with a variation in the level of intake. Their method of sampling could have lead to salivary contamination of the sample with a consequent effect on pH values.

Assuming a linear relationship over the range in d.m. intake of 15 to 32 lb per 24 hours, a change in d.m. intake of one lb resulted in a change of 0.03 units for rumen pH and 0.23 mM per 100 ml for VFA concentration. The relationship between the variables within treatments was confounded by cows,

days and squares which together with the restricted range of intake, contributed to the poor relationship obtained.

Despite the fact that the individual day regressions for d.m. intake and VFA concentration were based on three observations only and were confounded by cows, their slopes agreed comparatively well with the slope for line T (Figure 7). The variation in the height of the individual day regressions above the X-axis indicated the possible effects of changes in pasture composition and/or the pattern of feeding between days and squares (see Sections 7.1 (3) and 8.2 (1)). The day regressions indicated that d.m. intake was exerting a comparatively constant effect on rumen VFA concentration despite changes in the level of VFA concentration between days and squares. However the significant regression ($P < 0.05$) obtained between d.m. intake and VFA concentration for treatment C, showed that where the restriction in intake was greatest, the effects of days and squares on VFA concentration were less obvious.

The relationship between rumen VFA concentration and d.m. intake appeared to be equally as close for the 8 a.m. sampling time prior to feeding as it was at 8 p.m., the time of peak fermentation. A poorer relationship between the two variables might have been expected at 8 a.m. through a regression of VFA concentration towards a more uniform low figure, with time after feeding (Section 1.2 (1)). Evidently the time since the previous feed was insufficient for this to show.

The significantly steeper slope ($P<0.05$) and the highly significant regression ($P< 0.01$) obtained at 8 p.m. compared with the flatter slope and the significant regression ($P< 0.05$) at 8 a.m. lent support to the occurrence of a stronger relationship between d.m. intake and pH at the former sampling time, compared with the latter.

(3) Rumen VFA proportions and dry matter intake.

Where the data from all sampling times were considered, a decrease in the intake of pasture affected the proportion of butyric acid only, although there were minor non-significant changes in acetic and propionic acid. A minor change in the proportions of the VFA s with a decreased intake of hay alone was noted by Bath and Rook (1963) but changes were larger where a hay and concentrate ration was fed. It appeared from their work that the degree of change in VFA proportions with variation in the level of intake depended on the nature of the diet, the change being greatest with diets giving low acetic acid proportions. The acetic acid proportions obtained with pasture in the present experiment were similar to those obtained by Bath and Rook (1963) using hay alone. As such, the present results agreed with these workers for their results with hay as far as the absence of marked changes in acetic acid were concerned. The results also agreed with these workers in that an increase occurred in the proportion of butyric acid with increased intake. The change in the proportion of butyric acid in the present experiment whilst highly significant ($P < 0.01$), was nevertheless small and probably of little importance.

Small but significant decreases in acetic ($P < 0.05$) and increases in butyric acid proportions ($P < 0.05$) were obtained in the present experiment with increased intake, where the data for the 8a.m. 11 a.m. and 2 p.m. sampling times only were considered. Thus if sampling had been confined to this period only, rather different conclusions would have been drawn. It emphasised the need for adequate replication.

The highly significant ($P < .01$) Times x Treatment interaction for acetic acid is difficult to explain in physiological terms. At the 8p.m. sampling time the acetic acid proportions appeared to be high for treatment A in blocks three, four and eight compared with other treatments at the same time. Thus treatment A values for acetic acid were 2.7 per cent greater than either

B or C at the above times, whereas the mean difference between Treatments A and B for all blocks was one per cent at 8p.m. and between A and C the difference was 1.5 per cent. The similarity of the results for acetic acid proportions at 11a.m. and 2p.m. reinforce each other as do the similar results for 8a.m., 5p.m. and 11 p.m. (Figure 8). Thus the results for the 8p.m.sampling time vary from all other times and appear to be unexpected and contrary to the results obtained by Bath and Rook (1963); Terry and Tilley (1963) and Williams and Christian (1956). However any changes, even at the 11a.m. and 2p.m. sampling times, were comparatively small. The proportion of propionic acid appeared to vary partly with acetic and partly with butyric acid (Figure 8). The Times x Treatment interaction for propionic acid appeared to follow no definite pattern that was capable of explanation.

Figure 8 showed an apparent interaction between treatments and times of sampling for butyric acid. This does not conflict with the non-significant interaction for butyric acid, obtained by analysis of variance, as it was evident from Figure 8 that changes were minor.

(4) Molar quantities of the individual VFAs

The values for the individual VFAs expressed as mM per 100 ml of rumen liquor i.e. their concentration, are a reflection of the concentration of the total VFAs as well as the proportions of the individual acids. As the proportions of the acids did not vary greatly between treatments, the molar quantities of the acids largely reflected the differences observed in VFA concentration. This is apparent in a comparison of Figures 5 and 9. The exception to this was observed to a small extent with propionic acid where the changes in the proportions of this acid with time after feeding were sufficient to have modified the variations obtained in VFA concentration. This applied also, but to a lesser extent with butyric acid.

The results emphasised that differences between Treatments A and B for all acids were less than those between Treatments A and C or B and C.

4.4 Variations with Time After Feeding

(1) Rumen pH and VFA concentration

With pasture offered at fixed intervals twice daily, the rate of fermentation after feeding as assessed by rumen pH and VFA concentration was not constant. It was minimal immediately before feeding and maximal approximately three hours after feeding. The minimum and peak values, within the limits of the sampling times, coincided with all three treatments. The daily variations reported here were similar to those of other workers (review of literature Page 10), but not to Williams and Christian (1956). These latter workers observed little variation in pH and VFA concentration throughout the day with grazing sheep. This may have been because of the even pattern of grazing over the day. Bryant (1961) observed distinct diurnal variations over the day with a grazing cow, although he stated that the pattern of grazing was disturbed by yarding and sampling. Where sheep were fed twice daily Barnett and Reid (1961), in commenting on the results of various workers, noted that the total VFA concentration in the rumen was higher after the evening feed than after the morning feed. This agrees with the results obtained in the present experiment.

(2) The relationship between pH and VFA concentration of the rumen liquor

The relationship between pH and VFA concentration of the rumen liquor appeared to be unaffected by treatments (see Section 3.5). The relationship did not follow that of Williams and Christian (1956) (Page 5), who observed no change in rumen pH with changes in VFA concentration because of varying levels of intake.

Differences were noted in the regression slopes for pH on VFA concentration at different sampling times in the present experiment (Table 19). No differences were noted in the regression slopes at the two extreme sampling times at 8 a.m.

and 8 p.m. It might have been expected, from the statement of Kay and Hobson (1963) (Review of literature Page 4), that any variations in the pH - VFA relationship would have occurred at these times rather than at 11 a.m. At least an agreement might have been expected between the 11 a.m. and 8 p.m. sampling times when VFA concentrations were high.

That the relationship between rumen pH and VFA concentration was not an exact one is seen in the highly significant ($P<0.01$) Time x Treatment interaction for pH but not for VFA. The significance of the interaction was probably because of the similar pH values at 11 a.m. and 2 p.m. for Treatments A and B (Figure 5 and Section 3.4 (2)). At all other times distinct differences in pH were obtained between treatments.

(3) Proportions of the individual VFA s

The proportions of the individual VFA s varied over the day and the extent of this variation exceeded that for all other factors studied. As the concentration of the total VFA s increased there was a decrease in the ratio of acetic to propionic acid with the highest ratio occurring before feeding. This agrees with the results of Reid et al. (1957); Gray and Pilgrim (1951); El-Shazly (1952); Bath and Rook (1963) and Davey etal. (1962). As already noted in the review of literature (Page11), a number of workers observed no consistent changes in VFA proportions with time after feeding. In fact Shaw (1961) stated that this uniformity was of great practical importance as it could then be assumed that the proportions of the VFA s in the rumen reflect both their relative production and absorption and that for most feeding régimes, a sample of rumen fluid drawn at any time during the day would give a good estimate of the actual VFA proportions produced. Bath and Rook (1963), in commenting on the daily variations in the proportions of the VFA s, stated that sampling of the digesta at regular intervals throughout a feeding cycle appeared essential if gross errors were to be

to be avoided.

The changes observed by Williams and Christian (1956) in acetic acid were comparatively small over a wider range of intakes than were used in the present experiment. They based their sampling on a previous experiment with grazing sheep, where daily fluctuations in rumen fermentation products were small. This suggested that sampling at one time from a number of animals was the most suitable method. In their intake study however, the sheep were fed twice daily indoors and no account appeared to have been taken of possible diurnal variations occurring in rumen VFA proportions under these different conditions. It is essential that sampling be carried out at regular intervals and that sampling times be reported.

4.5 The Amounts of Ingesta and VFA s in the Rumen and the Dry Matter Per Cent of the Contents of the Rumen

Unlike the results of Hale et al. (1940) and Williams and Christian (1956), the present experiment showed that the level of intake had a significant influence ($P < 0.05$) on the d.m. percentage of the rumen ingesta. To this extent the present results agreed with those of Mäkelä (1956) and Burroughs et al. (1946).

Emptying the rumen at one time in each feeding cycle provided limited information of a quantitative nature. A close relationship was shown to exist between d.m. intake and the total d.m. in the rumen under the conditions of the experiment. In accord with the results of Burroughs et al. (1946), the amount of d.m. in the rumen preceeding feeding was approximately the same in amount as that consumed in the one feeding period. There was also a close relationship between the quantities of VFAs in the rumen and d.m. intake. The results suggest therefore that the outflow of particulate matter from the rumen and the absorption of organic constituents through the rumen epithelium were approximately proportionate to d.m. intake. The total water content and volume of ingesta in the rumen were less affected by changes in intake and between treatments A and

B particularly, little difference was seen in these values. The lowered d.m. per cent of the rumen ingesta at the lower intakes may have been because of a less than proportionate fall in water intake and salivary secretion compared with the loss of d.m. (Review of literature, Page16).

4.6 Suggested Reasons why Changes in the Level of Intake Affect Rumen pH and VFA Values

A number of possible reasons why changes in the level of intake affect rumen pH and VFA values are discussed as follows:-

(1) Rates of eating

It was thought by the author that rates of eating might influence the level of fermentation in the rumen. No relationship between rates of eating and rumen pH and VFA levels were found. The rate of d.m. intake was higher for treatment C, but subsequent to the first 30 min of eating, the intake of the treatment C animal appeared to be influenced by the restriction in the amount of its feed. This fact would limit the influence of rate of intake, as analysed, on rumen pH and VFA concentration. Further discussion on this point appears in Part II.

(2) Patterns of feeding

A possible explanation for the effect of intake on rumen pH and VFA concentration was provided by the different patterns of feeding between treatments (Figure 10) and by a consideration of Figure 5, showing the effects of time of sampling on the values determined. Differences between Treatments A and B were at a minimum at 11 a.m. and 8 p.m. and at a maximum for all treatments, at 8 a.m. and 5 p.m. At these two latter times the number of hours since feed was last ingested was least for Treatment A. The high VFA concentration and low pH values for Treatment A, at these times, could have been associated with more recent feeding. The true peak of VFA concentration for Treatment C may have occurred earlier than the peak for Treatment A but would remain

undetected because of the fixed times of sampling employed. The effects of changes in the pattern of feeding are further discussed in Part III, Chapter 10.

(3) Influence of pre-feeding levels of VFA concentration

It is possible that the levels of VFAs prior to feeding influenced peak concentration (Bryant, 1961). Levels of pH and VFA concentration in the present experiment were different at 8 a.m. and 5 p.m. for Treatments A and B but there were no differences at 11 a.m. and 8 p.m. This suggested that for these treatments, initial levels had no effect on peak concentrations of the VFAs. In contrast however, the fact that the P.M. values for VFA were consistently higher than A.M. values suggested that later values were probably affected by those occurring earlier.

(4) Absorption of the VFAs

Bath and Rook (1963) stated that variations in the proportions of the VFAs with different levels of intake may have been because of changes in their relative rates of absorption, induced by changes in rumen pH. Limited evidence (Page 13) suggested that pH can influence the relative rates of absorption from the rumen, but there was no experimental evidence in the work of Bath and Rook (1963) to substantiate this. The changes these workers obtained in pH were not large. Williams and Christian (1956) observed no changes in pH although they showed that the proportions of the VFAs could be varied to a small extent by intake differences. However their method of sampling by stomach tube was open to criticism. Where a hay and concentrate ration was increased (Bath and Rook, 1963), this may simply mean that a greater total amount of readily available carbohydrate was being fermented to produce a mixture of acids with a lower proportion of acetic acid.

(5) Dry matter per cent of the ingesta and rumen volume

If it is assumed that the d.m. per cent of the ingesta in the rumen is a measure of feed per unit volume, then the reduction in the concentration of the

VFA s with decreased intake, could be explained in terms of a dilution effect. As intake decreased there appeared to be a proportionate loss of d.m. from the rumen but probably a less than proportionate decrease in the intake of water through drinking or salivation.

4.7 Summary Part I

(1) Data supporting those of the main experiment were presented and discussed.

(a) With three lactating Jersey cows fed pasture indoors, sampling from the rumen and from the removed and mixed rumen contents gave only minor differences in VFA concentration between the two methods, prior to feeding.

(b) Recoveries of the VFAs by steam distillation, variability in the separation of the VFA s by gas-liquid chromatography and differences between duplicate pH readings were presented and discussed.

(c) A short preliminary period was conducted to accustom the animals to indoor feeding and to establish the experimental routine.

(2) The main experiment was concerned with the effects of three levels of intake of pasture, fed indoors, on the pH and the concentration and proportions of the VFA s in the rumen liquor of three lactating Jersey cows. The cows were arranged in a 3 x 3 latin square which was repeated in November, December and January, 1962 - 63.

Variation in rumen pH and the concentration and proportions of the VFA s, with time after feeding, were also studied.

(3) An increase in the daily dry matter intake of pasture was associated with a fall in pH ($P<0.01$) and an increase ($P<0.01$) in the VFA concentration of the rumen liquor.

(4) Where all data were considered, an increase in the daily dry matter intake of pasture was associated with minor non-significant changes in the rumen liquor. There was a highly significant ($P<0.01$) increase in the proportion of butyric acid in the rumen liquor, between the low and two highest levels of intake.

Where the data from the 8 a.m., 11 a.m. and 2 p.m. sampling times only, were considered, an increase in the dry matter intake of pasture was associated with a significant decrease in the proportion of acetic acid and a decrease in butyric acid ($P<0.05$). The changes were small but emphasized

the need for adequate replication of times of sampling.

(5) An increase in the dry matter intake of pasture was associated with a highly significant increase ($P < 0.01$) in the molar quantity of acetic acid between the three levels of intake. There was a highly significant increase ($P < 0.01$) in the molar quantities of propionic and butyric acids between the low and two highest levels of intake.

(6) The dry matter per cent of the rumen ingesta, determined prior to the morning feed, was positively related to the dry matter intake of pasture. This suggested that a dilution effect may have been partly responsible for changes in rumen VFA concentration with changes in intake.

(7) Part of the changes in rumen pH and VFA concentration associated with level of intake were attributed to variations in the pattern of feeding between treatments.

(8) Comparatively large variations in the proportions of the VFA s occurred with time after feeding. The proportion of acetic acid was highest and propionic lowest prior to feeding. After feeding the proportion of acetic acid in the rumen liquor fell with lowest proportions coinciding with peak VFA concentration. There was a corresponding rise in the proportion of propionic acid with time after feeding.

These changes with time after feeding were discussed in relation to the design of experiments and the need for serial sampling where the proportions of rumen VFA s are being obtained, was stressed.

PART II

(a) THE EFFECT OF CHANGES IN THE CHEMICAL AND BOTANICAL COMPOSITION OF PASTURE.

(b) THE EFFECTS OF GRAZING OR FEEDING PASTURE INDOORS.

(c) THE EFFECTS OF INDIVIDUAL COW DIFFERENCES.

INTRODUCTION

Most of the work on rumen fermentation has involved the use of mixed hay and concentrate rations and there is a singular lack of information on variations in rumen pH and VFA values associated with compositional changes in pasture. It is only comparatively recently that attention has been directed towards pasture and this work has been mainly confined to the United Kingdom. Studies in New Zealand have been mainly with sheep and few reports have been published. As far as the author is aware, only one New Zealand study involving rumen pH and VFA values in dairy cattle fed pasture has appeared (Bryant, 1961). Considering the established relationship between the proportions of the VFAs produced in the rumen and the composition of the milk of the cow, it appeared essential to obtain more information on the effects of changes in chemical composition, stage of growth and botanical composition of pasture, on rumen fermentation in the dairy cow.

The main objects of this section of the experiment were to obtain information on rumen fermentation with dairy cows fed pasture, at different stages of maturity, under both stall feeding and grazing conditions.

The information collected was as follows:-

Rumen pH and VFA concentration and proportions of the individual acids:-

(a) with three, lactating Jersey Cows fed pasture indoors at intervals over a period of three months, from November, 1962 to January, 1963. (See Section 2.2).

(b) with grazing cows, compared with cows fed similar pasture indoors.

(c) with cows grazing immature spring pasture, markedly different from that fed in (a) above.

Limited information on variations between cows in rumen pH and the concentration and proportions of the VFAs was also obtained. The results

and discussion on the above are presented in Part II.

CHAPTER 5.

REVIEW OF LITERATURE

Variation in Rumen pH, VFA Concentration and Proportions of the Individual Acids.

5.1 The Effect of the Diet (Pasture).

(1) Rumen pH and VFA concentration

Tilley, Deriaz and Terry (1960) obtained VFA concentrations ranging from 6 to 14 m - equiv per 100 ml rumen liquor from sheep fed different species of pasture plants or their conserved products. Rumen pH values ranged from 6.76 for New Zealand white clover, containing 5.4 per cent of water-soluble carbohydrate on a d.m. basis, to 5.90 for S24 perennial ryegrass containing 16.0 per cent water-soluble carbohydrate. They noted that high rumen VFA concentrations and low pH values were associated with high levels of water-soluble carbohydrate in the pasture. Rumen pH and VFA concentration with four cows fed various pasture species at different stages of growth were studied by Bath, Rook and Rowland (1962). Values given were the means of a 12-hour sampling period and ranged from 5.79 to 6.57 pH units and from 9 to 18 m-equiv VFA per 100 ml rumen liquor for two cows given cut herbage indoors. Corresponding values for two cows grazing pasture similar to that fed indoors were 5.20 to 6.50 pH units and 13 to 18 m-equiv VFA per 100 ml rumen liquor. Lower VFA concentrations were observed when mature grasses were grazed or given indoors.

