THE PRODUCTION OF VOLATILE FATTY ACIDS IN SHEEP ON DIFFERENT PASTURE TYPES

A thesis presented in partial fulfilment of the requirements for the degree of Master of Agricultural Science in Animal Science at Massey University

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PREFACE

The New Zealand economy depends largely on the efficient conversion of pasture to exportable animal products. The current emphasis on higher stocking rates increases the need for pastures best suited to animal production.

Pasture species used in New Zealand are recognised as differing in their effects on animal production, these differences being loosely attributed to variation in "pasture quality". If the level of output of saleable product is the accepted measure of pasture quality, then the principal factors governing this are the quantity of feed consumed and its subsequent utilisation.

A number of studies have shown that differences in food intake alone cannot account for the observed differences in animal performance. It has thus been considered important to investigate the factors affecting the utilisation of pasture by the animal.

Volatile fatty acids (VFA) produced in the rumen are generally considered to account for 70 to 80 per cent of the net energy requirements of ruminants, and their production must be a major determinant of feed utilisation. Differences in the ruminal concentrations and proportions of VFA have often been observed with pasture feeding and have been cited as possible reasons for differences in pasture quality.

The investigation described here was undertaken as a preliminary study of the role of VFA production in determining the quality of New Zealand pasture species.

TABLE OF CONTENTS

CHAPTER THREE RESULTS

CHAPTER FOUR DISCUSSION

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

APPENDICES

CHAPTER ONE

REVIEW OF LITERATURE

Introduction

The literature is reviewed in four sections:

(1) A review and summary of previous studies of the quality of the major New Zealand pasture species.

(2) Discussion of the importance of VFA in the metabolic processes of the ruminant and their possible role in causing differences in feed quality.

(3) Methods of measurement of VFA production.

(4) The problem of obtaining a representative sample of rumen contents.

1.1. The Quality of Major New Zealand Pasture Species

As early as 1931 Roberts at Aberystwyth found that the inclusion of wild white clover in a predominantly ryegrass pasture mixture increased the live weight gain of sheep by 35 per cent. Since then several workers in New Zealand and Australia have demonstrated consistently faster growth of sheep grazing white clover pastures than those grazing ryegrass species (Sinclair, Clarke and Filmer, 1956; Rae, Brougham, Glenday and Butler, 1963; Rae, Brougham and Barton, 1964; McLean, Thomson, Iverson, Jagusch and Lawson, 1962; McLean, Thomson, Jagusch and Lawson, 1965; Hight and Sinclair, 1965, 1967; Wilson, 1966; Gallagher, Watkin and Grimes, 1966; Joyce and Newth, 1967; Joyce and Rattray, 1969; Ulyatt, 1969). Differences in the growth rate of sheep grazing various ryegrass species have also been shown (McLean *et al.*, 1962, 1965; Rae *et al.*, 1963, 1964; Ulyatt, 1969). The live weight gains on these various pasture types, relative to those on perennial ryegrass observed in these experiments, are presented in Table 1.1.

Butler, Rae and Bailey (1968) reviewed the pasture quality trials conducted at Massey University over the last twelve years and arrived at two generalisations:

(1) "Less persistent ryegrass varieties give greater rates of live weight gain than more perennial varieties and

(2) White clover gives an additional benefit whether alone or in association with ryegrass."

Table 1.1.

Live Weight Gain of Sheep on Different Pasture Species Relative to Perennial Ryegrass (100)

C	C + S	C + A	C + P	I	S	A	P	Comments	Country	Reference
	239		74		157		100	Autumn-Summer 1957	N.Z.	Rae et al. (1963)
	192		135		153		100	Winter-Summer 1959		
	258		263		189		100	Winter 1961		Rae et al. (1964)
		151			138	124	100	Spring 1962		
		122		161		97	100	Spring 1963		
257					139		100	Spring 1961	N.Z.	McLean et al. (1962)
123					132		100	Autumn 1962		
212					116		100	Spring 1962		McLean et al. (1965)
188					152		100	Autumn 1963		
219					119		100	Spring 1963		
408							100	Autumn	N.Z.	Sinclair et al. (1956)
515			270				100	Autumn (Short Pasture)	N.Z.	Hight & Sinclair (1965)
531			282				100			
205			146				100	Autumn (Long Pasture)		Hight & Sinclair (1967)
146							100	Spring	Australia	Gallagher et al. (1966)
147							100	Summer		
185							100	Spring	Australia	Wilson A.D. (1966)
150							100	Summer		
439							100	Autumn		
191							100	Autumn (Indoors)	N.Z.	Joyce & Newth (1967)
127							100	(Restricted C)		
144			114				100	Ad lib Spring (Indoors)	N.Z.	Joyce & Rattray (1969)
152			121				100	Restricted		
147					119		100	Spring	N.Z.	Ulyatt (1969)

C White clover
S Manawa Short-rotation ryegrass
A Ariki ryegrass
I Paroa Italian ryegrass
P Perennial ryegrass

Some progress in defining the reasons for these differences between species has been made. Evidence to suggest that differences in food intake can account for part but not all of the observed differences in live weight gain has been produced by McLean *et al.* (1965), Grimes, Watkin and Gallagher (1967), Joyce and Newth (1967), Rattray and Joyce (1969), and Ulyatt (1969).

Differences in weight of rumen contents and in the concentrations and molar proportions of VFA in the rumen of sheep grazing pasture have been observed (Table 1.2). These observations have been interpreted to mean that pastures producing greater live weight gains are more readily fermented and give rise to more favourable end products of digestion (McLean *et al.*, 1962, 1965; Johns, Ulyatt and Glenday, 1963; Milford and Minson, 1965, 1966; Grimes *et al.*, 1967).

Bailey (1964) showed that short rotation ryegrass (S) had consistently lower cellulose and possibly higher soluble sugars than perennial ryegrass (P) and in general the lower cellulose contents correlated with higher live weight gains. Evans (1964) demonstrated that the leaves of P were physically stronger than S and that this difference was closely correlated with cellulose content. These observations suggested that the more annual ryegrass species might be more rapidly broken down in the rumen and move at a faster rate along the digestive tract than the more perennial species (Butler *et al.*, 1968).

Table 1.2.

Rumen Characteristics of Sheep on Different Pasture Species

	C	C + S	C + P	S	P	Reference
VFA Conc.		14.7	13.4	12.3	11.8	Johns et al. (1963)
(mMole/100ml)	14.3			8.2	8.8	McLean et al. (1962)
	15.0			10.5	10.7	
	14.4			8.9	7.3	McLean et al. (1965)
				14.8	14.4	Ulyatt (1964)
	20.4			13.9	13.1	Ulyatt (1969)
VFA molar		61-25-12			74-21-5	Johns et al. (1963)
proportions	59-25-16			61-23-16	63-22-15	Ulyatt (1969)
(acetic-	59-26-15				62-25-13	Grimes et al. (1967)
propionic-				58-26-16	60-25-15	Ulyatt (1964)
butyric)						
Weight of		5.9	8.4	6.7	12.0	Johns et al. (1963)
contents per						
100lb LW (lb)	6.4			14.7	17.1	McLean et al. (1965)

Ulyatt (1969) demonstrated that S has a lower rumen digestibility and a faster turnover time in the rumen than P and suggested that it might be less completely digested by the rumen microorganisms. This theory was supported by evidence that a higher proportion of mesophyll cells, containing much of the soluble plant components, passed to the intestines on S compared with P. This would suggest a greater digestion of S than P distal to the rumen (Evans, pers. comm.; cited by Ulyatt, 1969). The superiority of clover over the ryegrasses was suggested by Ulyatt (1969) to be due to its lower cellulose content and to a higher ratio of readily fermentable to structural carbohydrate. High rumen concentrations of VFA and ammonia, and a rapid turnover time for the organic matter of clover in the rumen, were thought to indicate a more intense fermentation and breakdown in the rumen. These circumstances could lead to a higher intake of organic matter, readily fermentable carbohydrate and protein compared with the ryegrasses.

Summary:

Sheep grazing different New Zealand pasture species show variation in live weight gain.

The less persistent ryegrass varieties give greater rates of liveweight gain than the more persistent varieties, and white clover is of additional benefit.

Differences in food intake cannot alone account for differences in live weight gain.

Rumen VFA differences, associated with variation in chemical composition of pasture species and resultant differences in digestion, have been suggested as a possible reason for variation in animal performance.

1.2. The Nutritional Significance of Volatile Fatty Acids

Large quantities of VFA, in particular acetic, propionic and butyric acids, are produced in the rumen as a result of the fermentation of dietary carbohydrate and protein by the rumen micro-organisms. Although the presence of VFA in the rumen had been known since the 1880s, their role in the animal remained uncertain until Barcroft, McAnally and Phillipson (1944) showed that they were readily absorbed and metabolised. Since then their nutritional importance has been widely recognised (Rook, 1964; Warner, 1964; Annison, 1965).