Changes in rumen VFA concentration and pH values were observed between months by Davey et al.(1962) with three grazing cows. Results for three months are summarised in Table 22.

TABLE 22. Mean pH and VFA Concentration in the Rumen Liquor from Three Cows Grazing Pasture at Different Stages of Growth. (Means are peak values on two days for each month). (Adapted from Davey et al 1962).

	October	November	December
VFA (mM/100 ml) rumen liquor	15.1	12.7	11.6
pH	5.9	6.2	6.5

Rainfall for the season was particularly low and by December the pasture was in a dry fibrous condition. Johns (1955b) observed no consistent changes in the rumen VFA concentration of grazing sheep with changing pasture composition throughout the year. The maximum range in peak VFA concentration was 10.1 to 18.7 mM VFA per 100 ml rumen liquor. Johns (loc.cit.) noted that peak VFA concentrations were generally higher with pasture than with hay, which contrasted with the results of Carrol and Hungate (1954). The latter workers obtained the greatest concentration of rumen VFA with grain fed steers. Hay gave the next greatest concentration and pasture the least. However sampling in the last case was by stomach tube with consequent difficulties in obtaining representative samples.

(2) Proportions of the individual VFAs

The proportions of the individual VFAs in the rumen are dependent, to a large extent, on the composition of the diet. Under most conditions of feeding, acetic acid predominates and the proportion of propionic acid usually exceeds butyric acid. Balch (1960) stated that with diets consisting entirely of hay it was usual to find some 60-70 per cent acetic, 18-20 per cent propionic

TABLE 23. PROPORTIONS OF RUMEN VFAs WITH SHEEP AND CATTLE FED PASTURE

Reference	Species (Number)	Feeding	Feed	Acetic Acid (%)	Propionic Acid (%)	Butyric Acid (%)	Higher Acids (%)	Sol.Carbohyd. (% of d.m.)	REMARKS
Tilley et al. (1960)	Sheep (2)	Stall Fed	S10 white clover	65	21	14		6.0	
			perennial ryegrass	63	22	15		7.9	
			S37 cocksfoot	61	25	14		10.2	
			S24 Perenn.ryegrass	53	30	17		17.6	
Armstrong (1960)	Sheep (2)	Stall fed	S23 Perenn.ryegrass	(1)61.0 ± 1.15	20.9 ± 0.71	13.9 ± 0.58		12.5	(1)Leafy: fibre 21.2 Cprotein 18.6
				(4)65.0	21.4	10.7		10.1	(4)Full seed: fibre 31.2 Cprotein 9.7
Bath et al.(1962)	Cows (2)	Stall fed	Italian Ryegrass Leafy	59.5	23.0	13.0	4.5	24.9	
			Mature	62.8	20.8	13.0	3.4	21.6	
Bath and Rook(1961)	Cattle(2)	Stall fed	S23 Perennial ryegrass at stage of maturity from 1-8	(1)65.8	18.6	12.4	3.2	8.4	Abstract article
				(4)58.5	23.9	15.0	2.6	18.8	
El-Shazly(1952)	Sheep	Stall fed	Frozen grass	(1)65.7	17.1	11.4	4.8		(1) Before feeding
			Clover mixture	(2)54.0	22.6	15.9	2.3		(2) 3-4 hr after
Johns (1955 b)	Sheep (2)	Restricted grazing	Mixed pasture throughout the year	50 - 62	21 - 30	12 - 17	3-10		4-6 hr after grazing Range over the year
Balch & Rowland (1957)	Cattle(2)	Grazing	'Old'permanent pasture	66.3	18.5	11.5	3.7		
Roffler(1961) and Murdoch	Cattle	Grazing	Ryegrass	63.5 ± 0.3	19.2 ± 0.4	10.6 ± 0.6	6.7 ± 0.5		Sampled by stomach tube Abstract article.
Annison et al. (1959)	Sheep(3)	Grazing	Lush pasture	57	26	16	1		Results for 3 sheep on one day
				53	27	16	4		
				60	24	12	4		
Bryant (1961)	Cattle (1)	Grazing	Mixed pasture	73.7	15.5	10.8			Mean of a number of samples
		Restricted Grazing	Mixed pasture	80.4	10.0	9.6			
				74.3	13.4	12.3			
Bath et al.(1962)	Cattle (2)	Grazing	Italian ryegrass Leafy	52.9	22.0	19.7	5.4	24.9	
			Mature	61.0	20.6	14.3	4.1	21.6	
			Perennial ryegrass Leafy	61.4	20.6	14.2	3.8	14.7	
Davey et al. (1962)	Cattle (3)	Grazing	Mixed pasture over different seasons	(1) 67.4	15.6	17.0			(1) Nov.1961
				(2) 69.2	15.6	15.2			(2) Feb/Mar 1962
				(3) 60.8	22.9	16.3			(3) June 1962
Johns, Ulyatt & Glenday (1963)	Sheep	Grazing	Perennial ryegrass	74.5	20.7	4.8			Mean of 8 observations determined at slaughter
			Short rotation ryegrass and white clover	61.4	25.3	12.3			
Bath & Rook (1961)	Cattle (2)	Grazing	S23 Perennial ryegrass at different stages of growth	(1-3)59.7	21.1	15.3	3.9		Mean of 8 observations
				(4-5)57.0	23.0	16.8	3.2		

and 8-10 per cent butyric acid in the rumen liquor and that the addition of concentrates to the hay lowered the proportion of acetic and raised the proportion of propionic acid. This change, where the hay was reduced below eight lb per day and replaced by concentrates, reached its greatest extent where the hay was ground and fed with a high proportion of cooked, starchy concentrates. Shaw (1961) in a review, quoted unpublished results of 44.7 per cent for acetic, 46.1 for propionic and 4.4 for butyric acid for such a ration. Mean values of 40.6 per cent for acetic, 36.5 for propionic and 10.7 for butyric acid were reported by Balch and Rowland (1957) with two cows each fed two lb hay and 24 lb of concentrates.

The limited amount of work with pasture, associated with rumen fermentation, has been chiefly concerned with changes in herbage composition with maturity and differences in the chemical composition between species and varieties. With the alteration in chemical composition of pasture because of increasing maturity, changes might be expected to occur in the nature of rumen fermentation as the season advanced. Card and Schultz (1953) feeding hay, silage and pasture cut at different stages of growth showed that the late cut material was associated with an increase in the proportions of acetic acid and a decrease in butyric acid. Results apparently conflicting with those of Card and Schultz (loc.cit.) were obtained in in vitro studies by Barnet and Reid (1957). They found that as the proportion of fibre in a mixed pasture increased with advance of the season, acetic acid proportions declined and propionic acid increased to the extent that at times it was present in greater proportions than acetic acid. The pH of the material was kept constant at about 6.5. Whilst the results of in vitro studies are likely to differ from those in vivo, Barnet and Reid (loc.cit.) did illustrate that marked differences do occur in rumen fermentation with changes in pasture composition. Johns (1955b) found no obvious change in rumen VFA proportions, with advance of the season, from sheep

grazing a mixed pasture. The fact that the pasture maintained a relatively constant low soluble carbohydrate content throughout the year may have contributed to this. The cows already referred to by Davey et al.(loc.cit.), showed increased rumen acetic acid proportions over the summer months compared with lower proportions when pasture was at a leafier stage earlier in the season. Bath and Rook (1961) in a brief report noted that the proportion of acetic acid in the rumen of two cows was inversely related to the water-soluble carbohydrate content of the S23 perennial ryegrass fed (Table 23). They classified stage of growth from one to eight but no further description was given. As stage of growth progressed, the soluble carbohydrate content of the ryegrass rose and a small decrease occurred in the proportion of acetic acid in the rumen. They also noted that the spring regrowth from the first cutting had a higher soluble carbohydrate content, giving lower rumen acetic acid proportions, compared with autumn regrowth at a comparable stage of growth. Armstrong (1960) fed artificially dried S23 perennial ryegrass at a young leafy or full seed stage to two sheep. Rumen acetic acid proportions were lower and butyric acid proportions were higher with the immature ryegrass (cut 1), compared with the mature material (cut 2) although differences in the VFA proportions were not large (Table 23). In contrast to the results of Bath and Rook (loc.cit.), the soluble carbohydrate content did not vary greatly with increased maturity of the ryegrass.

Results obtained both in vitro and in vivo by Tilley, Deriaz and Terry (1960) showed that increases in the soluble carbohydrate content of pasture gave lower ruminal proportions of acetic and higher proportions of propionic acid. Their results are summarised in Table 23. Later, Terry and Tilley (1961) stated that herbages high in soluble carbohydrate content produced an acid mixture rich in propionic acid. However, further work by these authors (Terry & Tilley, 1962) who fed seven pasture species at different stages of

maturity to a sheep, showed a relatively low correlation between the soluble carbohydrate content of the pasture species and rumen VFA proportions. They stated that factors other than the soluble carbohydrate content were exerting a major influence on rumen acid proportions and suggested that level of intake and frequency of feeding may be important. It should be noted that this latter work was carried out with one sheep, fed different pasture species over successive periods. This approach has obvious limitations on the possibility of interpreting the results obtained. However, Bath, Rook and Rowland (1962) fed various herbage varieties at different stages of maturity to four cows, two fed indoors and two grazing. The proportion of ruminal acetic acid was inversely related to the water-soluble carbohydrate content of the Italian ryegrass at different stages of growth but there was no close relationship with any of the other species fed. They observed that the ruminal proportion of acetic acid was lowest with the cows grazing leafy Italian ryegrass (Table 23). A percentage of acetic acid greater than 62 and of propionic acid less than 19 in the rumen of the grazing cows was observed only on cocksfoot and mature perennial ryegrass. Unfortunately times of sampling were not given. In fact they stated that, only some samples were examined for individual VFAs. The results of Tilley et al. (1960), summarised in Table 23, gave no indication of the higher acetic acid proportions with cocksfoot, indicated in the work of Bath et al. (1962). Johns, Ulyatt and Glenday (1963) claimed that perennial ryegrass gave higher acetic acid values and lower propionic acid values in the rumen of sheep than did a short rotation ryegrass, white clover pasture (Table 23).

Table 23 has been compiled mainly for comparative purposes and includes some references not discussed in the text. It is difficult to reconcile differences in VFA proportions obtained by the various workers

quoted. There is a real lack of standardisation of experimental methods and few reports are given of the level of intake involved, the methods of sampling used or the composition of the pasture. Mean proportions of the VFAs are more often given with no indication of their derivation or variability and many experiments involved only one or two animals. From the results summarised in Table 23, rumen VFA proportions found with pasture feeding under a wide variety of conditions, with both sheep and cattle, ranged from approximately 50 to 74 per cent for acetic, 10 to 30 per cent for propionic and 10 to 21 per cent for butyric acid.

5.2 Variations Between Days

Day to day variations in the pattern of rumen fermentation with two cows on a hay and concentrate diet were observed by Bath and Rook (1963). The results indicated that within-cow differences in the proportions of the acids from diet to diet could be considered meaningful if they exceeded about three per cent of the mean value for acetic and about 10 per cent for propionic and butyric acids. Day to day differences in the proportions of the VFAs were also noted with a grazing cow by Bryant (1961) and Davey et al. (1962). These variations in the proportions of the acids were associated with changes in rumen pH and VFA concentration and may have been due to a combination of factors, such as a change in the composition of the pasture eaten and differences between days in the level of intake and the pattern of feeding.

5.3 Differences Between Indoor Feeding and Grazing

Limited evidence is available on differences occurring in rumen pH and VFA values with animals stall fed or grazing pasture. Bath and Rook(1961), in an abstract, reported that rumen pH values were lower and VFA concentrations were higher with cows grazing pasture compared with cows fed the same material

indoors. They stated that these differences were possibly because of the greater intake and degree of selection of the pasture with the grazing animals. Bath et al.(1962) observed that with cows receiving cut herbage indoors, rumen butyric acid proportions were almost constant at approximately 13 per cent but a greater variation from 12.5 to 21.4 per cent occurred in grazing cows. They also observed that the rumen proportions of acetic acid were lower in the grazing cows (Table 23).

5.4 Variations Between Animals

Limited data are available on variations between animals. Highly significant differences ($P<0.01$) in VFA concentration were seen between cows grazing pasture by Davey et al.(1962), although part of these differences could have been because of differences in intake or degree of selection of the feed. Bath and Rook (1963) fed two cows an identical ration at the same d.m.intake twice daily. Day to day differences in pH and VFA concentration were evident but larger differences were obtained between cows. Some of the results are given in Table 24.

TABLE 24. Variations between Cows and Days in Rumen pH and VFA Concentration using a Mixed Hay and Concentrate Ration. (Adapted from Bath and Rook,1963)

		pH.	VFA (m.equiv/ 100 ml
Day 1	Cow A	6.26	8.34
	B	6.15	9.06
Day 4	Cow A	6.00	8.66
	B	6.01	10.02
Mean of 4 days	A	6.16	8.50
	B	6.11	9.59

Differences in rumen VFA proportions between cows were also noted by Bath and Rook (loc.cit.). The mean differences between cows were comparatively large as shown in Table 25.

TABLE 25. Variations Between Cows in VFA Proportions Using a Mixed Hay and Concentrate Ration. (Adapted from Bath and Rook, 1963).

	Proportions of the VFAs (%)		
	Acetic Acid	Propionic Acid	Butyric Acid
Cow A	61.7 ± 0.71	19.4 ± 0.78	15.3 ± 0.71
Cow B	67.1 ± 0.35	20.7 ± 0.17	9.2 ± 0.10

If such differences do in fact occur, then small differences in VFA proportions between diets would appear to be meaningless, where only one or two animals were being used. Bath and Rook (loc.cit.) stated that such differences may be accounted for by between cow differences in the rate of passage of ingesta from the rumen, which are particularly marked where the diet contains concentrate foods. Other workers have observed differences between animals in rumen pH and VFA values, e.g. Johns (1955b) and Terry and Tilley (1963), but these have been partly attributed to variations in the intake and selection of feeds, between animals.

5.5 The Effects of the Feeding Régime

Varying the frequency of feeding of a hay and concentrate ration to two cows from once to four times daily at a constant d.m. intake had little effect on the daily mean values for rumen pH, VFA concentration and VFA

proportions, although the range of all values was decreased (Bath and Rook, 1963). These results contrast with those of Knox and Ward (1961) who obtained higher mean VFA concentrations and lower acetic acid proportions in the rumen liquor where hay and concentrates were fed to cattle eight times daily, compared with twice daily feeding. With twice daily feeding, two distinct peaks of VFA concentration were obtained but small fluctuations occurred with the eight times feeding.

5.6 Summary - Review of Literature

(1) Experiments with both sheep and cattle showed that changes could occur in pH, VFA concentration and proportions of the individual acids in the rumen liquor with changes in the stage of growth of pasture. Limited evidence showed that the ruminal proportions of acetic acid were inversely related to the water-soluble carbohydrate content of the pasture although no close relationship was seen in two experiments. Generally higher proportions of acetic acid in the rumen liquor were associated with the feeding of mature compared with immature pasture, although limited evidence from one trial showed that the soluble carbohydrate content increased with the maturing of a strain of perennial ryegrass and small decreases in the proportions of rumen acetic acid were obtained. In one experiment no change occurred in the proportions of the VFAs with change in the botanical and chemical composition of pasture.

(2) Variations in the proportions of rumen VFAs, some marked, have been obtained between pasture species.

(3) There is a limited amount of evidence to show that rumen pH and VFA values from pasture, varied between stall feeding and grazing

(4) Rumen VFA proportions found under pasture feeding with both sheep and cattle ranged from approximately 50 to 74 per cent for acetic, 10 to 30 per cent for propionic and 10 to 21 per cent for butyric acid. It was difficult to reconcile the results of different workers because of the wide variation in the experimental methods adopted and the inadequate reporting of results.

(5) Limited evidence was presented on variations between cows and days in the rumen pH and VFA values.

(6) Some evidence showed that the feeding régime adopted may affect rumen pH and VFA values obtained.

CHAPTER 6.

MATERIALS AND METHODS

6.1 General Outline

The general outline of the experiment for both Parts 1 and II is given in Section 2.2 The outline of the experiment and the data to be presented in Part II are given in more detail, as follows:-

(a) The botanical and chemical composition of the pasture fed indoors or grazed.

(b) Variations between days and with advance of the season in rumen pH, VFA concentration and proportions of the individual acids from three cows fed pasture indoors.

(c) Rumen pH, VFA concentration and proportions of the individual acids from three cows grazing pasture (designated mature), similar to that fed indoors in (b) above.

(d) Rumen pH, VFA concentration and proportions of the individual acids from two cows grazing pasture in spring 1963 (designated immature).

(e) Variations between cows in rumen pH and the concentration and proportions of the VFAs.

6.2 Botanical Composition of the Pasture

For botanical composition in all experiments, representative samples of approximately 100 g of green material were taken and the main components of the pasture separated out and dried, to enable the comparison to be made on a dry weight basis. This was carried out in duplicate on the fourth day of each treatment period for the indoor experiment, i.e. on each rumen sampling day for the a.m. and p.m. feed combined. The dried material fed on the same day was

sampled and ground in a "Wiley" mill, placed in an air tight jar and stored at -10°C for chemical analysis.

6.3 Chemical Composition of the Pasture

Analyses of the pasture fed on rumen sampling days for moisture, ash, crude fibre, crude protein, either extract and nitrogen-free extract were made by methods recommended by the A.O.A.C. (1960) with the exception that a saturated boric acid solution was used to collect the ammonia distilled, in the determination of nitrogen (Meeker and Wagner 1933).

6.4 Variations Between Days and with Advance of the Season

The design of the experiment as three 3 x 3 latin squares with the squares repeated in each of three months from November, 1962 to January, 1963 inclusive, is given in Section 2.3. This design permitted the testing of differences between days within squares for rumen pH and VFA concentration.

The data obtained in the indoor feeding experiment for VFA proportions, were analysed as a randomised block design (Section 2.8). The blocks in the design corresponded to days in the latin squares. The calendar dates for each block and the corresponding period in the latin squares are given in Table 26. The materials and methods were those described in Part I.