1.2.1. Production of VFA in the Rumen of the Sheep

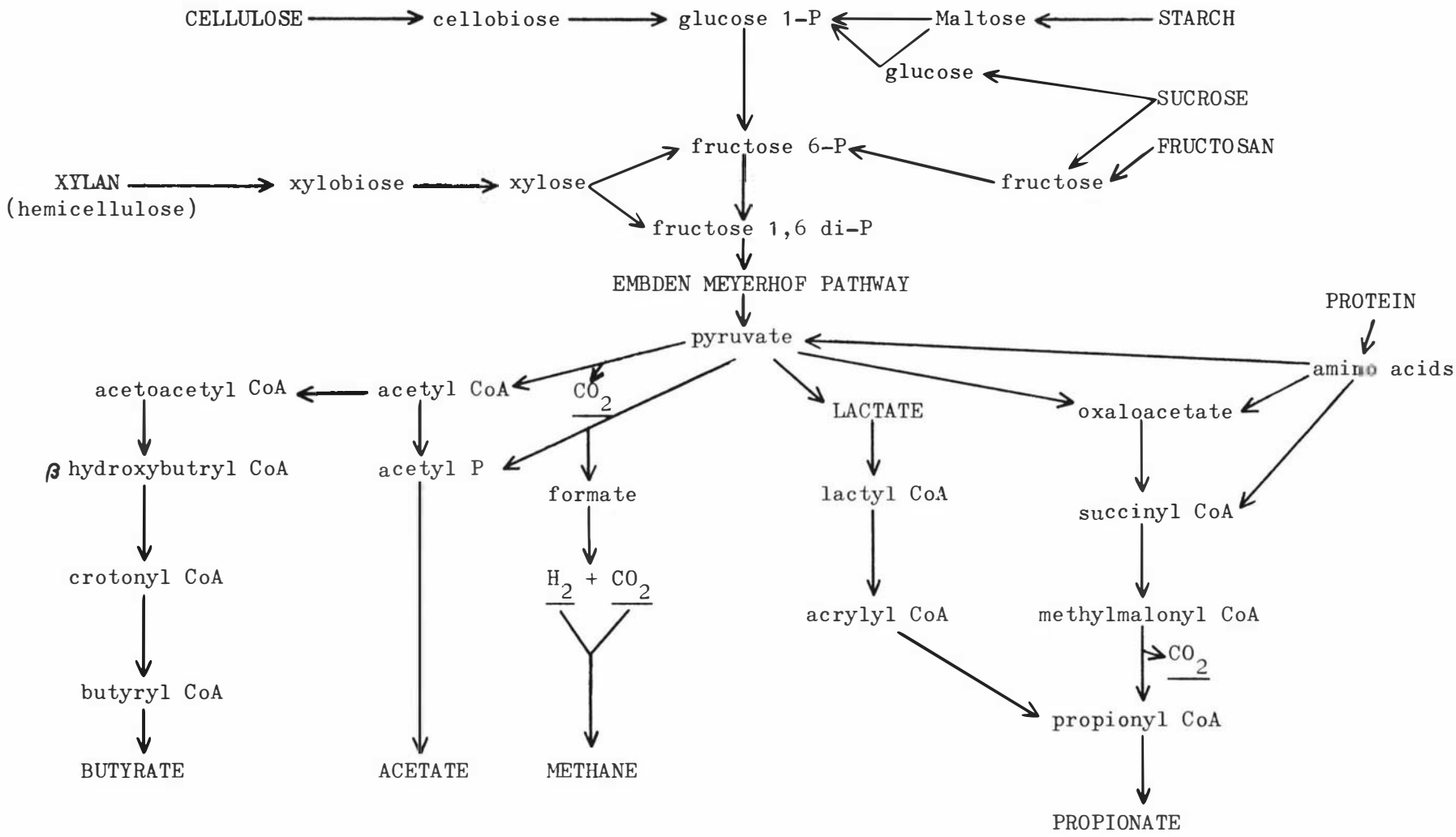
The dietary constituents giving rise to VFA are primarily cellulose, hemicellulose, soluble carbohydrate and protein. The proportions of the end products vary with the relative amounts of these substrates.

Pathways in the degradation of food carbohydrate and protein to the end-products VFA, lactate, methane, hydrogen and carbon dioxide are depicted in Fig. 1.1.

In general the substrates which are less rapidly fermented in the rumen, in particular cellulose (Bailey, 1964), give rise to more acetate (Annison and Lewis, 1959). Propionic acid does not seem to be a significant derivative of cellulose fermentation but is derived from succinic acid by the decarboxylation reactions of other bacterial species (Johns, 1951). When glucose and other mono- and oligosaccharides

Figure 1.1

Metabolic Pathways of VFA Production (adapted from Armstrong, 1965)



are fermented, propionate is produced in large amounts, usually via the reduction of lactate (Elsden, Volcani, Gilchrist and Lewis, 1956).

The hemicelluloses are more soluble than cellulose and hence more easily fermented. Butyrate appears to be a major product of their fermentation (Oxford, 1958).

Branched chain fatty acids produced in the rumen appear to be derived from proteins attacked by the rumen microbes e.g. isovaleric (El-Shazly, 1952), isobutyric, 2-methyl-butyric (Annison, 1954). A number of amino acids are degraded principally to acetic acid while aspartic acid produces mainly propionate (Lewis, 1955; Sirotnak, Doetsch, Robinson and Shaw, 1954).

The relative amounts of the end-products of rumen digestion are not simply determined by the chemical composition of the diet, however. The physical form of the foodstuff can influence the ratio of acetic to propionic acid in the fermentation products (Balch, 1960).

Because of the continuous production and absorption of the acids and the onward passage of digesta, measurement of the ruminal production of VFA has proved technically difficult but a number of approaches have been derived.

Warner (1964) suggested from a comparison of results from a large number of experiments that VFA probably provide 70-80 per cent of the net energy supplied by most feeds. More recently, improved techniques have been developed and estimates of the contribution of VFA to the animal's energetic needs made. Estimates made using sheep are summarised in

Table 1.3.

Published Estimates of VFA Production in Sheep

Numbers of Animals	Daily Ration	Moles/day Production Rate	% GE	% DE	% ME	Method of Measurement	Reference
2	Dried grass 801g DM	5.4	45	62		Isotope dilution	Bergman et al. (1965)
6	Lucerne chaff 549g DM	5.36	46	79 53* 45*		Isotope dilution	Leng & Leonard (1965)
3	Lucerne hay chaff Wheaten hay chaff 1000g DM	4.31	31	54	64	Isotope dilution	Gray et al. (1967)
3	Lucerne chaff 800g WM	8.77				Isotope dilution	Leng & Brett (1966)
4	Lucerne chaff & maize 600g WM	5.70					
	Lucerne & wheaten chaff 500g WM	2.50					
5	Phalaris tuberosa (grazing) 360g DOM	1.47				Isotope dilution	Leng et al. (1968)
8	Dried ryegrass 600-800g OM	3.11-5.52				Isotope dilution	Weston &Hogan (1968)
21	Dried forage oats 500-900g OM	2.4-6.3					
	Grass hay 850g DM	3.7		38**	46***	In vitro fermentation	Sutherland (1963)
2	Lucerne hay 850g DM	2.52		34	41	In vitro fermentation	Faichney (1968a)
2	Oat straw 472g DM	1.40		42	54		
1	Wheat & Lucerne chaff 700g WM	2.32		33	39		
3	Oat straw 522g DM	0.85		38	50	In vitro fermentation	Faichney (1968b)
3	Oat straw & urea 1122g DM	2.38		31	40		
3	Lucerne hay 680g DM once daily	2.23		36	46	In vitro fermentation	Faichney (1968c)
3	Lucerne hay 85g DM 3-hourly	2.32		39	51		
4	Hay & concentrates 440-1230g DM			50-54		Partition of digested energy	Nicholson & Sutton (1969)

* Recalculated by Leng et al., (1968) ** Faichney (1968a) *** Rook (1964)
GE Gross Energy DE Digestible Energy ME Metabolisable Energy

Table 1.3 (unless stated otherwise, data applies to pen-fed sheep).

It can be seen that production rate varies considerably with the type and amount of food eaten, and ranges from less than one mole per day on an oat straw diet to more than eight moles per day on a diet of dried lucerne chaff. There is also considerable variation in the contribution of VFA to digested energy. The very high figure of Leng and Leonard (1965) was criticised by Faichney (1968a) in that daily production was obtained by extrapolation from a four hourly period of infusion during which rumen concentrations were constant. Leng, Corbett and Brett (1968) recalculated the VFA production rates using the daily mean concentrations.

The results obtained by *in vitro* methods are consistently lower than those obtained by *in vivo* techniques but none of the workers have offered an explanation for this (Faichney, 1968a).

Bull, Johnson and Reid (1967) suggested that variation in data on VFA production might be due in part to such differences as levels of intake, rate of passage of food through the alimentary tract, chemical composition of the diet and methods of measurement employed.