6.5 Grazing Studies

(1) Three cows grazing mature pasture

As part of an independent project, three lactating dairy cows were grazed on the same area from which pasture was being cut for the indoor experiment. The three cows were those used in the indoor experiment. Grazing was carried out over two, one-week periods from 18 to 25 October and 21 to 28 November, 1962. The cows had free access to water. Sampling from the rumen for pH and VFA determinations took place on the last two days of each grazing period. The first sample was taken prior to grazing when the cows had been yarded for three hours during and after the a.m. milking. Thereafter the cows were yarded,

sampled and returned to pasture with a minimum of delay. Sampling times were determined by the routine already established in this independent project and were at two-hourly intervals throughout the day. Times of sampling are shown in Table 27. Sampling and chemical methods were as described in Chapter 2.

(2) Two cows grazing immature pasture

Both cows 98 and 69, used in the indoor experiment, were unavailable . The animals used were Cow 15 and Cow 45, the latter being one of those used in the indoor experiment. The objects were to obtain additional information on rumen fermentation with pasture differing markedly from that fed in 1962/63. The two cows were grazed on rapidly growing pasture in Spring 1963 over two one-week periods. Two areas were used, Field A being grazed from 18 to 25 September and Field B from 26 September to 4 October, 1963. Except for the sample taken in the yard prior to grazing in the morning, samples were taken in the field on the last day of each weekly period. The cows had free access to water.

Samples of pasture for chemical analysis were taken by plucking from random points within each field. Methods were as described in Chapter 2 and in Sections 6.2 and 6.3.

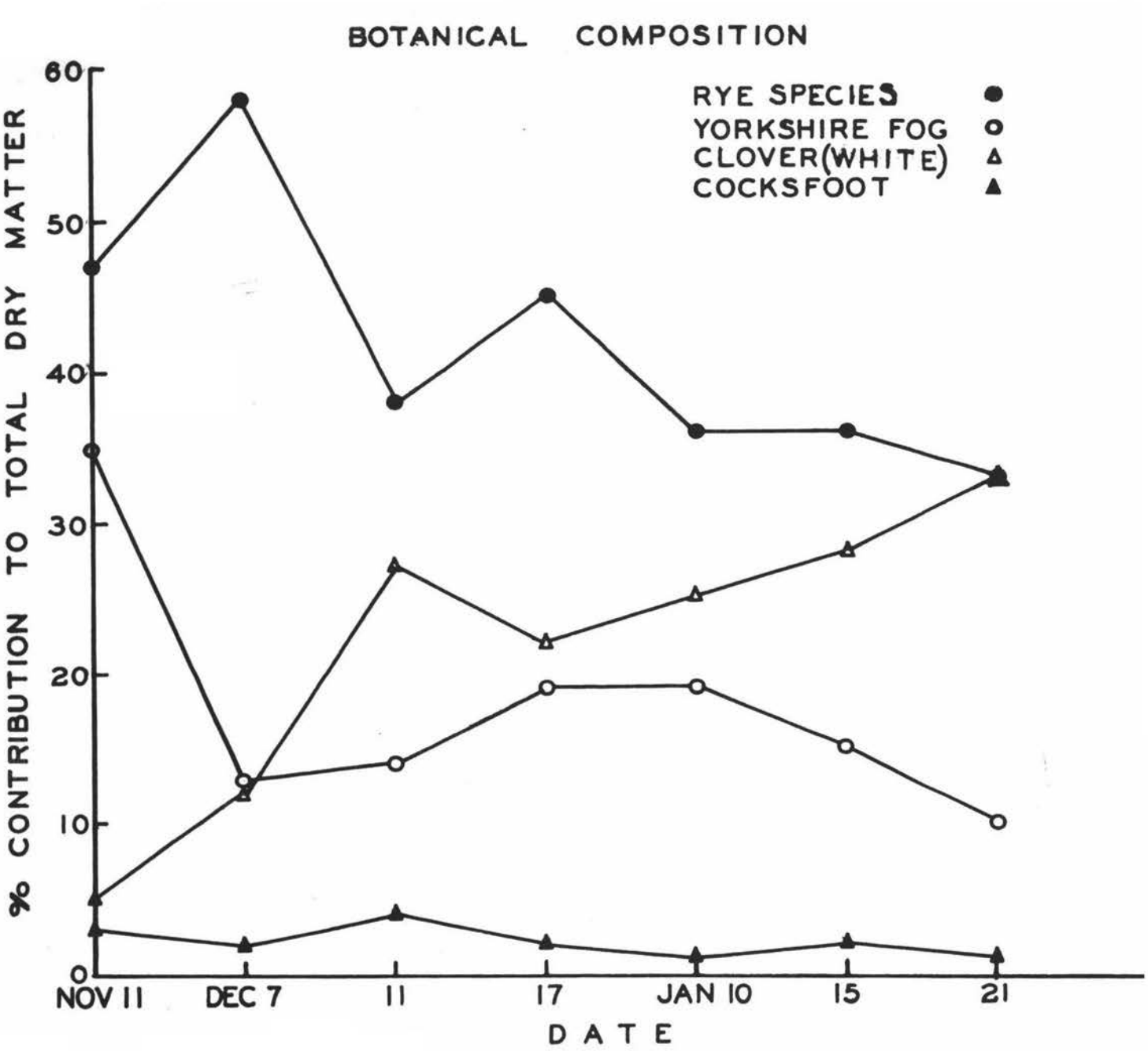


Fig.12: Botanical composition of the pasture fed indoors.

TABLE 26. (a) Percentage Composition (oven dried)of the Pasture Fed to Cows Indoors.

(b) Mean Rumen pH & VFA Concentration from Cows Fed Pasture indoors and their Variation Between Days and Squares.

(c) Mean Rumen VFA Proportions from Cows Fed Pasture Indoors and their Variation Between Blocks.

(d) Mean Molar Quantities of the Individual VFA's from Cows Fed Pasture Indoors and their Variation Between Blocks.

(e) Significance of Differences Between Blocks.

Squares	I			II			III			S.E.
Days in Squares	1	2	3	1	2	3	1	2	3	
Blocks	1	2	3	4	5	-	6	7	8	
Calendar Date	12/11/62	16/11	21/11	6/12	11/12	17/12	11/1/63	15/1	21/1	
(a)Ash (%)	9.6	9.6	9.1	8.4	9.3	9.2	9.6	9.5	9.8	
Crude Protein(%)	15.6	15.4	14.5	13.4	14.7	16.7	16.3	17.9	17.2	
Ether Extract(%)	4.8	4.1	3.9	2.6	4.1	3.1	3.7	4.1	3.6	
Crude Fibre (%)	27.4	28.7	31.7	29.4	28.3	26.9	27.8	26.1	25.0	
N.F.E. (%)	42.6	42.2	40.8	46.2	43.6	44.1	42.6	42.4	44.4	
Dry Matter % of Pasture Fed Indoors	16.7	17.2	17.6	18.0	20.5	20.6	19.4	21.1	27.5	
(b) pH		6.6			6.6			6.7		
	6.4	6.6	6.7	6.7	6.5	6.5	6.7	6.6	6.7	± 0.04 a
VFA (mM/100 ml)		10.40			10.79			10.19		
	10.84	10.66	9.69	9.75	11.32	11.31	9.37	10.28	10.93	± 0.24 a
(c)Acetic Acid (%)	68.9	68.6	67.1	71.2	70.7	-	70.6	71.3	71.2	± 0.50 b
Propionic Acid (%)	18.5	18.4	20.1	16.1	17.0	-	17.5	16.8	17.2	± 0.30 b
Ratio Acetic to Propionic Acid	3.7	3.7	3.3	4.4	4.2	-	4.0	4.2	4.1	
Butyric Acid (%)	12.7	12.9	12.7	12.7	12.6	-	12.3	11.9	11.6	± 0.25 b
(d)Acetic Acid(mM/100ml)	7.43	7.30	6.46	6.92	7.99	-	6.58	7.24	7.77	± 0.16 b
Propionic Acid (mM/100 ml)	2.03	1.97	1.98	1.59	1.94	-	1.68	1.79	1.90	± 0.06 b
Butyric Acid (mM/100 ml)	1.37	1.39	1.55	1.25	1.39	-	1.11	1.24	1.26	± 0.06 b
(e)Acetic Acid (%)	3	2	1	6	5	-	4	8	7	
Significance 1%										
Significance 5%										
Propionic Acid (%)	4	7	5	8	6	-	2	1	3	
Significance 1%										
Significance 5%										
Butyric Acid (%)	8	7	6	5	1	-	4	3	2	
Significance 5%										
Acetic Acid(mM/100ml)	3	6	4	7	2	-	1	8	5	
Significance 1%										
Propionic Acid (mM/100 ml)	4	6	7	8	5	-	2	3	1	
Significance 1%										
Butyric Acid (mM/100 ml)	6	7	4	8	1	-	2	5	3	
Significance 1%										

a. S.E. of days within squares means.
b. S.E. of block means.

CHAPTER 7

RESULTS

7.1 Variations Between Days and with Advance of the Season

(1) Botanical composition of the pasture

As shown in Figure 12 the pasture consisted mainly of ryegrass (Lolium species), white clover (Trifolium repens), Yorkshire Fog (Holcus lanatus L.) and small amounts of Cocksfoot (Dactylis glomerata). A change in botanical composition was evident with advance of the season, with ryegrass and Yorkshire Fog decreasing and white clover increasing. The dates given in Figure 12 also correspond to the rumen sampling days.

(2) Chemical composition of the pasture

The chemical composition and d.m.content of the pasture fed to the cows indoors are given in Table 26 (a). The most noteworthy changes with advance of the season occurred with the d.m.content of the pasture. No apparent relationship was seen between the d.m. content of the pasture and d.m. intake or rumen pH and VFA values.

Changes also occurred with advance of the season in crude fibre and crude protein. The range in either constituent was not great, being 25.0 to 31.7 per cent for crude fibre and 13.4 to 17.9 per cent for crude protein.

The pasture used contained a considerable proportion of stalky material throughout the experiment, regardless of the season. Lower crude fibre values were obtained in January when clover comprised an important fraction of the pasture. Highest crude fibre values occurred in late November and early December before the clover content had increased appreciably and whilst ryegrass was rapidly becoming more mature. However, a higher content of dead material occurred in January. Crude

protein values showed a decrease to December, then increased steadily as the proportion of white clover in the pasture mixture increased. Throughout the season rainfall was adequate for good pasture growth.

(3) Rumen pH and VFA concentration

The results for rumen pH and VFA concentration for days within squares and for squares are summarised in Table 26 (b). Small non-significant differences in rumen VFA concentration were obtained between squares. There were no differences in rumen pH values between squares. Larger differences in both rumen pH and VFA concentration occurred between days within squares. Estimates of the Mean Squares showed that 11 per cent and 8 per cent of the total variation was associated with differences between days within squares for pH and VFA concentration respectively (Appendices 3 and 4). The high crude fibre contents of the pasture on 21 November and 6 December were associated with comparatively low rumen VFA concentrations (Table 26 (a) and (b)). The possibility of a negative relationship occurring between crude fibre and VFA concentration was measured by analysis of regression (Appendix 17). The regression coefficient ($b = -0.179 \pm 0.12$) was not significant. The possibility of a relationship between the crude fibre content of pasture and rumen VFA concentration for treatment A at the 8 p.m. sampling time was also examined but the regression coefficient was not significant ($b = -0.142 \pm 0.13$).

(4) Proportions of the individual VFAs

Differences in the proportions of acetic and propionic acids were highly significant ($P < 0.01$) between blocks and were significant ($P < 0.05$) for butyric acid. Lowest acetic to propionic acid ratios occurred in November (Table 26 (c)). The Blocks x Times interaction was highly significant ($P < 0.01$) for acetic acid but was not significant for propionic and butyric acids. For information on significant differences between blocks see Table 26(e). The blocks in this section of the table were ranked from left to right in increasing proportions of

the VFAs.

Rumen acetic acid proportions were lowest in blocks one, two and three when the crude fibre content of the pasture was high. However the regression of the proportion of acetic acid in the rumen liquor on the crude fibre content of the pasture was non-significant (b = 0.525 $\pm$ 0.28, appendix 17).

(5) Molar quantities of the individual VFAs

Differences between blocks for the molar quantities of all three acids were highly significant ($P<0.01$, Appendix 12). The Blocks x Times interactions were highly significant ($P<0.01$) for each of the three acids. The results for blocks are summarised in Table 26 (d). For information on significant differences between blocks, see Table 26 (e). The blocks in this section of the table were ranked from left to right in increasing molar quantities of the VFAs.

7.2 Grazing Study - Mature Pasture

(1) Rumen pH and VFA concentration

Results for rumen pH and VFA concentration are summarised in Table 27. Differences between months and times of sampling were highly significant ($P<0.01$) for the VFA concentration of the rumen liquor of cows grazing mature pasture (Table 27). With rumen pH, differences between times of sampling were significant ($P<0.05$) but there were no significant differences between months. The Days by Months and Months by Times interactions were significant for pH ($P<0.05$) but there were no significant interations for VFA concentration. With both rumen pH and VFA concentration, no significant differences occurred between cows and days (Appendix 18).

TABLE 27. pH and VFA Concentration in the Rumen Liquor from Cows Grazing Mature Pasture

Grazing	Times of Sampling 9a.m.	11a.m.	1p.m.	3p.m.	5p.m.	Mean	S.E.
25/10/62 pH	6.9	6.5	6.2	6.1	6.1	6.4	
25/10/62 VFA (mM/100 ml)	7.77	10.17	12.40	12.27	12.45	11.01	
28/11/62 pH	6.7	6.3	6.1	6.0	6.2	6.3	
28/11/62 VFA (mM/100 ml)	10.07	12.83	14.31	14.59	14.07	13.17	
pH	6.8	6.4	6.2	6.1	6.1	6.3	± 0.09
Significance 5%		———					
VFA (mM/100 ml)	8.92	11.50	13.36	13.43	13.26	12.09	± 0.34
Significance 5%		———					

(2) Proportions of the individual VFAs

The proportions of the individual VFAs in the rumen liquor of cows grazing mature pasture are given in Table 28.

Chromatographic separations were made on four of the five daily sampling times from 9 a.m. to 3 p.m. inclusive. The separations were carried out independently of those of the main experiment. Unfortunately trouble was experienced with the equipment and many samples from the first day in each month were used without producing satisfactory results. The data for day two for each month are presented, although three separations, two 1 p.m.

samplings for October and one 3 p.m. sampling for November, were unsatisfactory and were omitted. The proportions of the VFAs showed a marked change throughout the day, with highest acetic acid proportions occurring in the 9 a.m. samples. Rather higher acetic and lower propionic acid proportions were obtained with the November samples but the data were too meagre to draw conclusions on this point.

TABLE 28. Proportions of the VFAs in the Rumen Liquor of Cows Grazing Mature Pasture. (21 determinations).

		Acetic Acid (%)	Propionic Acid (%)	Butyric Acid (%)
25/10/62	Mean	65.3	19.4	15.3
	Range within a day	62.5 - 68.5	17.7 - 21.1	13.1 - 16.9
28/11/62	Mean	66.5	17.6	15.9
	Range within a day	63.6 - 72.3	14.4 - 19.6	13.4 - 17.9
S.E. of Means		± 0.8	± 0.6	± 0.4

7.3 Grazing Study - Immature Pasture

(1) The botanical and chemical composition of the pasture

The botanical composition and d.m. content of the pasture grazed in September and October, 1963 are shown in Table 29 (a). The pasture was leafy and free from stalky material.

The chemical composition of the immature pasture is shown in Table 29 (b). The difference in chemical composition from the more mature pasture (Table 26(a)) fed in the main experiment is obvious.

TABLE 29. (a) Botanical Composition of Immature Pasture (% oven dried)

(b) Chemical Composition of Immature Pasture (% oven dried)

(a) Species	Field A25/9/63 (%)	Field 13. 4/10/63 (%)
Ryegrass Species (mainly short rotation)	73.4	57.2
White Clover	20.7	29.0
Prairie grass (Bromus Catharticus)	-	6.9
Other grasses	5.9	6.9
D.M. Content	15.4	13.9
(b)		
Ash (%)	11.8	13.4
Crude Protein (%)	27.0	27.1
Ether Extract (%)	7.5	5.5
Crude Fibre (%)	18.1	18.1
N.F.E. (%)	35.6	35.9

(2) Rumen pH and VFA concentration

Because of the differences in rumen pH and VFA concentration between cows, the data for both animals are presented separately in Table 30.

The ingesta of each cow was frothy from the 11 a.m. sampling period onwards, that for cow 15 being extremely so. This cow lost a considerable amount of ingesta when the fistula plug was removed at the 11 a.m. sampling, on both days. Rumen pH was higher and VFA concentration lower with cow 15 compared with cow 45.

TABLE 30. pH and VFA Concentration in the Rumen Liquor from Cows Grazing Immature Pasture

		9a.m.	11 a.m.	1 p.m.	3 p.m.	Mean	S.E.
25/9/63							
Cow 15	pH	6.9	6.3	6.4	6.2	6.5	
	VFA(mM/100 ml)	8.40	11.50	11.65	10.55	10.52	
Cow 45	pH	6.1	6.2	6.1	6.1	6.1	
	VFA(mM/100 ml)	13.78	12.56	14.82	13.79	13.74	
2/10/63							
Cow 15	pH	6.6	6.3	6.4	6.2	6.4	
	VFA(mM/100 ml)	10.18	10.43	10.40	13.11	11.30	
Cow 45	pH	6.6	6.3	6.3	5.9	6.3	
	VFA(mM/100 ml)	10.65	11.02	12.70	16.00	12.59	
Mean	pH	6.5	6.3	6.3	6.1	6.3	± 0.06
	VFA(mM/100 ml)	10.23	11.38	12.39	13.36	12.04	± 0.50

(3) Proportions of the individual VFAs

The proportions of the individual VFAs in the rumen liquor of cows grazing immature pasture are given in Table 31. Differences were noted between the cows, with the proportion of acetic acid being lower and propionic higher for Cow 45 compared with Cow 15.

TABLE 31. Proportions of the VFAs in the Rumen Liquor from Cows Grazing Immature Pasture (16 determinations).

		Acetic Acid (%)	Propionic Acid (%)	Butyric Acid (%)
29/9/63 Cow 15	Mean	67.5	18.2	14.3
	Range within a day	64.0 - 72.5	13.8 - 19.8	12.7 - 16.5
Cow 45	Mean	64.2	20.5	15.3
	Range within a day	60.8 - 70.5	16.5 - 22.8	13.0 - 16.4
2/10/63 Cow 15	Mean	67.2	17.8	15.0
	Range within a day	64.0 - 68.5	15.7 - 21.0	14.0 - 15.7
Cow 45	Mean	65.0	19.9	15.1
	Range within a day	61.5 - 71.0	16.5 - 22.0	12.5 - 16.5
S.E.		± 0.9	± 0.7	± 0.3

7.4 Comparison of Cows Grazing Pasture with Cows Fed Pasture Indoors.

Because of the fact that different sampling times were used; that days were different and that the comparison could only be made between single cows indoors on the A treatment for any one period, no statistical analysis comparing differences between the two methods of feeding was possible. Thus the comparison between the methods of feeding was limited.

Although the means given in Table 32 for rumen pH and the concentration and proportions of the VFAs have been presented elsewhere, they are included

here to facilitate comparisons between the methods of feeding. Mean pH and the concentration and proportions of the VFAs from the cows stall fed pasture in the main experiment are given for comparative purposes. The results are those for treatment A (see Part I), obtained during the period closest in time to the grazing experiment with mature pasture, i.e. over the period 12 November to 6 December, 1962.

TABLE 32. A Comparison of Rumen pH, VFA Concentration and Proportions of the Acids Obtained from Cows Grazing or Fed Pasture Indoors.

Experiment	pH	VFA (mM/100 ml)	Acetic Acid (%)	Propionic Acid (%)	Butyric Acid (%)
Indoors-Mature (Treatment A)	6.5±0.06	10.80±0.28	69.2 ± 0.7	18.1 ± 0.4	12.7 ± 0.1
Grazing-Mature	6.3±0.09	12.09±0.34	66.0 ± 0.8	18.4 ± 0.6	15.6 ± 0.4
Grazing - Immature	6.3±0.06	12.04±0.50	66.0 ± 0.9	19.1 ± 0.7	14.9 ± 0.3

7.5 Variations Between Cows

(1) Rumen pH and VFA concentration

The results for rumen pH and VFA concentration are summarised in Table 33. There were no significant differences between cows fed pasture indoors, for rumen pH and VFA concentration. However the mean pH value for cow 69 in Square 1 exceeded that for cow 98 by four S.Es. The corresponding value for VFA concentration, for cow 69, was approximately two S.Es in excess of the mean value obtained for cow 98. Data from Treatment A were also included in Table 33, but variations between cows were small.

TABLE 33. Variations Between Cows in Rumen pH and VFA Concentration

	45	69	98	S.E.
pH				
Square I	6.56	6.64	6.52	
II	6.56	6.59	6.58	
III	6.60	6.68	6.63	± 0.03[a]
Means	6.57	6.64	6.58	
Means for Treatment A.	6.54	6.48	6.43	
VFA (mM/100 ml)				
Square I	10.37	10.19	10.62	
II	10.78	10.70	10.89	
III	10.40	10.03	10.15	
Means	10.52	10.31	10.55	± 0.24[a]
Means for Treatment A.	11.46	11.11	11.45	

a S.Es of the means for cows within squares.

(2) Proportions of the individual VFAs

In Table 34 a summary is presented of the variations between cows fed pasture indoors in the proportions of the individual VFAs. Minor non-significant differences occurred between cows for all three acids where all data were considered. Where Treatment A data only were considered, the proportion of acetic acid was lower and propionic acid was higher by approximately four S.Es for cow 98 compared with cow 45. Variations occurring between cows grazing immature pasture have already been noted in Section 7.3 (2) and (3).

TABLE 34. VariationsBetween Cows in the Proportions of the VFAs

	45	69	98	S.E.
Acetic Acid % All treatments	70.3	70.1	69.7	
Treatment A	70.7	70.0	69.0	± 0.55 a
Propionic Acid % All treatments	17.5	17.7	17.9	
Treatment A	16.9	17.6	18.3	± 0.32 a
Butyric Acid % All treatments	12.2	12.2	12.4	
Treatment A	12.4	12.4	12.7	± 0.54 a

a. S.Es of the means for Treatment A

(3) Rates of eating

Highly significant differences ($P < 0.01$) were noted in the rates of eating for the first 30 mins of feeding, between cows, with cow 69 eating at a faster rate than either cow 45 or 98 (Appendix 13). Values obtained

for the first 30 min were 3.5, 3.7 and 4.3 $\pm$ 0.1 lb d.m. for cows 45, 98 and 69 respectively.

CHAPTER 8

. DISCUSSION

8.1 The Botanical and Chemical Composition of the Pasture

The recent data of Hutton (1961) on the chemical composition of dairy pastures appeared to be the most satisfactory, for comparative purposes. Other than Hutton's work, few results have been published on the chemical composition of dairy pastures in New Zealand and changes that occur in them over the season. Hutton (loc.cit.) observed similar changes in botanical composition over the season, to those obtained in the present experiment. The results obtained during the main experiment with mature pasture for crude protein and crude fibre levels were similar to those of Hutton (loc.cit.), obtained over the period November to January. The rather higher crude protein levels in January, in the present experiment (Table 26(a)), could have been because of the higher clover content of the pasture at this time. A small decline in crude protein and increase in crude fibre occurred in late November and early December, in the present experiment. This was probably because of the increased maturity of the grass fraction of the pasture before the clover increased to such an extent, that it influenced the levels of protein and fibre.

Ether extract values obtained in the present experiment were rather higher and more variable than those of Hutton (1961). In noting that his values for ether extract were rather lower than those of other workers, Hutton (loc.cit.) suggested that this may have been because of differences between sheep and cattle pastures and of variations between workers in the treatment and analyses of samples. Hawke(1963) extracted the lipids from

mature and immature ryegrass and obtained mean values of 5.1 per cent and 8.1 per cent of the dry weight respectively. These lipids were obtained in a purified form so that comparable ether extract values determined by the Henneberg method would have been rather higher. In light of Hawke's (loc.cit.) results, the values for ether extract obtained in the present experiment with the immature pasture (Table 29(b)) do not appear to be unreasonable, although they are considerably higher than any result reported by Hutton (loc.cit.). Crude fibre values were low and protein values were high for the immature, compared with the mature pasture. The values for the fibre and protein in the immature pasture were similar to the extreme values reported by Hutton (loc.cit.), and obtained by him in the March/April period.

8.2 Variations Between Days and with Advance of the Season

(1) Rumen pH and VFA concentration

The highly significant differences ($P<0.01$) between days within squares for rumen pH and VFA concentration were probably because of changes in the chemical composition of the pasture, although other factors such as variations in the pattern of feeding may have had an effect. The chemical composition of the pasture, determined by proximate analysis, failed to explain these differences between days. Limited information suggested that low rumen VFA concentrations may be associated with pasture with a high fibre content e.g. Davey et al. (1962). This was not apparent in the present experiment. That the water-soluble carbohydrate content of pasture affected rumen pH and VFA concentration was seen in the work of Tilley et al. (1960) and Bath et al. (1962). It is possible that changes in the water-soluble carbohydrate content of the pasture or changes in the pattern of feeding were responsible for variations in rumen pH and VFA concentration between days.

Nevertheless the differences in pH and VFA concentration were not great and were probably of little physiological importance. The investigation reported here failed to produce information on day to day variations in rumen pH and VFA concentration, uncomplicated by effects such as variations in the diet. The results were therefore not comparable with those of Bath and Rook (1963), who noted variations in rumen pH and VFA values between days, where cows were being fed a constant diet at a constant intake.

Differences between squares in rumen pH and VFA concentration were small. In view of the differences between days within squares for these values this is perhaps surprising, particularly as the period covered by the squares ranged over a considerable change in the nature of the pasture.

Lower rumen pH values and higher VFA concentrations than those of the present experiment were obtained by Tilley et al. (1960); Davey et al. (1962) and Bath et al. (1962), with sheep or cows fed leafy pasture (Review of literature, Page 84).

(2) Proportions of the individual VFAs

The lower acetic to propionic acid ratios obtained in November are difficult to relate to changes in the chemical composition of pasture, determined in the main indoor experiment. Thus, on 21 November, crude fibre was higher and crude protein lower than at any time and yet was associated with lowest acetic acid proportions in the rumen liquor. Low rumen acetic acid proportions have been associated with rations low in fibre e.g. Balch (1960) and Shaw (1961). However there is no evidence that the comparatively small changes in crude fibre of pasture such as were obtained in the present experiment are associated with changes in the proportions of acetic and propionic acid in the rumen. A tentative explanation for the variations in the proportions of acetic and propionic acids between blocks is possible

through the unsupported observation of Ferguson(1963), that the soluble sugar content of legumes was low compared with that for grasses. In addition Bailey (1964) obtained lower water-soluble carbohydrate levels in young white clover, compared with ryegrass. Thus as the clover increased the higher proportions of rumen acetic acid could have been because of a lowered soluble carbohydrate content in the pasture in the present experiment. Bailey (1964) observed that the cellulose content of white clover was considerably lower than that for ryegrass. However, despite the large increase in the proportion of white clover in January, in the present experiment, there was no great decrease in the crude fibre content. It is probable that the lower fibre content of the leafy clover was offset by the increasing maturity and high fibre content of the grass fraction of the pasture and by the greater proportion of dead material in January. It may have been these latter factors combined with a lower water-soluble carbohydrate content in the clover, that produced higher rumen acetic values as the season advanced.

The extent of the differences in the proportions of the VFAs must be kept in perspective. Thus the extreme range of values between blocks, for the proportion of acetic acid, was 67.1 to 71.3 per cent. This is not large, particularly when the errors involved in the separation of the VFAs are considered (Section 3.1 (4)). However the results are difficult to reconcile with those of Johns et al. (1963) (Table 23), except that the more mature grass fraction in January in the present experiment may have had a larger influence on rumen fermentation that its proportion in the pasture mixture would indicate. The results of Johns et al. (loc.cit.) can be criticised on the grounds that the rumen samples were taken after slaughter and there was one determination only for each animal, with no possibility of allowing for diurnal variations in VFA proportions.

In most blocks, the proportion of acetic acid in the rumen liquor decreased with time after feeding but in blocks two and eight the proportions remained comparatively constant over the day. In block three the proportion of acetic acid was comparatively high prior to the a.m. feed but fell to the lowest value for all blocks by 8 p.m. No explanation could be offered for these interactions. Changes with time after feeding were relatively constant between blocks for propionic acid and peak proportions of the acid occurred at 11 a.m. and 8 p.m. with the exception of block one and four when peak proportions occurred at 2 p.m. With butyric acid, changes in proportions with time after feeding were irregular between blocks. The non-significance of the interaction could only be attributed to the large Block x Treatment x Times interaction for this acid.

The mean proportions of the individual VFAs obtained in the present experiment, particularly for the first three blocks and for the grazing experiments, were in general agreement with some of the results summarised in Table 23, e.g. Armstrong (1960) with mature S23 perennial ryegrass; Davey et al. (1962) with mixed pasture and Balch and Rowland (1957) with "old" permanent pasture. Values obtained in New Zealand by Bryant (1961) with a mixed pasture and by Johns et al. (1963) with perennial ryegrass were the only values for acetic acid higher than those obtained in the present experiment. Failure to make a determination of the proportion of the higher acids, mainly valeric, in the present experiment would result in increases in the percentage of acetic, propionic and butyric acids. The increase would be small and insufficient to account for the high acetic acid proportions in the New Zealand results. A valid comparison of the results of the present experiment, with those of most other workers, was not possible because of the lack of published information on feeding and sampling techniques used and of the chemical composition of the pasture fed. Accepting these

limitations it is apparent that most overseas results show appreciably lower proportions of acetic acid in the rumen of sheep and cattle compared with those obtained in New Zealand (Table 23). Johns (1955a) noted that his values for the water-soluble carbohydrate content of pasture, obtained in New Zealand, were low compared with values quoted by Waite and Boyd (1953) and by Laidlaw and Reid (1952) in the United Kingdom. Bailey (1958) also noted that the total free sugar concentrations in red clover, obtained by workers in France, were higher than his own values for red clover obtained in New Zealand. It is possible that the lower ruminal acetic acid proportions obtained in the United Kingdom compared with New Zealand may be partly related to differences in the soluble-carbohydrate content of the pasture. It should be noted however that Johns (1955b) obtained relatively low proportions of ruminal acetic acid with two sheep grazing a mixed pasture in New Zealand.

(3) Molar quantities of the individual VFAs

The variation in the molar quantities of the individual acids between blocks, reflect variations in VFA concentration and proportions of the acids and are difficult to interpret.

There were no obvious trends in the quantities of acetic acid with advance of the season. For propionic and butyric acids, values were high in November and with one result in December. However the values for propionic acid for the first three blocks were not significantly higher than those for blocks seven and eight, obtained in January (Table 26 (e)).

The highly significant Blocks x Times interaction ($P < 0.01$) for all three acids appeared to be mainly because values for blocks six, seven and eight were lowest at 5 p.m. but highest at 8 p.m. compared with other blocks. That these results were confined to data obtained in January may be of significance, but no explanation was evident.

Most workers dealing with rumen fermentation refer to the individual acids as a percentage of the rumen liquor. It is possible that the results presented as quantities of VFA per 100 ml of the rumen liquor may be more informative in some cases in that they describe actual concentrations of the individual acids in rumen liquor.

8.3 Comparison of Cows Grazing Pasture with Cows Fed Pasture Indoors

(1) Grazing mature pasture

(a) Rumen pH and VFA concentration

Higher mean rumen VFA concentrations and lower mean pH values were noted with the grazing cows, compared with those fed ad libitum indoors. This agreed with the results of Bath and Rook (1961) and Bath et al. (1962). That these differences were likely to be real effects was indicated by the S.Es given in Table32 .

The comparison of the data from the grazing and stall feeding experiments, obtained at different dates, can be criticised and imposed a limitation on the conclusions that could be drawn. However this situation could only be improved with an experiment specifically designed to compare the two systems of feeding. The fact that the grazing period in November was preceeded and followed by a period of stall-feeding, both of which varied from the grazing results, lent support to the argument that real differences did exist between the two methods of feeding.

Except for pre-feeding samples, both rumen pH and VFA concentration were comparatively uniform throughout the day under grazing conditions. This contrasted with the results gained indoors, where a distinct depression in VFA concentration occurred at 5 p.m. The indications from this were that the grazing cows fed for longer periods over the day, compared with the stall fed animals. The significant ($P < 0.05$) Day x Months interaction for pH was probably because of one low unexplained value on day two in

October. The significant Times x Months interaction for pH was also probably because of one unexplained result.

(b) Proportions of the individual VFAs

The more important differences between the two methods of feeding were the lower mean acetic acid and higher mean butyric acid proportions obtained from the rumen liquor of the grazing cows, compared with the mean values obtained with the stall fed animals. There appeared to be little variation in the proportions of propionic acid between stall feeding and grazing. These results were in agreement with those Bath et al. (1962). However, butyric acid levels with the stall fed animals in the present experiment were not as constant as was implied by Bath et al. (loc.cit.). The differences in proportions of the acids could possibly be attributed to a higher intake of pasture with the grazing cows, or to a greater degree of selection of the pasture, or to a different pattern of feeding. In view of the results obtained in the present experiment (Part I), where intake differences of 40 per cent caused only minor changes in VFA proportions, it is likely that most of the effect was because of the greater opportunity for selection under grazing conditions or of the different pattern of feeding. The differences seen in rumen VFA concentration and proportions between grazing and stall feeding on pasture, points to the difficulty of relating results obtained indoors to the grazing situation. Work on this aspect by Hardison, Reid, Martin and Woolfolk (1954), showed that free-grazing steers ate pasture containing a higher proportion of protein and ether extract and a lower proportion of fibre than did animals fed the same pasture indoors.

(2) Grazing immature pasture

Despite the large difference in chemical composition between mature and immature pasture (Table 26(a) and 29(b), rumen pH, VFA concentration and

the proportions of the acids were not greatly different. Whilst the mature pasture was stalky, the bottom contained good leafy growth. The grazing cows may have selected more of this leafy portion of the pasture than was indicated by the sample analysed for chemical composition in the indoor experiment. The immature pasture was uniformly leafy and with the method of sampling used (Section 6.5 (2)), the sample taken for chemical analysis was more likely to be representative of that which was grazed. This argument, that the cows grazing the mature pasture were obtaining material likely to be lower in crude fibre and higher in crude protein (Hardison et al., 1954) than that sampled for chemical analysis, may help to explain the small differences in rumen VFA concentration and proportions observed between cows grazing mature and immature pasture. It does not explain the relatively small differences in rumen VFA values between cows grazing immature pasture and those fed indoors on the mature pasture. Under stall feeding conditions the sample of pasture taken for chemical analysis was likely to be representative of that which was eaten and the argument used above, based on the efficiency of the sampling of the pasture, would apply to only a minor extent. Comparatively small differences in the proportions of the VFAs in the rumen liquor of sheep fed mature and immature S23 ryegrass were observed by Armstrong (1960) (Review of literature Page 88), despite comparatively large differences in the crude fibre and crude protein content of the grass. The soluble carbohydrate content of the immature and mature ryegrass used by Armstrong (loc.cit.) did not differ greatly. These results support the possibility that soluble carbohydrate levels may not have been greatly different between the mature and immature ryegrass used in the present experiment.

8.4 Variations Between Cows

(1) pH and VFA concentration

The lack of variation between cows in rumen pH and VFA concentration in the present experiment was not supported by the limited results of other workers (Review of literature Page92). However differences between animals under grazing conditions reported by Davey et al.(1962) and Johns (1955b)may be complicated by factors such as variation in intake and degree of selection of the pasture. As far as the author is aware, the work of Bath and Rook (1963) is the only example where variations between cows in rumen VFA values have been specifically examined. Their experiment was limited to one feeding experiment with two cows. Sampling from the rumen was carried out over four days at the end of the feeding period. This illustrates the limited extent of the information available on differences in rumen VFA values between cows. As an investigation into variations in rumen pH and VFA concentration between cows, the present experiment gave inconclusive results because of the complication of changing pasture composition and the different treatments imposed.

(2) Proportions of the individual VFAs

Variations in the proportions of the VFAs between cows were of minor importance in this experiment. The variations observed between animals where Treatment A data only was considered, although significant ($P<0.05$), were not large. Moreover, two low and unexplained values for acetic acid of 62.6 and 60.3 per cent, for cow 69 on Treatment A, contributed largely to the lower mean value obtained for this animal. Comparatively large variations in the proportions of the VFAs between two cows were observed by Bath and Rook (1963). The limited extent of this data has already been mentioned. As the present experiment was not set up to examine critically,

variations between cows in rumen VFA proportions on a given diet, the results were inconclusive although they suggested that with pasture, differences may not be as great as those obtained by Bath and Rook (loc.cit.) who used a hay and concentrate ration. Variations were noted under controlled conditions in the present experiment with cows grazing the immature pasture. One of the cows was more prone to bloat and frothiness of the rumen contents was more marked. This cow had higher pH values, lower VFA concentrations and higher acetic acid proportions in the rumen liquor than the other animals used. However the evidence was insufficient to establish a relationship between bloat and VFA values in the rumen.