1.2.2. Production of VFA in the Hind Gut of the Sheep

Fermentation of food residues by microorganisms in the caecum of the sheep results in the production of some VFA. Hungate *et al.* (1959) estimated the amount to be less than 5 per cent of the total VFA production, but Faichney (1968d)

suggested it is closer to 12 per cent of the total. As the volume of the caecum is approximately 14 per cent of the rumen volume (Williams, 1965) and the mean concentration of VFA in the caecum about 80 per cent of that in the rumen, Faichney (loc. cit.) suggested that production from the caecum is probably limited to less than 14 per cent of that in the rumen.

Hogan (cited Gallagher, 1966) suggested that a value of 5 per cent might be too low for diets in which a considerable portion of the digestible organic matter is digested in the caecum and colon. Goodall and Kay (1965) have reported that with dried feeds more than 10 per cent of the total cellulose digested is degraded in the large intestine, and Gray (1947) put this figure as high as 30 per cent. Thomson et al. (1969) found 23-27 per cent digestion of cellulose in the caecum and colon of sheep fed dried lucerne diets.

Thus digestion in the hind gut could supply a significant proportion of the energy requirements of the ruminant. However there are no reports of the magnitude of VFA production in the hind gut of sheep fed fresh pasture.

1.2.3. Absorption of VFA

The VFA produced in the rumen become available for the metabolic processes of the animal by absorption into the blood stream, mainly through the rumen wall. Many workers have shown that in sheep receiving feed the rates of absorption of individual VFA are generally proportional to

their concentrations in the rumen (Sutherland, 1963; Perry and Armstrong, 1969; Weston and Hogan, 1968a) and it has been suggested that absorption is by simple diffusion (Dobson, 1961; Annison, 1965; Hungate, 1966; Leng and Brett, 1966; McDonald, 1969). Other workers however, have noted that the proportions of the individual acids in the VFA absorbed have not been the same as their proportions in rumen fluid (Danielli, Hitchcock, Marshall and Phillipson, 1945; Faichney, 1968a; Williams, Hutchings and Archer, 1968). The only direct evidence of active absorption has been the demonstration by Stevens and Stettlar (1967) of a slight movement of acetate against its electrochemical gradient across the rumen epithelium from the plasma to the lumen, and the biological implications of this are obscure.

The effective concentration gradients for the individual VFA may possibly be altered by a number of factors, including pH in the rumen (Danielli et al., 1945; Gray, 1948), interconversion of acetate and butyrate (Bergman, Reid, Murray, Brockway and Whitelaw, 1965; Leng and Brett, 1966) and metabolism of butyrate to ketone bodies in the rumen epithelium (Pennington, 1952; Sutton, McGilliard and Jacobsen, 1963). This may help to explain some of the differences reported in the literature in the relative rates of absorption of the VFA.

Williams et al. (1968) have suggested that up to 20 per cent of the VFA produced in the rumen can enter the omasum but probably little of this reaches the abomasum (Gray, Pilgrim and Weller, 1954; Stevens, Sellers and

Spurrell, 1960; Joyner, Kesler and Holter, 1963). However the epithelium of the abomasum appears to have a similar absorptive capacity to that of the rumen (Ash, 1961; Williams et al., 1968).

Absorption of the VFA produced in the hind gut appears to take place through the caecal epithelium by simple diffusion (Myers et al., 1967).

1.2.4. Metabolism of VFA

Acetate is the largest single contributor to the metabolisable energy of the ruminant. Only small amounts are metabolised in the rumen epithelium and liver, and large quantities appear in the peripheral blood (Annison, Hill and Lewis, 1957) although some is of endogenous origin (Annison and White, 1962). In tissues which utilise acetate, oxidation occurs via acetyl CoA and the citric acid cycle and there is no known mechanism for net synthesis of glucose. Acetate is taken up by adipose tissue for fat synthesis, but this requires the provision of $NADPH_2$ for reduction reactions, from the concurrent metabolism of glucose.

Annison and Lindsay (1961) have estimated the contribution of oxidised acetate to expired CO_2 production to be 35 per cent for fed sheep.

Propionic acid, together with the non-essential amino acids, provides the main source of glucose in the ruminant. Some propionate is oxidised to lactate by the rumen wall during absorption (Pennington and Sutherland, 1956) but most is removed from the blood stream by the liver (Annison

et al., 1957).

Propionate enters the citric acid cycle via succinyl CoA and proceeds to pyruvate via oxaloacetic acid. Pyruvate then enters the citric acid cycle via acetyl CoA. Estimates of the contribution of propionate to expired CO_2 vary from 25 per cent (Bergman, Roe and Kon, 1966) to 50 per cent (Smith, Osborne-White and Russell, 1965, 1967).

Alternatively, propionate can be used for glucose synthesis via succinate, oxaloacetate, phosphoenolpyruvate and reversal of the glycolytic pathway. The passage of glucose-6-phosphate throughthe pentose shunt could provide the necessary $NADPH_2$ for the synthesis of fat from acetyl CoA. The formation of oxaloacetate facilitates the oxidation of acetyl CoA derived from acetate, butyrate or β-hydroxybutyrate.

Butyric acid is almostly completely metabolised by the epithelium of the rumen and omasum (Pennington, 1952) and only traces appear in the peripheral blood (Annison et al., 1957). Oxidation of butyrate to ketone bodies accounts for approximately 70 per cent of the oxygen consumption of rumen epithelium, and up to 40 per cent for the liver (Hird and Weidemann, 1964). In many tissues the ketone bodies can substitute for glucose in oxidative metabolism via the citric acid cycle but do not give rise to a net synthesis of glucose (Annison, Leng, Lindsay and White, 1963)

Oxidation in the liver occurs via the crotonyl CoA pathway, entering the citric acid cycle as acetyl CoA. Butyrate, like acetate, is incorporated into long chain fatty acids via malonyl CoA with stepwise elongation by the addition

of 2-carbon units from acetyl CoA (Thompson and Jacobsen, 1966).

The major metabolic pathways of VFA utilisation are illustrated in Fig. 1.2.

1.2.5. The Efficiency of Utilisation of VFA

The theoretical efficiencies for VFA as energy sources have been presented by Armstrong and Blaxter (1957) for maintenance and by Bull et al. (1967) for lipogenesis. These are presented in Table 1.4. with the number of high energy phosphate bonds (ADP $\longrightarrow$ ATP) formed per mole of substrate oxidised.

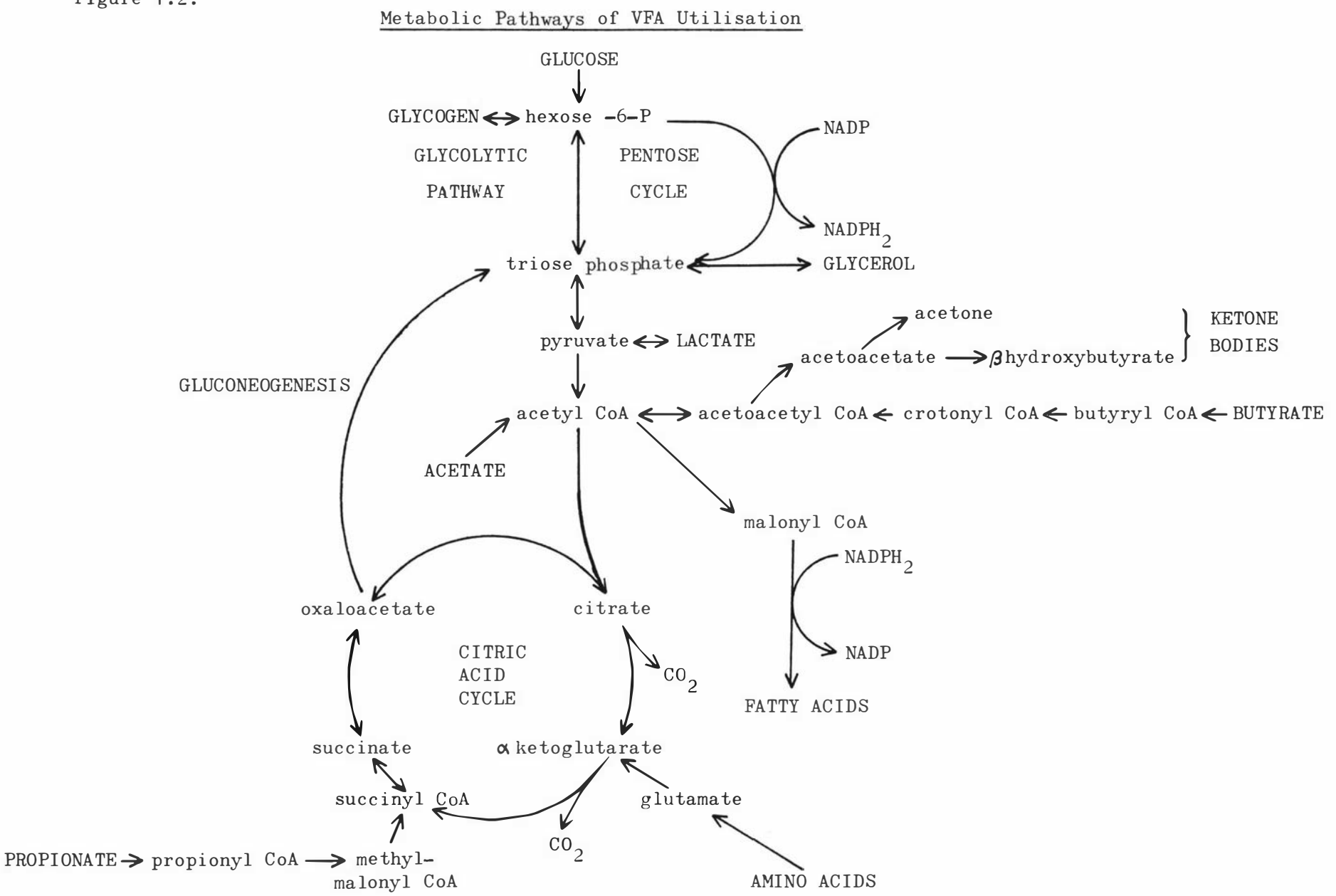
Table 1.4.