(3) Rates of eating

From the results obtained it appeared that rate of eating, as it applied to the first 30 min of feeding, had no influence on rumen pH, VFA concentration or VFA proportions in that variations between cows in rates of eating were not reflected in different rumen VFA values. No other work appears to have been carried out on the effect of rate of eating on rumen fermentation although a number of workers, in explaining variations in their results, have suggested that different rates of eating between animals may have been important.

8.5 Summary Part II

1. Variations in pH, VFA concentration and proportions of the VFAs in the rumen of lactating dairy cows, grazing or stall-fed pasture, which varied in its stage of maturity and chemical composition were studied. Variations in rumen pH and VFA values between cows, were also examined.

2. In the indoor experiment, pasture was fed at three levels to three lactating Jersey cows (See Part I). The design of the experiment of three 3 x 3 Latin Squares repeated in each of three months, from November, 1962 to January, 1963 inclusive, enabled an examination to be made of variations in rumen pH and VFA values with advance of the season.

3. Variations between squares in rumen pH and VFA concentration were small despite changes in the botanical and chemical composition of the pasture, as the season advanced. Differences between days within squares were highly significant ($P < 0.01$). Possible reasons for this are discussed.

4. There were no significant differences in rumen VFA proportions between cows. The cow effects were ignored and the data analysed as a randomised block design. Differences between blocks in the proportions of acetic and propionic acid were highly significant ($P < 0.01$) and for butyric acid they were significant ($P < 0.05$). Acetic acid proportions were lower and propionic acid higher in November compared with December or January. The chemical composition of the pasture, as determined by the Henneberg method, failed to explain these observed differences in the proportions of the VFAs. The differences in proportions of the acids were not large. The possible causes of the changes in the VFA proportions with advance of the season are discussed.

5. Molar quantities of propionic and butyric acids were higher in November than the other two months. Acetic acid varied between blocks but in an irregular manner.

6. Rumen pH, VFA concentration and proportions of the acids were similar for cows grazing pasture whether mature or immature. Possible reasons for this similarity are discussed.

7. Limited evidence showed that rumen pH was lower and VFA concentration was higher with cows grazing pasture, whether mature or immature, compared with cows fed pasture indoors. The proportions of acetic acid were lower and butyric acid higher in the rumen liquor of the grazing cows compared with those fed indoors. The differences were not great. Possible reasons for these differences are discussed.

8. With many overseas results, mainly in the United Kingdom, the ruminal proportions of acetic acid from pasture were lower than results obtained in New Zealand. However in one New Zealand experiment low acetic proportions were obtained.

9. Minor variations in rumen pH, VFA concentration and proportions of the individual acids occurred between cows, fed indoors or grazing mature pasture. Differences in all values were noted between two cows grazing immature pasture.

PART III

MILK PRODUCTION AND COMPOSITION AND RUMEN FERMENTATION

CHAPTER 9

RUMEN VFA PROPORTIONS IN RELATION TO MILK PRODUCTION AND COMPOSITION

INTRODUCTION

The association between the proportions of the VFAs in the rumen and the butterfat content of the milk of the dairy cow has been widely recognised. Limited evidence is also available for an association between the proportions of the VFAs in the rumen and the solids-not-fat (SNF) content of the milk of the cow. Lactating cows were used in the present experiment and the opportunity was taken to measure the production and composition of their milk. The collection of these data was subsidiary to the main aim of the experiment which was to examine some of the factors affecting rumen pH and VFA values with cows fed pasture. It was judged worthwhile however, to round off this thesis with a brief consideration of rumen VFA proportions in relation to the production and composition of the milk of the cows fed pasture in the indoor experiment and during the period of grazing in October and November, 1962. This is presented in Chapter 9. Chapter 10 is concerned with the general discussion and conclusions for the whole thesis.

9.1 Materials and Methods

Throughout the indoor trial and also when the cows were grazing in October and November, 1962, the milk yield was recorded for each cow at each milking and a sample of milk was taken for the estimation of the butterfat

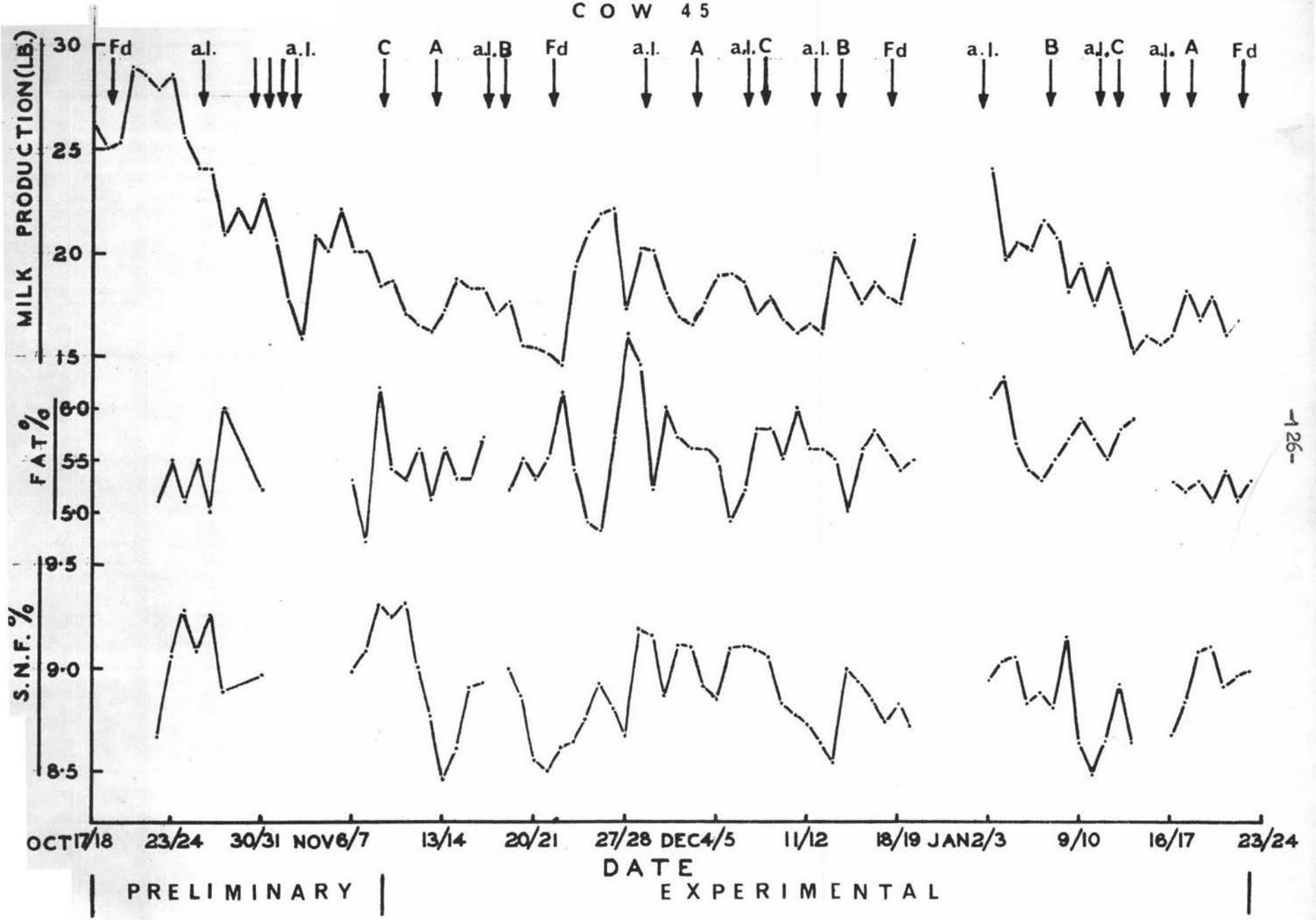


Fig.13: Yield and composition of the milk from cow 45 (A,B and C treatments; a.l. ad libitum feeding; Fd, grazing)

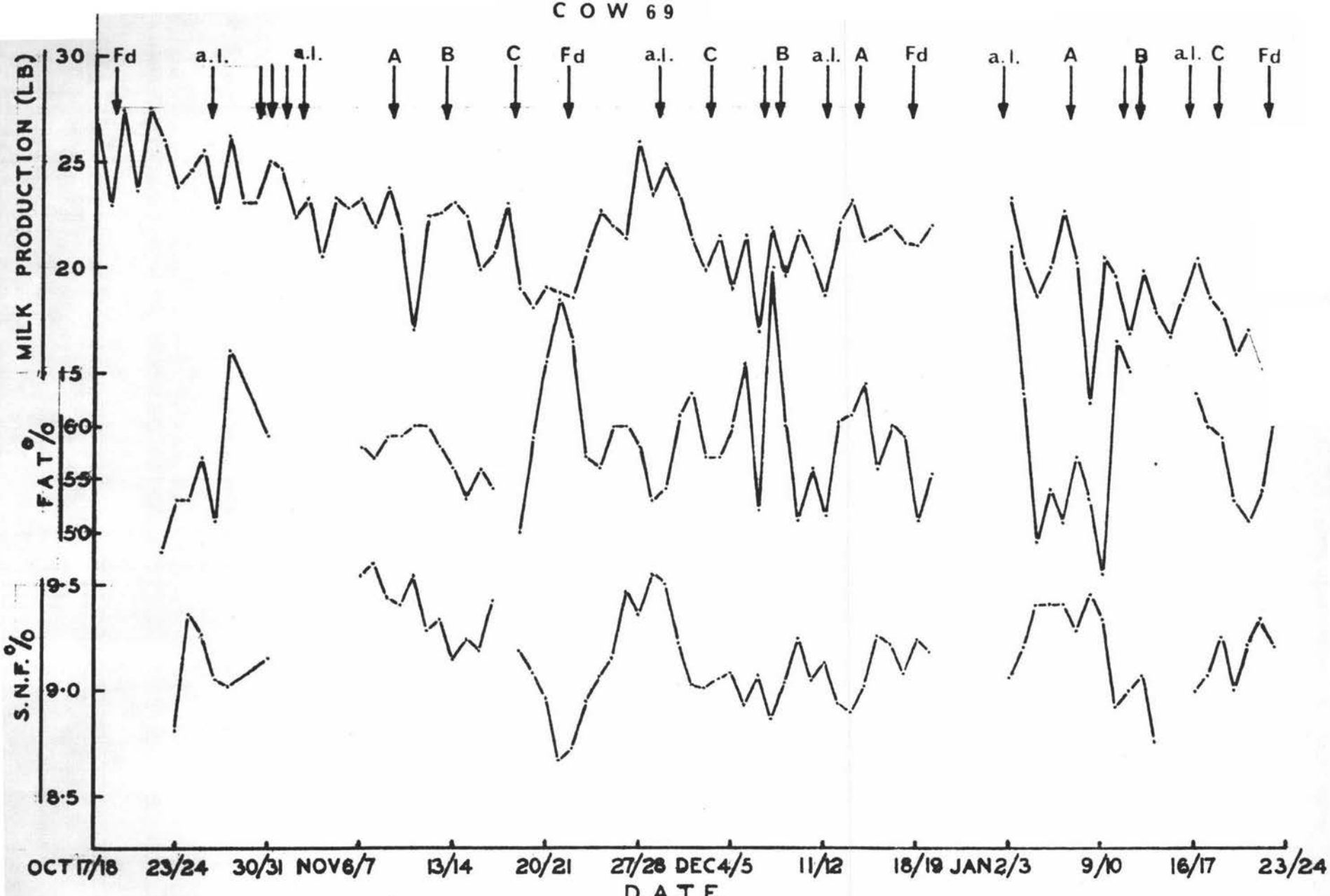


Fig.14: Yield and composition of the milk from cow 69 (A,B and C treatments; a.l. ad libitum feeding; Fd, grazing).

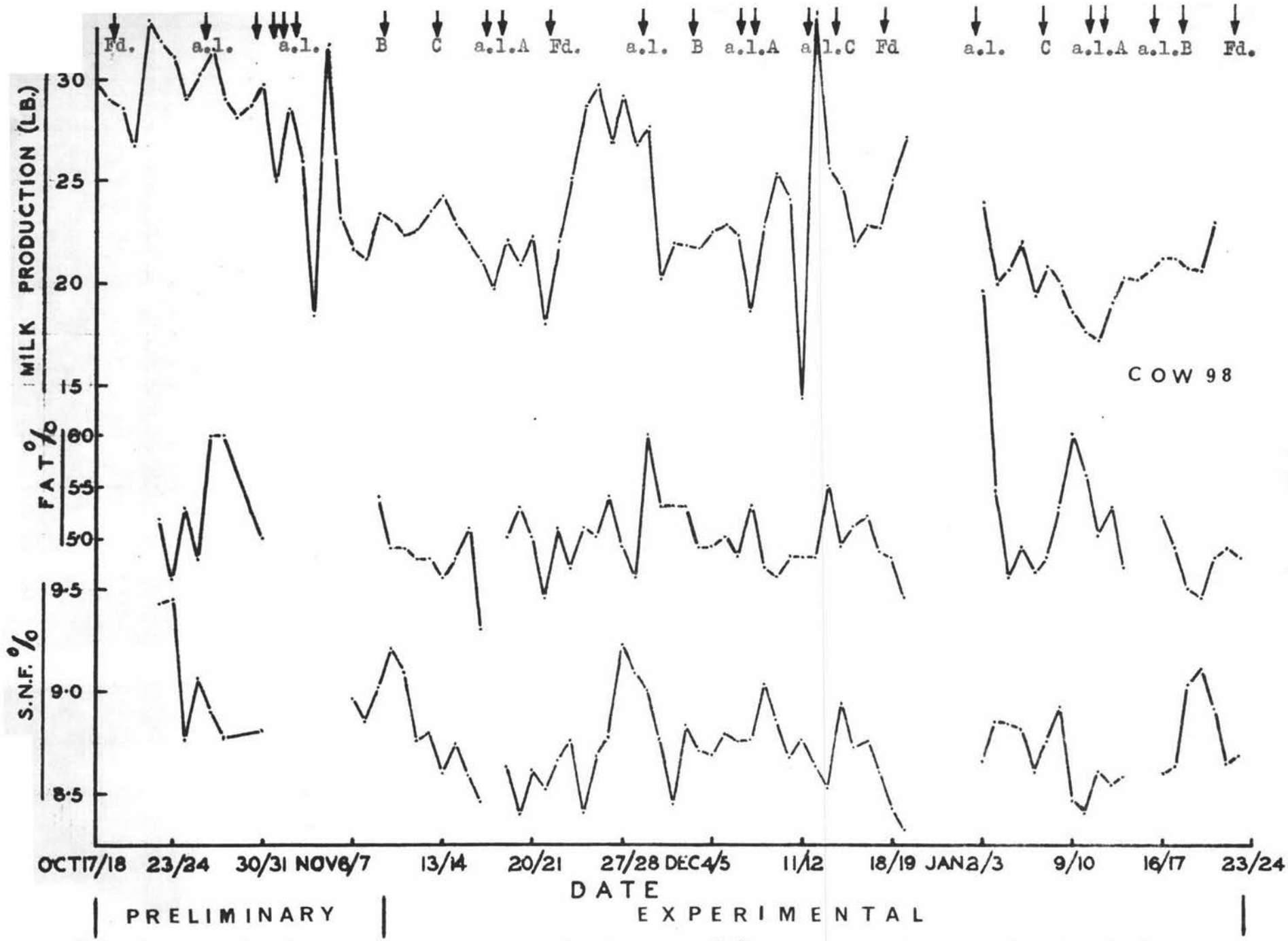


Fig.15: Yield and composition of the milk from cow 98 (A,B and C treatments; ad libitum feeding; Fd, grazing).

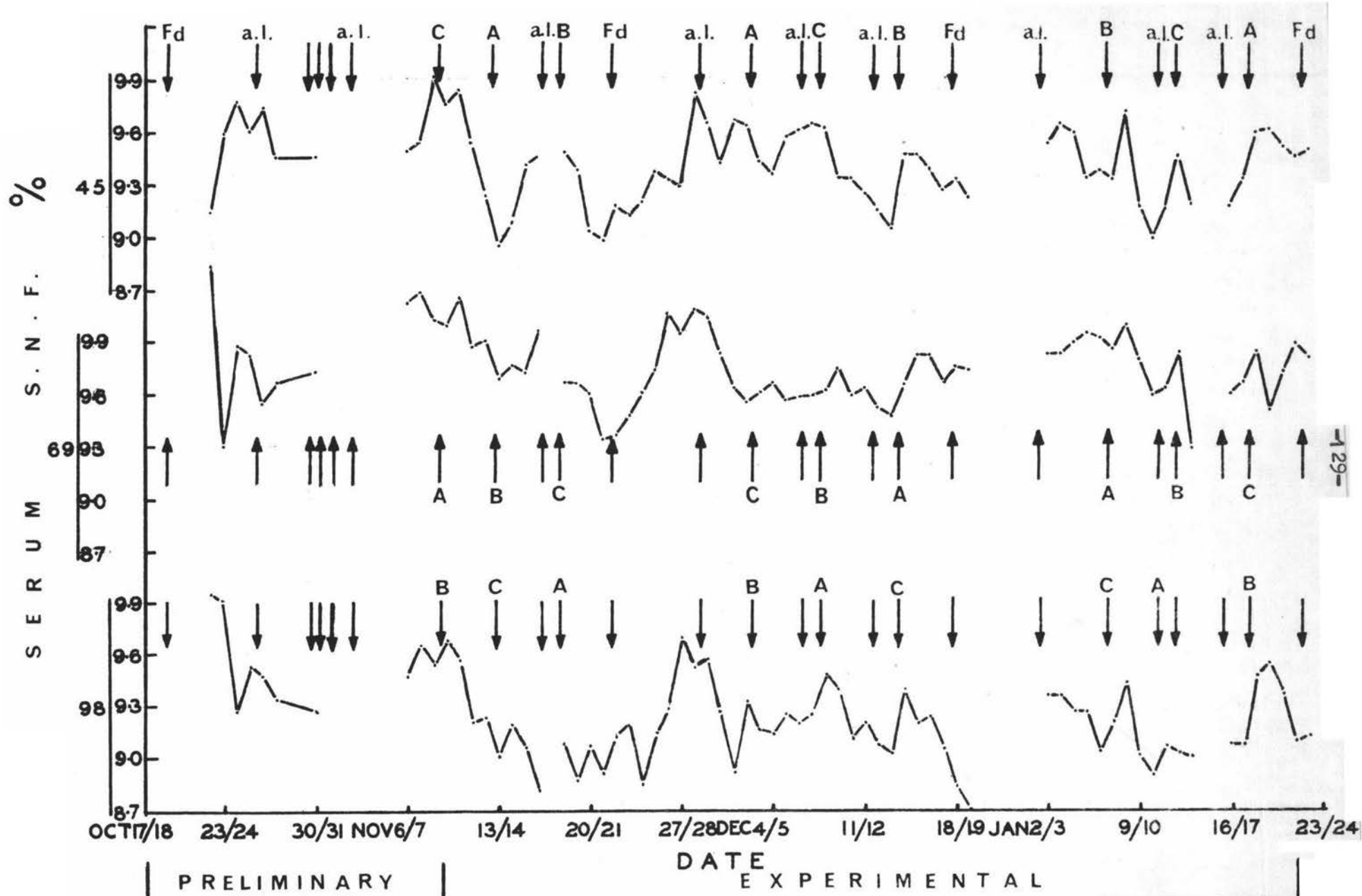


Fig.16: Serum SNF content of the milk of the three cows. (A,B and C treatments; a.l, ad libitum feeding; Fd, grazing.)

and SNF contents. The cows were milked twice daily at 6.30 a.m. and 4.30 p.m.