Theoretical Efficiency of Utilisation of Various Substrates

Substrate	Mole ATP/Mole Substrate	Theoretical Efficiency	
		Maintenance	Lipogenesis
Stearic acid	146	100	100
Glucose	38	105.8	
Acetic acid	10	88.6	77.0
Propionic acid	18	91.0	78.5
Butyric acid	27	95.7	83.0
Glutamic acid	23		

These values have been calculated using the intermediary energy transfers in the pathways illustrated in Fig.1.2. and stearic acid as the energy source in fat.

Figure 1.2.


Metabolic Pathways of VFA Utilisation
GLUCOSE
GLYCOGEN
hexose -6-P
NADP
GLYCOLYTIC PATHWAY
PENTOSE CYCLE
NADPH2
triose phosphate
GLYCEROL
acetone
KETONE BODIES
pyruvate
LACTATE
acetoacetate
βhydroxybutyrate
GLUCONEOGENESIS
acetyl CoA
acetoacetyl CoA
crotonyl CoA
butyryl CoA
BUTYRATE
ACETATE
malonyl CoA
NADPH2
oxaloacetate
citrate
NADP
CITRIC ACID CYCLE
CO2
FATTY ACIDS
succinate
α ketoglutarate
succinyl CoA
glutamate
CO2
PROPIONATE
propionyl CoA
methyl-malonyl CoA
AMINO ACIDS

(1) Efficiency of Utilization of VFA for Maintenance

(a) Individual VFA

When Armstrong and Blaxter (1957a) infused single VFA into the rumen of fasting sheep, heat increments calculated from carbon and nitrogen retentions and from respiratory exchanges revealed that acetic and butyric acids as the sole energy source were utilized very poorly relative to propionic acid as the sole energy source. This was explained on the basis that an impairment of glucose metabolism due to lack of oxaloacetic acid and or other carbohydrate metabolic products might occur; as evidenced by an accumulation of acids in the blood, occurrence of acidosis, decreased blood sugar levels, and increased urinary nitrogen (Armstrong and Blaxter, 1961). Blaxter (1962) suggested that part of the low energetic efficiency of acetic acid might be due to the use of protein as a glucose precursor, and further, that acetate might accumulate in body tissues and interfere with oxidative phosphorylation resulting in an increased, wasteful heat production.

The greater efficiency of utilisation of butyric acid than of acetic acid may be due to metabolism in the rumen epithelium producing mainly aceto-acetic acid which can be used in extrahepatic tissues (Armstrong and Blaxter, 1961).

Black, Luick, Moller and Anand (1966) have shown that butyrate metabolism effects gluconeogenesis by sparing pyruvate oxidation, so conserving a glucogenic precursor, and in addition by enhancing the conversion of pyruvate to oxaloacetate.

The efficiency of utilisation of propionate was much closer to the theoretical value because of its glucogenic and hence protein sparing nature (Bull et al., 1967).

(b) Different Proportions of VFA

Infusion of various mixtures of the three VFA into the rumen of fasted sheep (Armstrong et al., 1957) resulted in relatively high and similar efficiencies of utilisation of the individual acids. This was interpreted to suggest a marked synergism mainly due to the facilitation of acetate and butyrate oxidation by the presence of small amounts of propionate, a glucogenic substance, giving substance to the hypothesis put forward to explain the low efficiency of utilisation of acetate and butyrate i.e. even small quantities of propionate are probably a sufficient supply of carbohydrate intermediates to facilitate the oxidation of acetate and to prevent the breakdown of body protein to form the necessary glucogenic amino acids.

(2) Efficiency of Utilisation of VFA for Lipogenesis

Reported estimates of the utilisation of VFA by fattening animals based on short term infusion studies have shown rather different results from those based on recent long term feeding trials.

Continuous infusion of individual VFA into sheep for periods of up to 18 days (Armstrong and Blaxter, 1957b; Armstrong, Blaxter, Graham and Wainman, 1958; Armstrong and Blaxter, 1961) showed that the calorimetric efficiencies with which the VFA were used for lipogenesis were generally lower than those for maintenance but increased with increasing

chain length. With VFA mixtures the efficiencies were those expected from the calorimetric efficiencies of the individual acids comprising the mixture. It appeared that as the proportion of energy derived from acetate increased, the efficiency with which the metabolisable energy of food was used for synthesis of body fat declined. These authors attempted to explain the results in terms of the large energy requirement for the synthesis of fat from short chain VFA. By contrast in the fasting animal, VFA which spare body fat oxidation might be used more efficiently. No explanation for the very low efficiency of utilisation of acetate was given except for the comment that the rumen microbial population may have been disturbed. Methane production fell and energy loss increased but there was no indication of altered metabolic activity of the rumen flora. Further work with goats (Armstrong and Blaxter, 1964) confirmed the results shown with sheep.

In several experiments in which VFA were fed to sheep over a long period of time (Ørskov and Allen, 1966a, 1966b, 1966c; Ørskov, Hovell and Allen, 1966) no differences were demonstrated in the efficiencies of use of the individual acids. Rook, Balch, Campling and Fisher (1963) drew a similar conclusion from the results of infusing VFA intraruminally into growing heifers during 35 day periods. However the increased nitrogen retention was not significantly greater than that resulting from the control infusion of water. Ørskov discounted the use of calcium and sodium salts of VFA instead of acid solutions, feeding twice daily versus continuous infusion,

the stage of maturity of sheep used, and interaction between individual VFA fed and the proportions produced by rumen fermentation of the basal diet as possible reasons for differences between the results of these workers and those of Armstrong's group after further investigation (Ørskov et al., 1966, Ørskov and Allen, 1966b, 1966c).

Bull et al., (1967) carried out further trials designed to eliminate the possible effects of excess cation input and interaction with the basal diet, by the use of triacetin as the acetate source and two levels of each of four diets. Energy storage was determined from comparative slaughter studies. Results suggested that acetate was used for fattening with the same efficiency as other VFA. The effects of length of treatment period on energy retention was then studied by indirect calorimetry. Energetic efficiency at three days after the beginning of the treatment period was very low but increased to a value similar to the theoretical at fifteen days and it appeared that the temporary low efficiency had disappeared and metabolic mechanisms had adapted to the increased acetate load. The enzyme thiokinase was suggested by these workers to be the important limiting factor in acetate adaptation while other factors might play a secondary role. However, this would not account for the difference in utilisation of acetate when given singly or in a mixture to fasting sheep. The carbohydrate interactions mentioned previously might also be important here.

(3) Utilisation of VFA for Live Weight Gain

The evidence for a relationship between molar proportions of VFA in the rumen and live weight gain is inconclusive.

Negative correlations between the acetate to propionate ratio and daily gain have been shown for growing fattening steers by Weiss, Baumgardt, Barr and Brungardt, (1967), and for sheep by Milford and Minson (1965, 1966), Johns et al. (1963), and Grimes et al. (1967). Conversely Putnam (1956), Armstrong (1964), Thomson (1962, 1965), and Grimes (1967) were unable to demonstrate any relationship between molar proportions of VFA and efficiency of utilisation of energy for fattening.

Grimes (1967) suggested that :-

> "the relationship between molar proportions of VFA and parameters of body growth may change as animals mature because of the changing composition of body weight increments and the different metabolic pathways by which fat and protein are deposited."

Shaw, Ensor, Tellechea and Lee (1960) observed an increased efficiency of utilisation of digestible protein and an increase in body weight of steers following cooking and grinding of the ration which increased the ruminal production of propionic acid relative to that of acetic acid. However, Rook (1964) has indicated that although this type of food preparation favoured increased molar proportions of propionate, changes in the physical condition of the diet alters the digestibility of the dietary constituents and probably the proportion of energy absorbed as fatty acids. Moreover Blaxter and Graham (1956) observed no difference with sheep in the net energy value of dried

grass when it was finely ground or in the long form. The presence of such a relationship appears then to be dependant upon a number of factors such as age of the growing animal (Grimes, 1967), and protein content of the feed (Armstrong, 1964). Variation in total VFA production is a possible confounding factor where a relationship has been suggested.

Summary:

The importance of VFA production in the rumen in the provision of the energetic requirements of ruminants is well established. Estimates of the rate of production of VFA in the rumen vary widely with factors such as level of intake, chemical composition of food, rate of passage and methods of measurement.

Production of VFA in the hind gut may also be important in the provision of energy for the animal's requirements.

The availability of VFA to the animal is best measured by production in the alimentary tract because of the possible contribution of VFA metabolism in the ruminal and omasal epithelium to the animal's requirements.

The proportions of VFA produced are affected by the chemical composition of food and its physical treatment. However different mixtures of VFA are used with similar efficiency as a source of energy for maintenance.

For lipogenesis, short term infusion studies suggest the efficiency of utilisation of VFA mixtures is higher when they contain a higher proportion of propionic and butyric acids than when they contain a high proportion of acetic acid. Longer term feeding trials suggest however, that this low utilisation efficiency of acetate is only temporary and adaptation may occur, eventually resulting in similar energetic efficiencies of VFA.

Studies of the relationship between molar proportions

of VFA produced in the rumen and live weight gains are inconclusive because of variation in the composition of feeds and stage of maturity of animals used.

There is no reported evidence of a relationship between total VFA production and live weight gain.

1.3. Techniques for the Measurement of VFA Production Rate

Methods used to study the quantitative production of VFA in the rumen can be categorised into four major groups:

(1) Methods based on changes in concentration in the rumen after feeding.

(2) Methods involving the analysis of blood draining the rumen either *in vivo* or *in vitro* using isolated preparations.

(3) Methods using the *in vitro* incubation of rumen contents.

(4) Isotope dilution techniques.

The first three groups have been comprehensively reviewed by Rook (1964), Warner (1964), Annison (1965), and Dobson and Phillipson (1968), and only their main conclusions will be discussed here.

1.3.1. Concentration Changes in the Rumen

Attempts have been made to assess VFA production rates in the rumen under normal conditions by time-course studies on VFA concentrations after feeding (Emery, Smith and Huffman, 1956). Annison (1965) however, points to the variation in both total levels of VFA and molar proportions with time after feeding and the overriding problem of obtaining representative samples of rumen contents (See 1.4.). Warner (1964) states;

> "Both the principal and the application of the method seems of doubtful validity"

Individual acids have been infused into the rumen at a constant rate and the basal production of the infused acid calculated from the observed change in concentration of that acid relative to that of other acids (Bath, Balch and Rook, 1962). However the technique is dependent on the questionable assumption that relatively large quantities of extraneous VFA introduced into the rumen do not affect the fermentation processes (Warner, 1964).

1.3.2. VFA Production Rates *In Vitro*

The initial rates of increase of VFA upon anaerobic incubation of samples of rumen contents can be measured and then extrapolated to the estimated total rumen contents. Gray, Pilgrim, Rodda and Weller (1951) and Balch (1958) incubated feed samples with rumen liquor and applied the VFA production estimates obtained to the amount of organic matter fermented in the rumen.

Other authors (Carroll and Hungate, 1954; Stewart, Stewart, and Schultz, 1958; Hungate, Mah and Simeson, 1961; Giesecke and Lawlor, 1965; Faichney 1968a, 1968b, 1968c) have reported the use of integrated initial rates to calculate the total VFA production from estimations of the rate and rumen volume.

The maintenance of a normal rumen flora pattern and fermentation processes under *in vitro* conditions can be reasonably achieved using short term incubations. However major difficulties are experienced in representative sampling of rumen contents (see 1.4.), and accurately determining rumen volume at frequent intervals throughout the feeding cycle. Furthermore the differences observed are very small and analytical

procedures must be very accurate to avoid large errors.

1.3.3. Analysis of Blood Draining the Rumen

(1) In Vivo Procedure

Measurement of the concentration of VFA in portal venous blood relative to that in arterial blood, coupled with simultaneous measurement of the rate of portal blood flow, has been used to estimate VFA absorption from the rumen (McAnally and Phillipson, 1942; Barcroft, 1945; Schambye and Phillipson, 1949; Schambye, 1951, 1955; Annison et al., 1957; Conrad, Smith, Vandersall, Pounden and Hibbs, 1958; Bensadoun, Paladines and Reid, 1962). Other workers have attempted to use jugular blood because of the difficulty in maintaining portal vein catheters (Sutton et al., 1963), but Khouri (1966) was unable to consistently detect increases in jugular blood VFA levels after the introduction of preformed VFA into the fasted rumen, and concluded that jugular blood was unsuitable for the study of VFA absorption from calves.

Since both portal blood flow and VFA concentration vary with time after feeding (Bensadoun et al., 1962) the product must be integrated throughout a feeding cycle. Measurement of portal blood flow rates presents many difficulties (Pegler and Hill, 1958; Bensadoun and Reid, 1962; Waldern, Johnson and Blosser, 1963) and because of the low concentration of VFA in the blood (Annison et al., 1957) the error in estimation is high. In addition delay in mixing renders measurements of blood concentrations uncertain and furthermore, according to the precise blood vessels used, the estimate may or may not

include VFA produced in the rumen but absorbed from other parts of the gastro-intestinal tract (Warner, 1964).

The metabolic activities of the rumen wall markedly alter the relative concentrations of the VFA absorbed from the rumen. Since the acids metabolised by the rumen mucosa may well spare other substrates (Warner, 1964; Black et al., 1966) the measurement of VFA absorbed into the blood is not a true representation of their importance to the overall energy metabolism of the animal.

Warner (1964) reports, "These technical difficulties probably account for the fact that nearly all the estimates obtained by this method for production of VFA are somewhat lower than those found by most theoretically satisfactory methods."

(2) In Vitro Procedure

Several groups of workers have studied VFA absorption from the isolated, perfused rumen (McCarthy, Holter, Shaw, Hueter and McCarthy 1957; McCarthy, Shaw, McCarthy, Lukshmanan and Holter, 1958; Brown, Davis, Staubus and Nelson, 1960). Annison (1965) pointed to the difficulties of maintaining normal physiological function in a large isolated organ, particularly the blood supply which is generally inadequate, leading to anoxia. Warner (1964) observed that the absence of mixing and inadequate diffusion of end products can be harmful to the micro-organisms in an artificial rumen. McCarthy et al. (1957) demonstrated a shift from VFA fermentation to one producing some lactate immediately after the commencement of perfusion.

A number of other factors would have to be taken into

consideration if the results were to lead to valid conclusions about the intact animal.

1.3.4 Isotope Dilution Methods

(1) Single Injection Technique

Tracer amounts of isotopically labelled VFA of high specific activity are introduced into the rumen. After an interval to allow mixing to take place, samples of rumen contents are removed and the specific activity of the acid determined. The decline in specific activity gives a measure of dilution by unlabelled acid i.e. of production. However this method depends on the assumption that the total amount of acid under consideration in the rumen contents remains constant, and Boyne, Campbell, Davidson and Cuthbertson, (1956) have shown that the maximum and minimum volumes of rumen contents are likely to coincide with maximum and minimum VFA concentrations. Adequate mixing of the isotopic substrates with rumen contents and representative sampling of the whole rumen pool are also necessary, and for accuracy interconversion of the acids should be allowed for. The method has been used by Shepperd, Forbes and Johnson (1959), Gray, Jones and Pilgrim (1960) with sheep and by Brown and Davis (1962) with calves, with questionable results in light of the conditions mentioned.

Warner (1964) suggested that the anomalies in the results of Sheppard et al. (1959) and Gray et al. (1960) could be most readily explained by incomplete mixing in the time period under consideration. Inert substances added to the rumen of sheep or cattle take at least an hour to become thoroughly mixed with the contents (Emery, Smith and Lewis, 1958; Gray et al., 1960; Hyden 1961). Warner (1964)

further suggested that the need for the amount of acid in the rumen to be constant could be avoided if the specific activity and concentration of an acid together with the volume of rumen contents could be measured sufficiently often or if the labelled acid was continuously infused at a rate which would keep the specific activity constant.

(2) Continuous Infusion Method

A number of workers have used continuous infusion of labelled VFA often preceeded by a large priming dose, to overcome this problem of variation in the substrate content of the rumen. Bergman et al. (1965) used a priming dose followed by continuous infusion of the same acid for 4-5 hours. A rumen pump (Sutherland, Ellis, Reid and Murray, 1962) was used to mix and sample the rumen contents and the rumen volume was estimated after the injection of polyethylene glycol. To achieve nearly constant concentrations of material in the digestive tract they used continuous feeding of a dried ration over the 24 hour experimental period. Nearly constant specific activities, indicative of steady state conditions, were reached after 2-3 hours of infusion. Gross production rates of the individual VFA were estimated from a series of experiments using each acid separately, and net production rates were calculated from a series of simultaneous equations utilising the data on gross production and rates of interconversion from these experiments.