The total solids content of the milk was estimated by the gravimetric method and the butterfat content by the Gerber method. The SNF content was obtained by difference and the serum SNF was calculated by :

$$\frac{\text{SNF} \times 100}{100 - \text{Butterfat \%}}$$

9.2 Results

The results for each cow for milk yield , SNF, butterfat and serum SNF content are shown in Figures 13,14,15 and 16. The following points can be observed from these figures. The milk yield and the SNF content of the milk increased noticeably when the cows were changed from indoor feeding to grazing. A depression in milk yield, SNF content and serum SNF content occurred when the cows were changed from the A to the C treatment. The butterfat content of the milk appeared to respond in a variable manner to changes in the level of feeding.

9.3 Discussion

(1) Experimental design

The design of the indoor experiment was inadequate for a study of changes in milk production and composition with changes in the level of feeding, in that the interval between treatments of four to five days was insufficient to allow carry-over effects to disappear. Periods in a change-over design should be of sufficient length for treatments to produce their effects and to allow for the elimination of the major portion of the residual effects (Patterson and Lucas,1962). The periods in the latin squares in the present experiment appeared to be of a sufficient length to comply with these conditions for rumen pH and VFA values. To comply with these requirements for milk production and composition would have imposed a physical limitation on

the time available for the design used, which was aimed at obtaining information on some factors affecting rumen pH and VFA values in the cow.

(2) The solids-not-fat content of the milk

There is some evidence that the proportion of propionic acid in the rumen is associated with the SNF content of the milk of the dairy cow (Balch, Balch, Bartlett, Bartrum, Johnson and Turner,1955; Rook and Balch,1961). It appeared that changes in the SNF content of the milk occurred only in response to large changes in rumen propionic proportions.

A reduction in the energy plane of nutrition lowered the SNF content of the milk of dairy cows (Riddet, Campbell, McDowall and Cox,1942; Campbell, Flux and Patchell,1955). Rook (1961) postulated that the effect of plane of nutrition on the SNF content of milk was mediated through its influence on propionic acid production. No great reliance can be placed on the data on milk production and composition obtained in the present experiment but it appeared that changes in the SNF content of the milk with changes in the level of intake were not associated with parallel changes in the proportions of propionic acid in the rumen.

Although the data were limited and some were missing, the serum SNF content of the milk increased when the cows were changed from indoor feeding to grazing, particularly for the period 21 to 28 November (Figure 16). This increase was associated with a decrease in the proportion of acetic acid and an increase in the proportion of propionic acid in the rumen liquor of the cows (Part II). However this was not sufficient evidence for a causal relationship between the proportion of propionic acid in the rumen and the SNF content of the milk. It is possible to speculate that lowered total amounts of propionic acid in the rumen of cows on a restricted intake, rather than the proportions of the acid, may be associated with the fall in the SNF content

of milk.

(3) The butterfat content of milk

A depression in the butterfat content of the milk of the cow with diets low in roughage and high in starchy concentrates or when the roughage is finely ground have been amply confirmed, e.g. Balch, Balch, Bartlett, Cox and Rowland (1952); Balch et al. (1955) and Balch and Rowland (1959). This depression in butterfat content was associated with low acetic and high propionic acid proportions in the rumen. Blaxter (1962) stated that depressions in the butterfat content of milk can be detected when the proportion of propionic acid rises to 25 per cent and the effects are severe with proportions greater than 30 per cent. This statement appeared to be based on the results of Ensor, Shaw and Tellechea (1959). In feeding various combinations of ground or long hay and cooked and uncooked maize, they obtained small decreases in the butterfat content of milk with rations giving 61.9 per cent acetic and 25 per cent propionic acid. Larger decreases in butterfat content (approximately 50 per cent) were obtained with rations giving 53.9 per cent acetic and 31.1 per cent propionic acid. There appeared to be no other evidence supporting the contention that the butterfat content of milk is depressed when the proportion of propionic acid in the rumen was about 25 per cent. Even so, from the VFA proportions determined in the present experiment, it is unlikely that the pasture used would produce acetic acid levels low enough or propionic acid levels high enough to influence the butterfat content of milk.

(4) The efficiency of milk secretion

It has been suggested by Blaxter (1962) that the proportions of acetic acid in the rumen may have a bearing on the efficiency with which digested energy is transformed to milk in dairy cattle. The data in support of

this contention are meagre, being confined to two feeding trials using various feeds to induce variations in the proportions of the rumen VFAs. In one of the experiments (Elliot and Loosli 1959), sampling from the rumen was by stomach tube, apparently taken at one time four to five hours after feeding. This in itself would impose a serious limitation on the validity of the results. Nevertheless Blaxter (1962) discussed these results in relation to the efficiency of milk secretion in the dairy cow. The figures in Table 35 have been taken from graphs which Blaxter (1962) used in this discussion.

TABLE 35. The Energetic Efficiency of Milk Secretion

(Adapted from Figure 42. Blaxter,1962).

Proportion of acetic acid in rumen (%)	Efficiency of use of Metabolizable energy (%)
38	52
55	72
60	72
65	65
68	59

Many of the results for acetic acid obtained in the present experiment were in the region of 68 per cent or even higher. It would appear that the efficiency of milk secretion under these conditions would be comparatively low. However, the suggestions put forward by Blaxter (loc. cit.),based as they are on such limited results, cannot as yet be considered seriously in anywhere near exact terms. It does however point the direction to future work.

CHAPTER 10

GENERAL DISCUSSION AND CONCLUSIONS

In the absence of satisfactory routine procedures for the measurement of VFA production in the rumen, work has been largely confined to variations in rumen pH, VFA concentration and proportions of the individual acids with different diets. This work has increased since it was shown that there was an association between the proportions of the VFAs in the rumen and some aspects of animal production. A consideration of VFA concentration and the proportions of the individual acids is capable of limited interpretation because of the dynamic conditions within the rumen. The concentration of the VFAs appeared to be important in determining absorption although pH has an effect (Review of literature Page13). Evidence on the relative rates of absorption of the individual VFAs is conflicting (Review of literature Page13) and there appears to be little possibility of using the concentration or proportions of the VFAs to measure the production of VFAs in the rumen. A relationship between total VFA concentration and d.m.intake has been demonstrated but even so it is unlikely to be a good measure of VFA production in the rumen because of the changing rate of flow of digesta to the omasum throughout the feeding cycle.

Whilst it is the total quantity of each of the individual VFAs produced in the rumen that are of major interest, the relative proportions of the VFAs do characterise, to some extent, the type of rumen fermentation, from diet to diet.

A number of factors other than the diet have been shown to affect the VFA values obtained and where comparisons between diets are being made it appears essential to standardise experimental procedures.

The method of sampling from the rumen and the problems of obtaining a representative sample were discussed in Part I. Further work on sampling methods is needed.

Despite the contention of Shaw (1961) to the contrary, serial sampling of the rumen ingesta is essential where fluctuations in the proportions of the VFAs are likely to occur with time after feeding. This also has a bearing on the method of feeding adopted. The variations in the proportions of the VFAs may not only reflect a change in the relative production of the acids because of the diet but a change in the equilibrium between the concentration of the acids and their absorption. It was noted in Part I, Section 4.6 (2) that variations in the pattern of feeding at different levels of intake may have partly contributed to the changes observed in rumen pH and VFA concentration. It is possible that with more marked variations in the pattern of feeding than were observed in the present experiment and with diets giving lower proportions of acetic acid (Bath and Rook,1963), changes in VFA proportions at different levels of intake would have been greater. Despite the small differences in VFA proportions between treatments, obtained in the present experiment, it would appear essential to standardise intake in rumen fermentation studies or at least to appreciate the possible effects of changes in the level of feeding. It would also appear essential to standardise the pattern of feeding between treatments and animals in rumen fermentation studies, particularly in relation to times of sampling. A technique adopted by a number of workers, including Johns (1955b), involves starvation of the animals overnight and feeding them for a fixed period on the rumen sampling day. This may lessen the complication of different VFA concentrations and proportions prior to feeding, affecting the results over the experimental period. It also ensures that rumen sampling time in relation to feeding can be standardised. The method is unsatisfactory in studies involving milk

production where the restricted feeding involved would influence milk yield and composition. The nature of the present experiment, involving different levels of intake, made variations in the pattern of feeding difficult to avoid.

Although differences in rumen pH and VFA values were small between animals in the present experiment, the results were inconclusive and the possibility of animal differences occurring cannot be ignored in planning experiments.

The chemical composition of the pasture, as determined in the present experiment, failed to explain variations, or the lack of them, in rumen VFA proportions. This applied not only to the main indoor experiment but also to the grazing results, particularly those obtained in Spring, 1963. The comparison between the cows grazing spring pasture and those stall-fed mature pasture can be criticised in that experimental techniques were not standardised. Further work comparing the pattern of rumen fermentation between mature and immature pasture and between different species under similar conditions, is indicated. Nevertheless differences in rumen VFA proportions were comparatively small between the immature and mature pasture in the present experiment, despite large differences in chemical composition. Any work involving rumen fermentation studies needs additional information on the chemical composition of the pasture. This may be provided, in part, by a determination of the water-soluble carbohydrate content, although there are likely to be other constituents and factors affecting the pattern of rumen fermentation.

Under the conditions of the experiment reported here, a restriction in the intake of pasture did not appear to affect the SNF content of the milk of three dairy cows through a change in the proportion of the VFAs in the rumen liquor. However the data on milk composition were capable of only a limited interpretation. The experiment would need to be designed

specifically towards obtaining information on the effects of low levels of intake on the SNF content of milk and relating this to changes in rumen fermentation. Problems associated with changes in the stage of growth in pasture would make interpretation difficult and the use of a diet of constant chemical composition is indicated.

In view of the results obtained by Tilley et al. (1960) with S24 perennial ryegrass; Annison et al. (1959) with lush pasture, and Bath et al. (1962) with leafy Italian ryegrass and leafy perennial ryegrass (Table 23), the possibility of certain pasture species at a particular stage of growth affecting the butterfat content of milk should not be ignored and further work in this direction is indicated, despite the rather high proportions of acetic and low proportions of propionic acid obtained in the present experiment.

In conclusion adequate reporting of the results obtained in rumen fermentation studies involving VFA proportions is essential and errors of estimate should be given. It must also be appreciated that small differences in VFA proportions are often difficult to interpret because of the number of variables, other than the diet, which are involved.

REFERENCES

ANNISON, E.F.(1954). Biochem.J. 57, 400

ANNISON, E.F., LEWIS, D. & LINDSAY, D.B.(1959). J.agric.Sci. 53, 34.

A.O.A.C.(1960). 'Official Methods of Analysis of the Association of Official Agricultural Chemists' 9th edn. p.p.233 et seq. Menasha, Wisconsin: George Banta.

ARMSTRONG, D.G. (1960). Proc. 8th Int.Grassl.Congr., 485.

BAILEY, C.B. (1961) Brit. J.Nutr. 15, 443.

BAILEY, R.W. (1958). J.Sci.Fd.Agric. 9, 748

BAILEY, R.W. (1964).(In Press)

BALCH, C.C. (1950). Brit. J.Nutr. 4, 361

BALCH, C.C. (1958). Brit.J.Nutr. 12, 330

BALCH, C.C. (1959). Proc. Nutr.Soc. 18, 97

BALCH, C.C. (1960). Proc. 8th Int.Grassl.Congr., 528.

BALCH, C.C., BALCH, D.A., BARTLETT, S., COX, C.P.& ROWLAND, S.J. (1952). J.Dairy Res. 19, 39

BALCH, C.C., BALCH, D.A.,BARTLETT, S., BARTRUM, M.P.,JOHNSON, V.W., & TURNER, J. (1955). J.Dairy Res. 22, 270.

BALCH, C.C., BALCH, D.A., JOHNSON, V.W., & TURNER, J. (1953). Brit.J.Nutr. 7, 212.

BALCH, D.A. & ROWLAND, S.J. (1957). Brit.J.Nutr. 11, 288.

BALCH, C.C. & ROWLAND, S.J. (1959). J.Dairy Res.26, 162

BARCROFT, J., McANALLY, R.A. & PHILLIPSON, A.T. (1944).J.exp Biol. 20, 120.

BARNETT, A.J.G. & REID, R.L. (1957). J.agric.Sci. 48, 315

BARNETT, A.J.G. & REID, R.L. (1961). 'Reactions in the Rumen' P.153 London: Edward Arnold

BATH, I.H. & ROOK, J.A.F. (1961). Proc.Nutr.Soc. 20, xv.

BATH, I.H. & ROOK, J.A.F. (1963). J.agric.Sci. 61, 341.

BATH, I.H., ROOK,J.A.F. & ROWLAND, S.J. (1962). 16th Int.Dairy Congr., 49.

BLAXTER, K.L. (1962). 'The Energy Metabolism of Ruminants'. pp.213,255 & 258 London: Hutchinson.

BLAXTER, K.L., WAINMAN, F.W. & WILSON, R.S. (1961). Anim. Prod. 3, 51.

BRIGGS, P.K., HOGAN, J.P. & REID, R.L. (1957). Aust.J.agric.Res. 8, 674

BRYANT, A.M. (1961). M.Agr.Sci. Thesis. (Lodged in Massey University of Manawatu Library).

BURROUGHS, W., GERLAUGH,P., SILVER, E.A. & SCHALK, A.F. (1946). J.Anim.Sci. 5, 338.

CAMPBELL, I.L., FLUX,D.S. & PATCHELL, M.R.(1955). Proc. N.Z.Soc.Anim. Prod. 15, 132.

CARD, C.S. & SCHULTZ, L.H. (1953). J.Dairy Sci. 36, 599.

CARROL, E.J. & HUNGATE, R.E. (1954). Appl.Microbiol. 2, 205.

COOP, I.E. (1949). N.Z.J.Sci. & Tech. 31A, 1

DANIELLI, J.F., HITCHCOCK, M.W.S., MARSHALL, R.A. & PHILLIPSON, A.T.(1945). J.exp.Biol. 22, 75.

DAVEY, A.W.F., ROBINSON, G.S. & CAMPBELL, I.L. (1962). Unpublished.

DAVEY, A.W.F. & WILSON, G.F.(1960). Unpublished.

DOBSON, A.(1961). In 'Digestive Physiology & Nutrition of the Ruminant' P.68. ed.Lewis, D. London: Butterworths.

DUNCAN, D.B. (1955). Biometrics. 11, 1.

ELLIOT, J.M. & LOOSLI, J.K. (1959). J.Dairy Sci. 42, 843.

EL-SHAZLY, K. (1952). Biochem.J. 51, 640.

ENSOR, W.L. (1959). Ph.D.Thesis, Univ. Maryland. Cited by Shaw J.C.(1961) in Proc. 8th Int. Congr. Hamburg, 29.

ENSOR, W.L., SHAW, J.C. & TELLECHEA, H.F. (1959).J.Dairy Sci. 42, 189.

FERGUSON, W.S. (1963) In 'Animal Health, Production and Pasture' P.92. Eds. Worden, A.N.,Sellers, K.C. & Tribe, D.E. London: Longmans.

FISHER, R.A. & YATES, F. (1957). Statistical Tables for Biological, Agricultural & Medical Research. Edinburgh: Oliver & Boyd.

GRAY, F.V., JONES, G.B. & PILGRIM, A.F. (1960). Aust. J.agric.Res. 2, 382

GRAY, F.V. & PILGRIM, A.F. (1951). J.exp.Biol. 28, 83.

HALE, E.B., DUNCAN, C.W. & HUFFMAN, C.F. (1940). J.Dairy Sci. 23, 953.

HARDISON, W.A., REID, J.T., MARTIN, C.M. & WOOLFOLK,P.G. (1954). J.Dairy Sci. 37, 89.

HAWKE, J.C. (1957). J.Dairy Res. 24, 366.

HAWKE, J.C. (1963). J.Dairy Res. 30, 67.

HUTTON, J.B.(1961) N.Z.J.agric.Res. 4, 583

HUTTON, J.B. (1963). Proc. N.Z.Soc.Anim. Prod. 23, 39.

HYDÉN, S. (1961). In 'Digestive Physiology & Nutrition of the Ruminant'. P.35. ed.Lewis, D. London: Butterworths.

JAMES, A.T. & MARTIN, A.J.P. (1952). Biochem J. 50, 679.

JOHNS, A.T. (1955 a). N.Z.J.Sci.& Tech. 37A, 301.

JOHNS, A.T. (1955 b) N.Z.J.Sci. & Tech. 37A, 323.

JOHNS, A.T., ULYATT, M.J. & GLENDAY, A.C. (1963). J.agric. Sci. 61, 201.

KAY, R.N.B. & HOBSON, P.N. (1963). J.Dairy Res. 30, 261

KNOX, K.L. & WARD, G.M. (1961). J.Dairy Sci. 44, 1550.

LAIDLAW, R.A. & REID, S.G. (1952). J.Sci.Fd Agric. 3, 19

MÄKELÄ, A. (1956). Acta.Agral.Fenn. 85, 1.