Gray, Weller, Pilgrim and Jones (1962) and Gray, Weller and Jones (1965) continued their investigation into the use of the isotope dilution technique to measure the production

of more than one acid simultaneously by setting up an *in vitro* fermentation closely parallel to the rumen in terms of the mixture of VFA formed. Using ^{14}C-labelled acids they were able to demonstrate that although transfer of label between acids occured, particularly between acetate and butyrate, little of the ^{14}C label was lost from the acid mixture, and they concluded that with a mixture containing appropriate concentrations of labelled acids, each individual acid could retain nearly all of its original amount of ^{14}C. This method was then applied to the artificial rumen and estimates of production agreed closely with direct measurements when due allowance was made for small losses of the radioactive labels from the respective acids. The method was then applied to the rumen itself using the losses of ^{14}C measured in the same material fermented in an artificial rumen as correction factors, and rates of production of the three acids in sheep measured. The findings were in close agreement with those of Stewart *et al.* (1958) who used an *in vitro* technique.

The necessity of measuring rumen fluid volumes before the amounts of VFA formed could be measured was then considered by Gray, Weller, Pilgrim and Jones (1966). Dilution methods for measuring rumen volumes are time consuming, and low in accuracy when applied continuously through the feeding cycle in normally fed animals (Ulyatt, 1964; Gray *et al.*, 1966). In an effort to avoid this problem these workers developed two related isotope dilution procedures to determine total VFA production. One of these determined the distribution of

production through the feeding cycle as suggested by Warner (1964).

Both procedures involved the continuous infusion of labelled acid mixtures in proportions similar to those existing in the rumen, and regular sampling throughout the 12 hour feeding cycle. In Procedure A ("Matching infusion") the rate of infusion was varied to follow the diurnal pattern of VFA concentration so that a nearly constant concentration of ^{14}C was maintained in the total VFA throughout the feeding cycle. The amount of acid produced in any sampling interval, or during the whole cycle, was determined from

$$\text{Acid produced (moles)} = \frac{^{14}C \text{ added in time under consideration } (\mu c)}{^{14}C \text{ in rumen acid } (\mu c/\text{mole})}$$

With Procedure B ("constant rate infusion") infusion rate was maintained constant, and samples taken at 15 minute intervals were composited for the 12 hour infusion period. Production from this period was then calculated from

$$\text{Acid produced (moles)} = \frac{^{14}C \text{ added to the rumen } (\mu c)}{^{14}C \text{ in pooled sample of rumen acid } (\mu c/\text{mole})}$$

The results obtained by these two methods were in close agreement with those obtained from an artificial rumen under very similar conditions.

Further infusion experiments were then performed (Weller, Gray, Pilgrim and Jones, 1967) using single labelled VFA at a constant rate. Composite samples of rumen fluid were collected during the 12 hour feeding cycle using an automatic sampling device operating every minute. Calculated productions of total

VFA were close, regardless of the particular acid infused, and agreed well with the results of previous work (Gray et al., 1966) and that of Bergman et al. (1965). These results gave a clear picture of the transfer of ^{14}C from each acid to the others enabling the calculation of interconversion rates. Using this information they were able to select the proportions in an acid mixture which would show no change in the distribution of ^{14}C between them. The net production of all VFA could then be measured simultaneously without having to correct for transfer of label between them.

They further noted that these and previous results (Gray et al., 1962, 1965; Bergman et al., 1965) showed that the mixture of acids finally produced in the rumen had a composition very similar to that of the mixture present in the rumen fluid. This suggested that where there is little change in the composition of the rumen acid mixture during the day (Gray et al., 1966), the value for the total VFA produced could be measured using a single labelled acid infusion and the values for the individual acids alloted according to their molar proportions in the rumen. Application of this technique to the data of Bergman et al. (1965), using the mean values for single acid infusions, resulted in the same rates as those of Bergman et al. using infusion of the three acids separately and the correction of "gross" rates to net rates for each of them.

Gray, Weller, Pilgrim and Jones (1967) and Weller, Pilgrim and Gray (1969) investigated the possible effects of the regularity and frequency of feeding

using the single acid infusion technique. Although increased variation in VFA concentration occurred as the time between feeds was extended, very similar amounts of VFA were produced under different frequency of feeding regimes whether steady or irregular. Quantities produced on successive days varied considerably, but the extent of the variation was similar to that occurring in faecal output and they concluded that the results for a considerable number of days should be used when VFA production from grazed pastures is being compared.

A further check on the validity of the isotope dilution method when the VFA pool is not constant (as with irregular feeding) was made using an equivalent marker procedure in a model rumen (Weller et al., 1969). The findings again supported the view that an isotope dilution method would be applicable under irregular intake conditions and hence to grazing sheep, provided mean rates are determined over a sufficiently long period.

Gray et al., (1967) devised a method to allow continuous sampling of rumen acids by dialysis and applied this to sheep grazing a mixed pasture (Weller et al., 1969). VFA production rates indicated the changing value of the pasture as the seasons progressed.

Leng and Leonard (1965) also used a continuous infusion technique to measure VFA production rates in sheep fed lucerne hay. Production was calculated on the basis of constant specific activities in the rumen acids achieved by feeding the animals at hourly intervals. Total production was estimated by extrapolation as the measured rates applied

only to a part of the feeding cycle. Single acid infusions were used to enable measurement of interconversion of the acids and "effective production rates" of individual acids calculated as the measured production rates from the mean, specific activity and rate of infusion of radioactivity, minus the sums of the rates of conversion into the other major acids.

Simultaneous measurement of production rates of the individual acids and hence the estimation of total VFA production, was then achieved using a constant infusion of the three acids, corrected for interconversion rates from single acid infusion experiments (Leng and Brett, 1966). The primary aim of this investigation was to establish a relationship between the concentration of individual VFA and their rates of production in the rumen and a clear general correlation was established despite large changes in the rumen fluid volume.

Using the same procedures to measure the rates of production of VFA in grazing sheep (Leng et al., 1968), similar relationships between entry rates and VFA concentration in the rumen to those measured in penned sheep were demonstrated. Infusion of the acid mixture was carried out using portable pumps over a six hour period and samples withdrawn every hour after the third hour of infusion. Production rates could not be measured continuously for 24 hours at pasture because the method depends on steady state conditions during the measurement period. Infusions at pasture were all made during the time when it could be expected that VFA concentrations would

be steady. However Leng et al. (1968) suggested that the regression equations relating entry rates and ruminal concentrations could be used to obtain valid estimates of VFA produced in 24 hours, by determining acid concentrations at regular intervals over 24 hours using an automatic collection technique of the type used by Weller et al. (1967). Applying this calculation to these and earlier results (Leng and Leonard, 1965) produced estimates of the same order of magnitude as that reported by Gray and his colleagues and Bergman et al. (1965).

Weller et al. (1969) tested the usefulness of the relationship between concentration and production of VFA in the rumen for calculating production rates as suggested by Leng et al. (1968). Although results indicated a somewhat different slope in the regression to that of Leng et al. (loc. cit.) these authors concluded that "the findings have similar implications". The relationship held good under conditions of irregular intake of food, providing experimental evidence to support the assumption of Leng et al. (loc. cit.).

Weston and Hogan (1968) also showed a clear relationship between total VFA production and their concentration in rumen liquor. These authors measured the ruminal production of VFA for diets of ryegrass and forage oats using a method similar to that of Bergman et al. (1965), with the modification of employing ^{51}Cr EDTA as the marker to measure rumen volume. The data obtained supported the conclusion of Weller et al. (1967), that when ^{14}C labelled VFA were infused either singly or in mixtures, the total production of VFA could be estimated

from the ^{14}C content of the total VFA in the rumen, using the formula:-

$$\text{Total VFA production (Moles/day)} = \frac{\text{Rate of infusion of } ^{14}C \text{ (}\mu c\text{/day)}}{^{14}C \text{ in total VFA (}\mu c\text{/mole)}}$$

The isotope dilution method of Gray et al. (1967) has thus been demonstrated to be satisfactory for measuring entry rates of VFA into the rumen. This is so both when the VFA pool is constant in size and when it varies with irregular feeding as during grazing.

(3) Interconversion of Fatty Acids

The isotope dilution method measures the gross production rates of VFA and these may appreciably exceed their net production rates, particularly for acetate and butyrate which undergo further metabolism in the rumen (Warner, 1964). Appreciable interconversion between acetate and butyrate has been shown, but propionate appears to be stable under most conditions Gray et al. (1960).

Reported estimates of interconversion of VFA are given in Table 1.5.

The assumption that all carbon atoms of the acids reacted similarly (Leng and Leonard, 1965) was considered by Leng and Brett (1966) to be invalid in the case of the interconversion of acetic and butyric acids. This would explain the comparatively low value for the proportion of butyric acid produced from acetic acid evident in the work of Leng and Leonard (1965).

Table 1.5.

Interconversion of VFA in the Rumen of Sheep

% propionic acid produced from acetic acid	% butyric acid produced from acetic acid	% acetic acid produced from propionic acid	% butyric acid produced from propionic acid	% propionic acid produced from butyric acid	% acetic acid produced from butyric acid	Feed	Reference
14	61	4.5	4.8	4.8	20	Dried Grass	Bergman et al. (1965)
4.5	16.9	3.2	0.9	3.4	14.7	Lucerne chaff	Leng & Leonard (1965)
4.3	44.6	0.45	0.7	2.7	10.7	Maize, lucerne chaff, wheaten straw chaff	Leng & Brett (1966)
	38.0				11.4	Phalaris tuberosa pasture	Leng et al. (1968)

Summary:

The isotope dilution technique for the measurement of VFA production in the rumen has several advantages over other available methods:

(1) It can be applied to animals which are physiologically more normal.

(2) It measures the total VFA potentially available to the ruminant from rumen fermentation.

(3) It can be successfully applied to the grazing animal.

However the method does not allow the measurement of VFA production in other parts of the alimentary tract.

1.4. Sampling from the Rumen of the Sheep

When it is necessary to take samples of rumen contents for analysis, two sources of error must be accounted for. The occurence of layering of food residues in the rumen and the resultant concentration gradients is well documented (Smith, Sweeney, Rooney, King and Moore, 1956; Davison, 1965; Bryant, 1964; Lane, Cummings, Noller and Collenbrander, 1966; Lane, Noller, Collenbrander and Cummings, 1968). Diurnal variation in concentration and proportions of rumen metabolites is also widely recognised (Bryant, 1964; Ulyatt and Henderson, 1968).

Bryant (1961) has reviewed the variations in rumen VFA concentration and pH arising through the method of sampling but little evidence is available pertinent to sheep.

1.4.1. The Distribution of VFA in Rumen Contents

In general in cattle a dorso-ventral gradient occurs in the rumen ingesta, the dorsal region being higher in VFA concentration (Smith *et al.*, 1956; Lampila and Poutiainen, 1966). No evidence for an anterior-posterior gradient was obtained by Bryant (1961). He noted a number of factors which affect the former gradient however, in particular type of feed and time after feeding. Layering of food particles was less evident as the time from feeding increased.

In sheep however, layering formation appears to be less pronounced. Boyne, Eadie and Raitt (1957) found non-significant differences in protozoan organisms between the dorsal and ventral regions of the rumens of sheep fed hay and concentrate.

The distribution of polyethylene glycol and a number of inorganic ions in the rumen fluid was studied by Hyden (1961). He concluded that the rumen fluid was on average of the same concentration in all parts of the rumen and reticulum. Gray et al. (1965) reached a similar conclusion for VFA concentration in different parts of the rumen of sheep fed chaffed roughage diets.

There appear to be no reports on concentration differences in the rumen of sheep fed fresh pasture.

Lane et al. (1966, 1968) found no differences among locations in the bovine rumen in the ratio of VFA, either before or after feeding.

1.4.2. Rumen Sampling Procedures

Sampling procedures based on material from slaughtered animals (Gray, 1947; Paloheimo, Makela and Salo, 1955; Turner and Hodgetts, 1955), cud (Davey and Briggs, 1959), and rumen puncture, although useful in certain situations are obviously very limited in application.

Sampling by stomach tube (Pounden, 1954; Hobson, 1961) may introduce errors through salivary contamination and the uncertainty of locating the end of the tube in a known position within the rumen (Davey, 1965a).

Sampling by fistula is the most commonly used method but the problem of sample withdrawal remains. Some degree of abnormality may be induced in the rumen environment (Bryant, 1961) but no major abnormalities are present (Smith, 1956; Hungate, 1966).

Thorough mixing of the whole of the rumen contents either with or without removal from the rumen has been used by a number of workers to reduce the errors in sampling.

Mixing *in situ* is feasible when solid food particles are small enough for a slurry to be formed, for example in fasted animals or animals fed ground or low fibre diets. Danielli *et al.* (1945) attempted to improve mixing by vigorous massage through the abdominal wall, with questionable results however. Circulating pumps have been developed for mixing and sampling (Sutherland *et al.*, 1962; Englehardt, Hoeller and Hoernicke, 1963) but are limited to use with finely ground diets.

Removal of rumen digesta for thorough mixing ("bailing") has been used in both cattle (Reid *et al.*, 1967; Bailey, 1965, 1967) and sheep (Watanabe and Umezu, 1962, 1963), but can be practised only for infrequent sampling because of aeration of the ingesta and mechanical breakdown resulting in temporary disruption of the normal digesta distribution pattern (Reid, 1965; Bryant, 1961).

Where serial sampling is involved, taking samples from a fixed location in the rumen is common (Bryant, 1964; Davey, 1965b) or from a number of sites to obtain a representative sample (Lane *et al.*, 1968; Bryant, 1964). Although doubt has been expressed as to the reliability of this method in the bovine animal (Reid *et al.*, 1967) evidence for satisfactory sampling from a fixed position in the ovine rumen has been documented by Hyden, (1961), Canaway, Terry and Tilley (1965), and Gausseres (1965), Gray *et al.* (1965, 1967) using automatic

sampling devices.

However again there is no information on the accuracy of the method in pasture-fed sheep.

1.4.3. Diurnal Variation in Rumen Metabolites

Composition of ingesta in the rumen is subject to marked diurnal variation, being affected by such factors as rate of fermentation, gastric motility, and ingestion of food and water.

Changes in VFA concentration with time after feeding have been well documented for pen-fed animals, both cattle (Bryant, 1964; Davey 1965a, 1965b; Davison, 1965; Lane et al., 1966, 1968) and sheep (Gray et al., 1965, 1967; Canaway et al., 1965; Weller et al., 1967). Changes in VFA proportions with changes in VFA concentration have also been noted on a variety of diets (Bryant, 1964; Davey, 1965a; Ulyatt and Henderson, 1968; Kingsbury, 1965).

To enable sampling of rumen contents over the whole day, or appropriate feeding period, automatic sampling devices have been developed to sample at frequent intervals (Canaway et al., 1965; Weller et al., 1967) or continuously (Gray et al., 1967) using a dialysis probe.

Summary;

Stratification of food residues and so metabolite concentrations occurs in the bovine rumen with most diets. In the ovine the position is less certain, particularly with regard to pasture diets. If layering does not occur then sampling rumen contents from a fixed position within the rumen should provide a representative sample of the whole contents of the reticulo-rumen. If this is so, the use of a continual or continuous sampling technique should enable accurate sampling under any feeding conditions to be done.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Animals

Nine Romney Marsh wether hoggets were prepared with rumen fistulae by the technique of Jarrett (1948). These sheep were trained to wear faecal collection harnesses and accustomed to confinement in metabolism crates.

The sheep had constant access to water and a mineralized salt lick (Na, 24.25%; Ca, 4.5%; Mg, 0.7%; on DM basis) and regular care of fistulae to guard against infection. During the experimental period they were vaccinated against salmonellosis and received regular treatment with the anthelmintic thiabendazole ("Thibenzole", Merck, Sharp and Dohme).

2.2. Pastures

The pasture types used in this investigation and their sowing dates were as follows:

Grasslands Ruanui perennial ryegrass (*Lolium perenne* L.)	(P)	Autumn 1958
Grasslands Manawa ryegrass (formerly short rotation ryegrass) (*L. perenne* x *L. multiflorum*)	(S)	Autumn 1967
Grasslands 4700 white clover (*Trifolium repens* L.)	(C)	Autumn 1966

These particular pasture types were chosen for this investigation because they represent the main improved species used in New Zealand.

All swards received dressings of 2 cwt of superphosphate per acre annually, and the ryegrasses 1 cwt of nitrolime per acre monthly. Excess growth was controlled by topping to a height which is normal for grazed pasture at this time of year.

2.3. Experimental Design

The general outline of the experiment is given as follows:

(a)	14th September to 1 November 1968	- sheep fistulated and grazing on mixed pasture
(b)	1 November to 11 December	- preliminary indoor feeding period
(c)	11 to 14 December	- test run for infusion and sampling techniques, and faecal collection
(d)	18 to 24 December	- experimental infusion and sampling (Period I)
(e)	24 to 30 December	- experimental infusion and sampling (Period II)

The objectives of the preliminary feeding period were threefold; to allow the animals to adjust to the feed, to accustom them to the metabolism crates and faecal collection harnesses, and to establish a satisfactory feeding routine. Success in achieving these aims was gauged from the behaviour and feed intakes of the animals.

During the test period methods of faecal collection, and infusion and sampling techniques were put into operation. The successful timing and maintenance of these operations was achieved.

Table 2.1 illustrates the general plan followed during the experimental periods. On each sampling day during Period I one sheep from each treatment group was moved to a separate room

Table 2.1.