MARKHAM, R. (1942).Biochem.J. 36, 790.

MASSON, M.J. & PHILLIPSON, A.T. (1951). J.Physiol. 113, 189.

MATSCHER, R., BORGHI, G. & BEGHELLI, V. (1957). Arch.Vet.Ital. 8, 513

McANALLY, R.A. (1944). J.exp.Biol. 20, 130.

McCARTHY, R.D., SHAW, J.C., McCARTHY, J.L., LAKSHMANAN,S. & HOLTER,J.B.(1958). Proc.Soc. exp.Biol. Med. 99, 556.

MEEKER, E.W. & WAGNER, E.C. (1933) J.Ind.Eng.Chem. (Anal.Edn.) 5, 396

PATTERSON, H.D. & LUCAS, H.L. (1962). Tech.Bul. No.147. Nth.Carolina agric. Expt. Sta. and U.S.D.A.

PHILLIPS, G.D. (1961). Res.vet.Sci. 2, 209

PHILLIPSON, A.T. (1942). J.exp.Biol. 19, 186

PHILLIPSON, A.T. & McANALLY, R.A. (1942). J.exp.Biol. 19, 199

REID, R.L., HOGAN, J.P. & BRIGGS, P.K. (1957). Aust. J.agric.Res. 8, 691.

RIDDET, W.,CAMPBELL, I.L., McDOWALL, F.H. & COX, G.A.(1942). N.Z.J.Sci.& Tech. 23, 80A.

ROFFLER, R.E. & MURDOCH, F.R. (1961). J.Dairy Sci. 44, 1204.

ROOK,J.A.F. (1961) Dairy Sci.Abstr. 23, 251.

ROOK, J.A.F. & BALCH, C.C. (1961). Brit.J.Nutr. 15, 361.

SHAW, J.C. (1961). Proc. 8th Int.Congr.Anim.Prod. Hamburg, 29.

SMITH, V.R. (1941). J.Dairy Sci. 24, 659.

SNEDECOR, G.W. (1961) 'Statistical Methods' 5th Edn.Ames: Iowa State College Press.

TERRY, R.A. & TILLEY, J.M.A. (1961). Exp.Grassl.Res.Inst.,Hurley No.13, 79.

TERRY, R.A. & TILLEY, J.M.A. (1962) Exp.Grassl.Res.Inst., Hurley. No.14, 58.

TERRY, R.A. & TILLEY, J.M.A. (1963). Exp. Grassl.Res.Inst., Hurley.No.15, 47.

TILLEY, J.M.A., DERIAZ, R.E. & TERRY, R.A. (1960). Proc. 8th Int.Grassl.Congr. 533.

TURNER, A.W. & HODGETTS, V.E. (1955). Aust.J.agric.Res. 6, 115.

WAITE, R. & BOYD, J. (1953). J.Sci.Fd Agric. 4, 257.

WILM, H.G. (1945). Biomet. Bul. 1, 16.

WILLIAMS, V.J. & CHRISTIAN, K.R. (1956). N.Z.J.Sci. & Tech. 38A, 403.

WILSON, A.D. (1963). Aust. J.agric.Res. 14, 680.

APPENDICES

A COPY OF THE DATA USED IN THIS THESIS HAS BEEN LODGED WITH THE DAIRY HUSBANDRY DEPARTMENT, MASSEY UNIVERSITY OF MANAWATU.

Appendix

1 Analysis of Variance:
(a) Rumen sampling
(b) pH duplicate readings
(c) Live-weights of the cows

2 Analysis of Variance:
(a) Dry matter intake - Preliminary period
(b) & (c) Dry matter intake - Experimental period

3 Analysis of Variance:
Rumen pH

4 Analysis of Variance
Rumen VFA concentration

5 Analysis of Regression of pH on d.m. Intake

6 Analysis of Regression of pH on d.m. Intake for 8 a.m. 11 a.m. and 8 p.m. Sampling Times

7 Analysis of Regression of VFA Concentration on d.m. Intake

8 Analysis of Regression of VFA Concentration on d.m. Intake for 8 a.m. 11 a.m. and 8 p.m. Sampling Times

9 Proportions of the Individual VFAs - Analysis of Variance of Squares I and III

10 Proportions of the Individual VFAs - Analysis of Variance of the Randomised Blocks

11 Proportions of the Individual VFAs - Analysis of Variance for the 8 a.m. 11 a.m. and 2p.m. Sampling Times

12 Molar Quantities of the Individual VFAs - Analysis of Variance

13 Rates of Eating - Analysis of Variance

Appendix

APPENDIX I

(a) Rumen Sampling - Sample taken from the middle of ingesta and from the removed and mixed contents.

VFA (mM/100 ml rumen liquor).

Analysis of Variance.

Source	d.f.	S.S.	M.S.	F	Result
Samples	1	0.2242	0.2242	< 1	N.S.
Error	22	34.4376	1.5653		
Total	23	34.6618			

(b) pH Duplicate readings -

Analysis of Variance.

Source	d.f.	S.S.	M.S.	F	Result
Duplicates	1	0.0409	0.0409	< 1	N.S.
Error	370	40.8449	0.1104		
Total	371	40.8858			

(c) Live-Weights (lb)

Analysis of Variance.

Source	d.f.	S.S.	M.S.	F	Result
Cows	2	100888.5	50444.2	99.2	* *
Days	9	9087.2	1009.7	2.0	N.S.
Error	18	9156.2	508.7		
Total	29	119131.9			

APPENDIX 2

(a) Dry matter intake (lb) - Preliminary period.

Analysis of Variance.

Source	d.f.	S.S.	M.S.	F	Result
Cows	2	6.6472	3.3236	6.58	*
Days	6	59.2470	9.8745	19.54	* *
C x D	12	6.0654	0.5054		
Times	1	8.6770	8.6770	7.58	*
DXT	6	69.3450	11.5575	10.10	* *
Error	13	14.8791	1.1445		
Total	40	164.8607			

(b) Dry matter intake (lb) - Experimental period.
(Mean over four days)

Analysis of Variance.

Source	d.f.	S.S.	M.S.	F.	Result
Squares	2	156.0967	78.0484	1.86	N.S.
Periods in Squares	6	251.6736	41.9456	16.1	* *
Cows in Squares	6	11.8993	1.9832	< 1	N.S.
Treatment	2	1243.7875	621.8938	239.2	* *
Error	10	26.0031	2.6003		
Total	26	1689.4602			

(c) Dry matter intake (lb) - Experimental period.
(For the 4th day of each period)

Analysis of Variance.

Source	d.f.	S.S.	M.S.	F	Result
Squares	2	27.8934	13.9467	< 1	
Periods in Squares	6	118.7197	19.7866	15.35	* *
Cows in Squares	6	19.9298	3.3216	2.58	N.S.
Treatment	2	338.7551	169.3776	131.40	* *
Error	10	12.8901	1.2890		
Total	26	518.1881			

APPENDIX 3

(a) Rumen pH

Analysis of Variance

Source	d.f.	S.S.	M.S.	F	Result
Squares (s)	2	0.1207	0.0604	< 1	
Cows in Squares (C:S)	6	0.2215	0.0369	1.47	N.S.
(Cows (C)	2	0.1513	0.0757	3.02	N.S.
(C x S	4	0.0702	0.0176	< 1	
Days in Squares (P:S)	6	1.5579	0.2597	10.35	* *
Treatments (T)	2	1.8231	0.9116	38.96	* *
T x S	4	0.0938	0.0234	< 1	
Error (CP:S)	6	0.1508	0.0251		
Total 1	26	3.9678			
Times (Ti)	5	9.4114	1.8823	73.82	* *
Ti x T	10	0.2552	0.0255	1.30	N.S.
Error (CP:STTi)	120	2.3476	0.0196		
Total 2	161	15.9820			

(b) Rumen pH - Components of Variance

	CP:STTi	TiT	Ti	CP:S	TS	T	P:S	C:S	S	Estimate	%
S	1			6			18	18	54	-0.0032	-
C:S	1			6				18		0.0007	0.6
P:S	1			6			18			0.0131	10.9
T	1	9			18	54				0.0166	13.8
TS	1	9			18					-0.0001	-
CP:S	1			6						0.0008	0.7
T	1	9	27							0.0688	57.2
TiT	1	9								0.0007	0.6
CP:STTi	1									0.0196	16.2
										0.1203	

APPENDIX 4

(a) Rumen VFA Concentration (mM/100 ml)

Analysis of Variance.

Source	d.f.	S.S.	M.S.	F	Result
Squares (S)	2	20.0892	10.0446	<1	
Cows in Squares(C:S)	6	6.5318	1.0886	<1	
(Cows (C)	2)	3.9138)	1.9569	<1	
(C x S	4)	2.6180)	0.6545	<1	
Days in Squares(P:S)	6	130.4550	21.7425	8.68	* *
Treatments (T)	2	222.9118	111.4559	207.80	* *
T x S	4	4.8274	0.5364	<1	
Error 1	6	15.0350	2.5058		
Total 1	26	399.8502			
Times (Ti)	5	1055.0740	201.0148	115.12	* *
(AMVPM	1	119.9998	119.9998	53.08	* *
(Ti after feeding	2	884.0974	442.0487	195.53	* *
(Interaction	2	0.9768	0.4884	<1	
Ti x T	10	17.4596	1.7460	<1	
(T x (AMVPM)	2	0.4230	0.2115	<1	
(T x (Ti after feeding)	4	15.4276	3.8569	1.71	N.S.
(Interaction	4	1.6090	0.4023	<1	
Error 2	119	269.0393	2.2608		
Duplicates within samples	161	0.4861	0.0030		
Total	321	1691.9092			

(b) Rumen VFA Concentration (mM/100ml)

Components of Variance.

	Dupli-cates	CP:STTi	TiT	Ti	CP:S	TS	T	P:S	C:S	S	Estimate	%
S	1	2			12			36	36	108		
C:S	1	2			12				36		-0.0395	-
P:S	1	2			12			36			0.5343	8.4
T	1	2	18			36	108				1.0111	15.8
TS	1	2	18			36					-0.0478	-
CP:S	1	2			12						0.0207	0.3
Ti	1	2	18	54							3.6825	57.7
TiT	1	2	18								-0.0029	-
CP:STTi	1	2									1.1289	17.7
Dupli-cates	1										0.0030	0.1

APPENDIX 5

Analysis of Regression of pH on d.m. Intake

pH, $\bar{y}$ = 6.59

d.m.Intake, $\bar{x}$, = 21.07

Source	d.f.	SSx	SSy	SPxy	b
Squares	2	27.8934	0.0185	0.4787	0.0172
Cows in squares	6	19.9298	0.0372	0.0488	0.0024
Periods in squares	6	118.7197	0.2550	-3.1403	-0.0264
Treatments	2	338.7551	0.3035	-10.0931	-0.0300
Error	10	12.8901	0.0405	-0.2674	-0.0207
Total	26	518.1881	0.6547	-12.9733	-0.0250

Tests for significance of b.

Source	d.f.	Lin.Reg.	Error	E.M.S.	F	Results	S.E.b
Squares	1	0.0082	0.0103	0.0103	<1	N.S.	
Cows in squares	5	0.0002	0.0370	0.0074	<1	N.S.	
Periods in squares	5	0.0829	0.1721	0.0344	2.4	N.S.	
Treatments	1	0.3028	0.0007	0.0007	433	*	0.0014
Error	9	0.0055	0.0350	0.0039	1.4	N.S.	
Total	25	0.3243	0.3304	0.0132	24.6	* *	0.0025

APPENDIX 6

Analysis of Regression of pH on d.m. Intake for 8 a.m., 11 a.m., and 8 p.m. Sampling Times.

(a) 8 a.m.

SSy = 0.8146 SSx = 515.2381 SPxy = -6.0490

b = -0.0117 S.E.b= $\pm$ 0.008

$\bar{y}$ = 7.00 $\bar{x}$ = 20.71

S.S. due to regression = 0.0605

Test for significance of b.

Source	d.f.	S.S.	M.S.	F	Result
Lin.Reg.	1	0.0605	0.0605	2.00	*
Error	25	0.7541	0.0302		
Total	26	0.8146			

(b) 11 a.m.

SSy = 0.4474 SSx = 518.1881 SPxy = -7.6983

b = -0.01486 S.E.b = $\pm$ 0.005

$\bar{y}$ = 6.55 $\bar{x}$ - 21.07

S.S. due to regression = 0.1144

Test for signifance of b.

Source	d.f.	S.S.	M.S.	F	Result
Lin.Reg.	1	0.1144	0.1144	8.60	* *
Error	25	0.3330	0.0133		
Total	26	0.4474			

(c) 8 p.m.

SSy	=	1.4550	SSx	=	518.1881	SPxy	= -17.6535
b	=	-0.0341	S.E.b	=	$\pm$ 0.008		
$\bar{y}$	=	6.30	$\bar{x}$	=	21.07		

S.S. due to regression = 0.6015

Test for significance of b.

Source	d.f.	S.S.	M.S.	F	Result
Lin.Reg.	1	0.6015	0.6015	17.64	* *
Error	25	0.8535	0.0341		
Total	26	1.4550			

(d) Test for significance of differences between regressions.

Source	d.f.	SSx	SPxy	SSy	SSy[1]	d.f.[1]	M.S.[1]	F	Result
A 8 a.m.	26	515.2381	-6.0490	0.8146	0.7541	25			
B 11a.m.	26	518.1881	-7.6983	0.4474	0.3330	25			
C 8 p.m.	26	518.1881	-17.6535	1.4550	0.8535	25			
Deviations from individual regressions					1.9406	75	0.0259		
A + B + C	78	1551.6143	-31.4008	2.7170	2.4030	77			
Difference between regressions					0.4624	2	0.2312	8.93	* *

APPENDIX 7

Analysis of Regression of VFA Concentration on d.m. Intake

VFA $\bar{y} = 10.46$

d.m. Intake $\bar{x} = 21.07$

Source	d.f.	SSx	SSy	SPxy	b
Squares	2	27.8934	1.6706	0.4585	0.0164
Cows in Squares	6	19.9298	0.3326	-1.3569	-0.0681
Periods in Squares	6	118.7197	10.8583	19.1763	0.1615
Treatments	2	338.7551	18.6180	79.1023	0.2335
Error	10	12.8901	1.8784	1.3745	0.1066
Total	26	518.1881	33.3579	98.7547	0.1906

Tests for significance of b.

Source	d.f.	Lin.Reg.	Error SS	E.M.S.	F	Result	S.E.b.
Squares	1	0.0075	1.6635	1.6635			
Cows in Squares	5	0.0924	0.2402	0.0480	1.92	N.S.	
Periods in Squares	5	3.0969	7.7614	1.5523	1.99	N.S.	
Treatments	1	18.4704	0.1476	0.1476	125.14	+	$\pm$0.021
Error	9	0.1465	12.7436	1.4160	< 1		
Total	25	18.8226	14.5353	0.5814	32.37	* *	$\pm$0.034

+ Significant at the 10% level

Regression Coefficients for VFA Concentration on d.m. Intake for Each Individual Day in the Latin Squares.

Squares	Days		
	1	2	3
I	0.110	0.353	0.170
II	0.178	0.346	0.181
III	0.282	0.154	0.327

APPENDIX 8

Analysis of Regression of VFA Concentration on the d.m. Intake for 8 a.m., 11 a.m. and 8 p.m. Sampling Times.

(a) 8 a.m.

SSy = 69.0996 SSx = 515.2381 SPxy = 146.0804

b = 0.2835 S.E.b = $\pm$ 0.05

$\bar{y}$ = 7.55 $\bar{x}$ = 20.71

S.S. due to regression = 41.4138

Test for significance of b.

Source	d.f.	S.S.	M.S.	F	Result
Lin. Reg.	1	41.4138	41.4138	37.40	* *
Error	25	27.6858	1.1074		
Total	26	69.0996			

(b) 11 a.m.

SSy = 49.5667 SSx = 518.1881 SPxy = 91.5546

b = 0.1767 S.E.b = $\pm$ 0.050

$\bar{y}$ = 11.34 $\bar{x}$ = 21.07

S.S. due to regression = 16.1777

Test for significance of b.

Source	d.f.	S.S.	M.S.	F	Result
Lin.Reg.	1	16.1777	16.1777	12.11	* *
Error	25	33.3890	1.3356		
Total	26	49.5667			

(c) 8 p.m.

SSy = 52.2919 SSx = 518.1881 SPxy = 104.7854

b = 0.2022 S.E.b = ± 0.049

$\bar{y}$ = 12.68 $\bar{x}$ = 21.07

S.S. due to regression = 21.1876

Test for significance of b.

Source	d.f.	S.S.	M.S.	F	Result
Lin.Reg.	1	21.1876	21.1876	17.03	* *
Error	25	31.1043	1.2442		
Total	26	52.2919			

(d) Test for significance of difference between regressions.

Source	d.f.	SSx	SPxy	SSy	SSy[1]	d.f.[1]	M.Si	F	Result
A 8 a.m.	26	515.2381	146.0804	69.0996	27.6858	25			
B 11 a.m.	26	518.1881	91.5546	49.5667	33.3890	25			
C 8 p.m.	26	518.1881	104.7854	52.2919	31.1043	25			
Deviation from individual regressions					92.1791	75	1.2290		
A + B + C	78	1551.6143	342.4204	170.9582	95.3908				
Difference between regressions					3.2117	2	1.6058	1.31	N.S.

APPENDIX 9

Proportions of the Individual VFA s.

Analysis of Variance of Squares I and III

(a) Acetic Acid (%)

Source	d.f.	S.S.	M.S.	F	Result
Squares	1	1126.54	1126.54	40.70	* *
Cows in Squares	4	122.44	30.61	<1	
Periods in Squares	4	110.61	27.65	<1	
Treatments	2	52.56	26.28	<1	
Error	6	253.35	42.22		
Total	17	1665.50			

(b) Propionic Acid (%)

Source	d.f.	S.S.	M.S.	F	Result
Squares	1	564.48	564.48	10.21	*
Cows in Squares	4	84.68	21.17	2.32	N.S.
Periods in Squares	4	221.14	55.28	6.05	*
Treatments	2	5.67	2.83	<1	
Error	6	54.85	9.14		
Total	17	930.82			

(c) Butyric Acid (%)

Source	d.f.	S.S.	M.S.	F.	Result
Squares	1	140.00	140.00	37.77	* *
Cows in Squares	4	8.57	2.14	<1	
Periods in Squares	4	15.89	3.97	<1	
Treatments	2	106.27	53.13	5.02	N.S.
Error	6	63.52	10.59		
Total	17	334.25			

APPENDIX 10

Proportions of the Individual VFA s

Analysis of Variance - Randomised block design, for all sampling times.

(a) Acetic Acid (%)

Source	d.f.	S.S.	M.S.	F	Result
Treatments (T)	2	12.90	6.45	1.74	N.S.
Blocks (B)	7	300.62	42.95	7.21	* *
T x B	14	51.81	3.70		
Times (Ti)	5	320.19	64.04	46.74	* *
T x Ti	10	38.84	3.88	2.83	* *
B x Ti	35	208.87	5.96	4.35	* *
T x B x Ti	70	95.85	1.37		
Total	143	1029.08			

(b) Propionic Acid (%)

Source	d.f.	S.S.	M.S.	F	Result
Treatments (T)	2	1.58	0.79	< 1	
Blocks (B)	7	202.19	33.17	12.11	* *
T x B	14	25.78	1.84		
Times (Ti)	5	248.08	49.62	22.87	* *
T x Ti	10	46.24	4.62	2.13	*
B x Ti	35	96.09	2.74	1.22	N.S.
T x B x Ti	70	152.23	2.17		
Total	143	772.19			

(c) Butyric Acid (%)

Source	d.f.	S.S.	M.S.	F	Result
Treatments (T)	2	21.45	10.72	9.32	* *
Blocks (B)	7	28.46	4.07	1.84	N.S.
T x B	14	16.15	1.15		
Times (Ti)	5	34.94	6.99	4.31	* *
T x Ti	10	14.50	1.45	< 1	
T x Ti	35	77.54	2.21	1.36	N.S.
T x B x Ti	70	113.35	1.62		
Total	143	306.39			

(d) **Components of Variance**

	BTTi	BTi	TiT	Ti	TB	B	T	Estimates		
								Acetic Acid	Propionic Acid	Butyric Acid
T	1				6		48	0.09	0.02	0.22
B	1	3				18		2.37	1.83	0.21
TB	1				6			6.39	-0.05	-
Ti	1	3		24				2.60	2.06	0.28
TiT	1		8					0.29	0.55	-
BTi	1	3						1.53	0.19	0.19
BTTi	1							1.37	2.17	1.62

APPENDIX 11

Proportions of the Individual VFA s.

Analysis of Variance - Randomised block design for the 8 a.m., 11 a.m. and 2 p.m. sampling times.

(a) Acetic Acid (%)

Source	d.f.	S.S.	M.S.	F	Result
Treatments (T)	2	28.48	14.24	5.78	*
Blocks (B)	7	172.32	24.62	3.43	*
T x B	14	34.56	2.47		
Times (Ti)	2	143.83	71.92	20.85	* *
Tr x Ti	4	6.09	1.52	<1	
B x Ti	14	100.24	7.16	2.08	*
T x B x Ti	28	96.56	3.45		
Total	71	582.11			

(b) Propionic Acid (%)

Source	d.f.	S.S.	M.S.	F	Result
Treatments (T)	2	5.35	2.68	2.46	N.S.
Blocks (B)	7	127.59	18.23	4.76	* *
T x B	14	15.23	1.09		
Times (Ti)	2	86.19	43.09	18.89	* *
Tr x Ti	4	15.47	3.87	1.69	N.S.
B x Ti	14	53.58	3.83	1.68	N.S.
T x B x Ti	28	63.88	2.28		
Total	71	367.29			

(c) Butyric Acid (%)

Source	d.f.	S.S.	M.S.	F	Result
Treatments (T)	2	10.84	5.42	5.74	*
Blocks (B)	7	23.95	3.42	1.48	N.S.
T x B	14	13.23	0.95		
Times (Ti)	2	17.99	8.99	4.65	*
T x Ti	4	2.02	0.51	<1	N.S.
B x Ti	14	32.37	2.31	1.20	N.S.
T x B x Ti	28	54.10	1.93		
Total	71	154.49			

APPENDIX 12

Molar Quantities of the Individual VFA s.

Analysis of Variance - Randomised block design.

(a) Acetic Acid (mM/100ml rumen liquor)

Source	d.f.	S.S.	M.S.	F	Result
Treatments (T)	2	46.2420	23.1210	49.66	* *
Blocks (B)	7	36.5750	5.2250	4.04	* *
T x B	14	6.5190	6.4656		
Times (Ti)	5	170.5000	34.1000	143.46	* *
T x Ti	10	2.8460	0.2846	1.20	N.S.
B x Ti	35	45.2620	1.2932	5.44	* *
T x B x Ti	70	16.6424	0.2377		
Total	143	324.5864			

(b) Propionic Acid (mM/100ml rumen liquor)

Source	d.f.	S.S.	M.S.	F	Result
Treatments (T)	2	2.7810	1.3905	23.57	* *
Blocks (B)	7	3.0930	0.4419	4.15	* *
T x B	14	0.7826	0.0590		
Times (Ti)	5	27.4489	5.4898	172.09	* *
T x Ti	10	0.8873	0.0887	2.78	* *
B x Ti	35	4.5651	0.1304	4.09	* *
T x B x Ti	70	2.2341	0.0319		
Total	143	41.7920			

(c) Butyric Acid (mM/100ml rumen liquor)

Source	d.f.	S.S.	M.S.	F	Result
Treatments (T)	2	3.7605	1.8805	9.86	* *
Blocks (B)	7	2.2680	0.3240	4.49	* *
T x B	14	2.6705	0.1907		
Times (Ti)	5	9.3033	1.8607	82.70	* *
T x Ti	10	0.3954	0.0395	1.76	+
B x Ti	35	2.5237	0.0721	3.20	* *
T x B x Ti	70	1.5779	0.0225		
Total	143	22.4993			

APPENDIX 13

Rate of Eating (d.m. lb per 30 min.)

Analysis of Variance (First 30 min. of eating) for Squares II and III.

Source	d.f.	S.S.	M.S.	F	Result
Squares	1	0.8756	0.8756	2.49	N.S.
Cows in Squares	4	2.1830	0.5457	13.81	* *
Periods in Squares	4	1.4092	0.3523	8.92	*
Treatments	2	0.7256	0.3628	9.18	*
Error	6	0.2369	0.0395		
Total	17	5.4303			

APPENDIX 14

Regression Analysis of pH on VFA Concentration

y = pH x = VFA

$\bar{y}$ = 6.60 $\bar{x}$ = 10.46

Source	d.f.	SSx	SSy	SPxy	b	S.E.b
Squares	2	10.0446	0.1207	- 0.8077	-0.0804	
Cows in Squares	6	3.2659	0.2215	- 0.7467	-0.2286	
Periods in Squares	6	65.2275	1.5579	- 7.0901	-0.0187	
Treatments (T)	2	111.4559	1.8231	-14.2547	-0.1279	
Error 1	10	9.9312	0.2446	- 1.4389	-0.1449	±0.0201
Times (Ti)	5	502.5370	9.4114	-67.1950	-0.1337	±0.0146
T x Ti	10	8.7298	0.2552	- 1.0951	-0.1254	
Error 2	119	134.9521	2.3476	-10.6177	-0.0737	±0.0096
Total	160	846.1440	15.9820	-103.2459	-0.1220	±0.0050

Tests for significance of b.

Source	d.f.	Lin.Reg.	Error S.S.	E.M.S.	F	Result
Squares	1	0.0649	0.0558	0.0558	1.16	
Cows in Squares	5	0.1707	0.0508	0.0102		
Periods in Squares	5	0.7707	0.7872	0.1574		
Treatments (T)	1	1.8232	0.0000	0.0000		* *
Error 1	9	0.2085	0.0361	0.0040	52.1	* *
Times (Ti)	4	8.9840	0.4274	0.1069	84.04	* *
T x Ti	9	0.1373	0.1179	0.0131		
Error 2	118	0.7825	1.5651			
Total	159	12.5960	3.3860	0.0213		

Regression Analysis of pH on VFA Concentration for Separate Times of Sampling

(a) 8 a.m.

x	=	VFA	y	=	pH		
$\bar{x}$	=	7.55	$\bar{y}$	=	7.00		
SSx	=	69.0996	SSy	=	0.8146	SSxy	= -6.6427
b	=	-0.0961	S.E.b	=	$\pm$0.010	bSPxy	= 0.6386

Test for significance of b.

Source	d.f.	S.S.	M.S.	F	Result
Lin.Reg.	1	0.6386	0.6386	90.71	* *
Error	25	0.1760	0.0070		
Total	26	0.8146			

$\hat{y}$ = 7.73 -0.0961 x

(b) 11 a.m.

$\bar{x}$	=	11.34	$\bar{y}$	=	6.55		
SSx	=	49.5667	SSy	=	0.4474	SSxy	= -2.8983
b	=	-0.0585	S.E.b	=	$\pm$0.015	bSPxy	= 0.1695

Test for significance of b.

Source	d.f.	S.S.	M.S.	F	Result
Lin.Reg.	1	0.1695	0.1695	15.24	* *
Error	25	0.2779	0.0111		
Total	26	0.4474			

(c) 8 p.m.

$\bar{x}$ = 12.68 $\bar{y}$ = 6.30

SSx = 52.2919 SSy = 1.4550 SSxy = -6.4005

b = -0.1224 S.E.b = $\pm$0.023 bSPxy = 0.7834

Test for significance of b.

Source	d.f.	S.S.	M.S.	F	Result
Lin.Reg.	1	0.7834	0.7834	29.17	* *
Error	25	0.6716	0.0269		
Total	26	1.4550			

$\hat{y}$ = 7.21 -0.1224 x

(d) Test for significance of differences between regressions.

Source	d.f.	SSx	SPxy	SSy	SSy'	d.f.'	M.S.'	F	Result
A 8 a.m.	26	69.0996	- 6.6427	0.8146	0.1760	25			
B 11 a.m.	26	49.5667	- 2.8983	0.4474	0.2779	25			
C 8 p.m.	26	52.2919	- 6.4005	1.4550	0.6716	25			
Deviations from Individual Regressions					1.1255	75	0.015		
(A+B+C)	78	170.9582	-15.9415	2.7170	1.2305	77			
Difference between regressions					0.1050	2	0.052	3.46	*

APPENDIX 15

(a) Changes in the d.m. Content of the Rumen Liquor According to Treatments and Time of Sampling.

The possibility of variations in the d.m. content of rumen liquor as sampled for VFA determinations being influenced by treatments or times of sampling was investigated to allow for corrections in estimating the quantities of VFAs in the rumen. A total of 54 observations in one complete Latin square were obtained. The small error degrees of freedon (two) did not allow a satisfactory analysis of the main effects, though differences between treatments were significant at the 10% level. Highly significant differences ($P < 0.01$) were obtained between times of sampling, the mean values being given in Table A.

TABLE A: Rumen Liquor Dry Matter Content According to Times of Sampling and to Treatments.

Times	8a.m.	5p.m.	2p.m.	11a.m.	11p.m.	8p.m.	S.E.	Significance of Difference
R.L.D.M.(%) 1%	3.0	3.2	3.3	3.4	3.6	4.2	±0.11	* *
Treatments	A	B	C					
R.L.D.M.(%)	3.6	3.4	3.2					+

(b) **Total amount of VFAs in the rumen 15-16 hours after commencement of feeding.**

The amounts of VFAs in the rumen were calculated as follows:-

$$\frac{\left[\text{R.C.(lb)-D.M.(lb)}\right]\left[\text{mM VFA/100 ml R.L.}\right)\ 2.2\ (100\text{-D.M.\% of R.L.}\left.\right]\ \frac{1000}{}}{1000}\ \text{moles}$$

R.C. (lb) = total rumen contents (lb)

D.M. (lb) = weight of contents in terms of dry matter (lb)

R.L. = rumen liquor

Allowance was made for differences in the R.L. dry matter content, although differences were small and at a minimum at the time of emptying the rumen (8 a.m.)

(c) Rumen Liquor d.m. Per Cent

Analysis of Variance.

Source	d.f.	S.S.	M.S.	F	Result
Cows (C)	2	0.83	0.42	5.25	N.S.
Periods	2	0.36	0.18	2.25	N.S.
Treatments (T)	2	1.53	0.77	9.63	+
Error 1	2	0.15	0.08		
Time (Ti)	6	9.19	1.53	13.08	* *
T x Ti	12	0.44	0.04		
Error 2	36	4.21	0.12		
Total	62	13.84			

(d) Total Amount of VFAs in the Rumen 15-16 hours after commencement of feeding (mM/100ml)

Analysis of Variance.

Source	d.f.	S.S.	M.S.	F	Result
Treatments	2	7.8924	3.9462	34.65	* *
Blocks	4	3.3769	0.8442	7.41	* *
Error	8	0.9113	0.1139		
Total	14	12.1806			

APPENDIX 16

(a) Total Rumen Ingesta (d.m. lb)

Analysis of Variance.

Source	d.f.	S.S.	M.S.	F	Result
Blocks	4	19.0563	4.7641	3.89	* *
Treatments	2	60.6637	30.3318	24.79	* *
Error	8	9.7893	1.2237		
Total	14	89.5093			

(b) Total Rumen Ingesta (Wet Matter lb)

Analysis of Variance.

Source	d.f.	S.S.	M.S.	F	Result
Blocks	4	992.27	248.07	1.23	
Treatments	2	2328.94	1164.47	5.76	*
Error	8	1617.73	202.22		
Total	14	4938.94			

(c) Dry Matter Per Cent of the Rumen Ingesta

Analysis of Variance.

Source	d.f.	S.S.	M.S.	F	Result
Blocks	4	8.5729	2.1432	2.57	+
Treatments	2	13.5363	6.7681	8.13	*
Error	8	6.6592	0.8324		
Total	14	28.7684			

(d) Total Water in the Rumen (lb)

Analysis of Variance.

Source	d.f.	S.S.	M.S.	F	Result
Blocks	4	833.74	208.435	1.16	N.S.
Treatments	2	1642.54	821.270	4.55	*
Error	8	1443.46	180.433		
Total	14	3919.74			

APPENDIX 16

(e) Regression Analysis - Total d.m. in the Rumen on the pm d.m. Intake for Day Four.

x	=	Intake	y	=	d.m. in rumen		
$\bar{x}$	=	10.19	$\bar{y}$	=	9.48		
SSx	=	92.2537	SSy	=	89.5093	SSxy	= 77.5894
b	=	0.8410	S.E.b	=	$\pm$0.14	bSPxy	= 65.2527

Test of significance of b.

Source	d.f.	S.S.	M.S.	F	Result
Lin.Reg.	1	65.2527	65.2527	34.97	* *
Error	13	24.2566	1.8659		
Total	14	89.5093			

(f) Regression Analysis - Total d.m. in the Rumen on the 24 hr. d.m. Intake.

x	=	Intake	y	=	d.m. in rumen		
$\bar{x}$	=	20.56	$\bar{y}$	=	9.48		
SSx	=	331.3114	SSy	=	89.5093	SSxy	= 156.2754
b	=	0.4719	S.E.b	=	$\pm$0.06	bSPxy	= 73.7464

Test of significance of b.

Source	d.f.	S.S.	M.S.	F.	Result
Lin.Reg.	1	73.7464	73.7464	60.82	* *
Error	13	15.7629	1.2125		
Total	14	89.5093			

APPENDIX 17

(a) **The Regression of VFA Concentration on the Crude Fibre Content of the Pasture.**

$\bar{y}$ = 10.46 $\bar{x}$ = 27.92

SSx = 30.396 SSy = 4.185 SSxy = -5.457

b = -0.1795 S.E.b = $\pm$0.12 bSPxy = 0.9795

Test for significance of b.

Source	d.f.	S.S.	M.S.	F	Result
Lin.Reg.	1	0.9795	0.9750	2.13	N.S.
Error	7	3.2054	0.4579		
Total	8	4.1849			

(b) **The Regression of VFA Concentration for Treatment A at 8 p.m on the Crude Fibre Content of the Pasture.**

$\bar{y}$ = 11.34 $\bar{x}$ = 27.92

SSx = 30.396 SSy = 4.061 SSxy = -4.321

b = -0.1422 S.E.b = $\pm$0.13 bSPxy = 0.6144

Test for significance of b.

Source	d.f.	S.S.	M.S.	F	Result
Lin. Reg.	1	0.6144	0.6144	1.25	N.S.
Error	7	3.4466	0.4923		
Total	8	4.0610			

(c) **The Regression of the Proportion of Acetic Acid on the Crude Fibre Content of the Pasture.**

$\bar{y}$ = 69.95 $\bar{x}$ = 28.05

SSx = 29.220 SSy = 16.980 SSxy = -15.330

b = -0.5246 S.E.b = $\pm$0.28 bSPxy = 0.8042

Test for significance of b.

Source	d.f.	S.S.	M.S.	F	Result
Lin. Reg.	1	8.0420	8.0420	3.54	N.S.
Error	6	16.1758	2.2695		
Total	7	16.980			

APPENDIX 18

(a) Rumen pH - Cows Grazing Mature Pasture

Analysis of Variance.

Source	d.f.	S.S.	M.S.	F	Result
Cows (C)	2	0.3556	0.1778	6.68	N.S.
Days (D)	1	0.4318	0.4318	3.13	N.S.
Months (M)	1	0.1073	0.1073	<1	
Times (Ti)	4	4.5088	1.1272	12.64	*
C x D	2	0.0532	0.0266	<1	
C x M	2	0.0181	0.0090	<1	
C x Ti	8	0.1898	0.0237	<1	
D x M	1	0.1378	0.1378	4.67	*
D x Ti	4	0.0894	0.0223	<1	
M x Ti	4	0.3569	0.0892	3.02	*
Error	30	0.8866	0.0295		
Total	59	7.1353			

(b) VFA Concentration - Cows Grazing Mature Pasture

Analysis of Variance.

Source	d.f.	S.S.	M.S.	F	Result
Cows (C)	2	6.1350	3.0675	2.04	N.S.
Days (D)	1	9.4800	9.4800	4.15	N.S.
Months (M)	1	70.4380	70.4380	104.82	* *
Times (Ti)	4	182.0420	45.5105	19.93	* *
C x D	2	3.0041	1.5020	1.09	N.S.
C x M	2	0.0996	0.0498	<1	
C x Ti	8	9.6640	1.2080	<1	
D x M	1	0.6720	0.6720	<1	
D x Ti	4	9.1320	2.2830	1.66	N.S.
M x Ti	4	1.9580	0.4895	<1	
Error	30	41.2970	1.3767		
Total	59	333.9217			