General Plan of the Experiment

Period	Date of Sampling	Sampling Group	Set of Apparatus					
			(1)		(2)		(3)	
			Treatment	Sheep Number	Treatment	Sheep Number	Treatment	Sheep Number
I	18 December	1	S	79	C	25	P	3
	20 December	2	S	94	C	88	P	122
	22 December	3	P	113	S	29	C	7
II	24 December	1	P	3	S	79	C	25
	27 December	2	C	88	P	122	S	94
	29 December	3	C	7	P	113	S	29

and there the infusion and sampling operations performed. This process was repeated in Period II using the same groups and sequence but arranging the sheep about the infusion room so that each infusion and sampling apparatus was used twice with each treatment. This enabled the comparison of treatments to be carried out independant of sets of apparatus.

2.4. Feed Organisation

Material from each plot was cut daily at 7 a.m. using an Allen motor scythe (John Allen and Sons Ltd, Oxford) to avoid any unnecessary bruising of the plant material. Rapid determination of the dry matter content (DM %) of this material was obtained from two samples of approximately 100g of each feed, placed in large gauze-bottomed trays in a forced-draught oven for an hour at 90°C. The weight of wet feed required to provide each sheep with approximately one kg of dry matter was calculated from the formula:

$$\text{Wet herbage fed (lb)} = \frac{220.5}{\text{rapid DM\%}}$$

Half this amount was fed to all sheep at 10 a.m. The remainder was stored in plastic bags in the anteroom of a freezing chamber at 5°C and fed at 10 p.m. A further sample of approximately 400g of each feed was dried at 90°C for 24 hours to provide an accurate measurement of dry matter percentage. In addition, daily samples of 100g were frozen, bulked for each grass over the seven day faecal collection period, and subsequently dried in a freeze-drier.

During the second experimental period feed samples taken at the 10 p.m. feed were frozen, bulked and dried for comparison with the a.m. feeds to determine the effect of storage.

2.5. Apparent Digestibility Determination

Faecal collections from all sheep were made each morning and the total wet faeces weighed. Samples of approximately 100g were weighed and dried at 90°C for 24 hours for determination of DM content. Further aliquots of 10% were frozen, bulked over the seven day experimental period, and a subsample dried by freeze-drying.

The freeze-dried samples of feed and faeces were finely ground through a 1mm mesh in a Wiley mill and stored in airtight glass jars. To allow calculation of the results on an organic matter basis, samples of feeds and faeces were ashed at 600°C for 4 hours.

The gross energy contents of the feed and faeces samples were determined in a Gallenkamp Automatic Adiabatic Bomb Calorimeter.

2.6. Infusion Technique

The single acid infusion method of Gray et al. (1967) was chosen for its simplicity and apparent accuracy (See 1.3). Sodium acetate -2-^{14}C was used as infusate because of its low cost (Radiochemical Centre, Amersham), although it had the possible disadvantage of a greater degree of interconversion than other ^{14}C-labelled VFA (Weller et al., 1967).

0.5mc of sodium acetate -2-^{14}C, specific activity 464 µc/mg, was eluted into 200 ml of deionised water and stored at -10°C. For each infusion 8 ml samples of this solution were diluted to 750 ml with deionised water and delivered at the rate of 50 ml/hour, i.e. 1.335 µc/hour, to the rumen. A sample of each infusate was stored at -10°C for later analysis.

The infusate was delivered from a glass container at floor level, into the rumen using a D.C.L. infusion pump (See Plate I). Pumping against a head of pressure should have prevented the fluctuation in flow rate suffered by gravity-feed systems.

Daily Routine:

8 a.m.	Infusion begun to allow mixing and equilibration in the rumen before the commencement of sampling.
10 a.m.	Sampling begun. Animals fed.
10 p.m.	Infusion terminated. Sampling stopped. Animals fed.

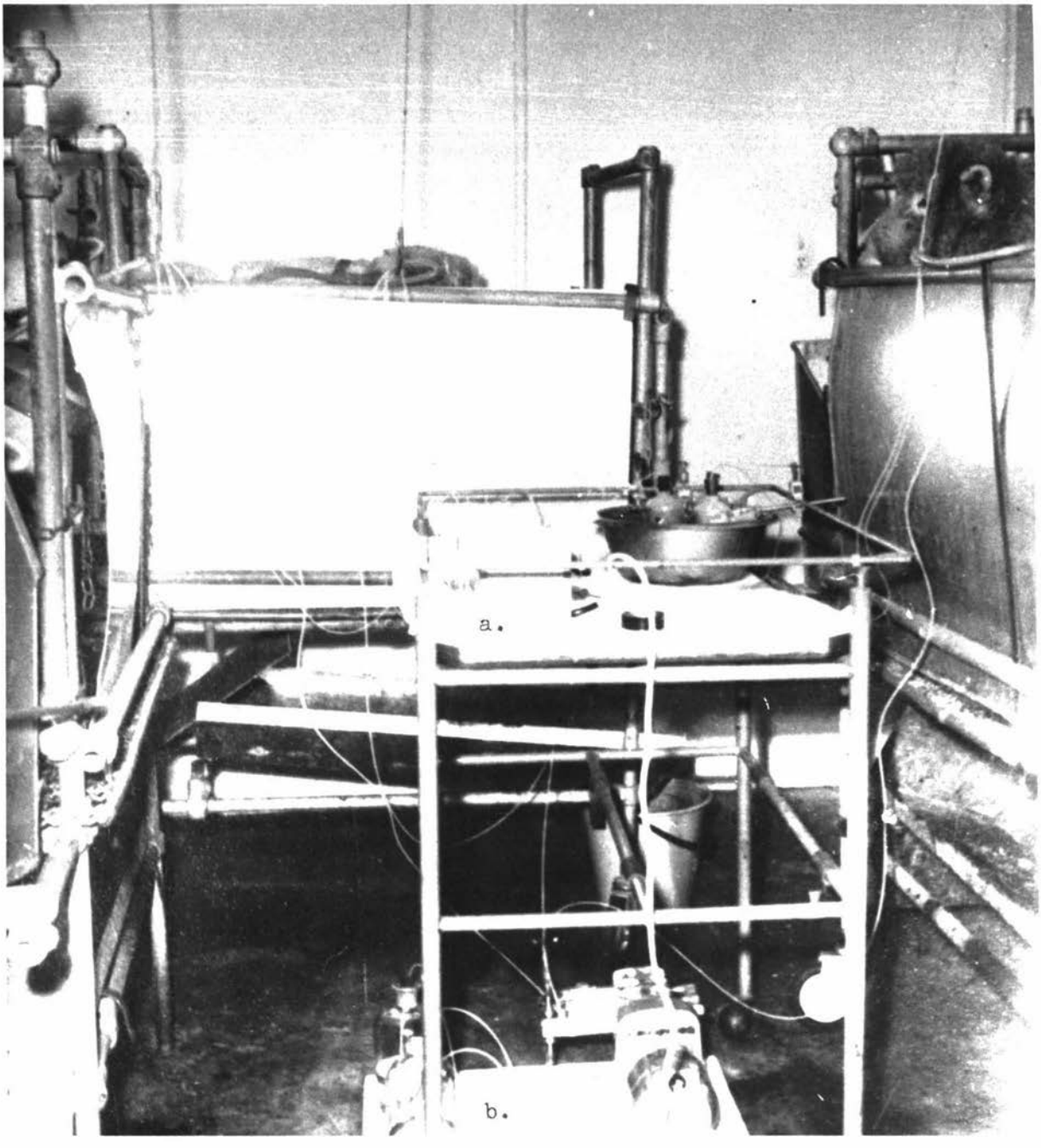


Plate I. Infusion and Sampling Apparatus in Operation

a. Sampling apparatus

b. Infusion apparatus

2.7. VFA Sampling Procedure

VFA present in the rumen during infusion were sampled by means of the dialysis system described by Gray et al. (1967).

A number of probes were made from 1/4 inch teflon rod, 4.5cm long, by turning a spiral channel along three quarters of the length of probe. The ends of the channel thus formed were connected to metal tubes through holes drilled the length of the probe (See Plate II).

Visking cellulose tubing (Union Carbide Corp.), 1/4 inch in diameter, was knotted at one end, pulled over the probe and sealed to it by a wire band. Dialysing medium was forced through the system at a rate of 40 ml per twelve hours, from syringes connected to the worm drive of a Palmer infusion pump (C.F.Palmer Ltd, London). Care was taken to ensure the point of infusion of acids was well above the position of sampling (See Plate III). Deionised water was used as the dialysing medium (Gray et al., 1967).

Dialysates were collected into plastic bottles in an ice bath to prevent loss of acid by volatilisation. The bottles contained 10 ml of 10 NH_2SO_4 saturated with $MgSO_4$ to prevent enzymic changes in the rumen contents. The stable acid solutions were stored at -10°C until required for analysis.

Testing of Dialysis Sampling Method

In order to determine the usefulness and accuracy of the dialysis sampling method, a number of investigations were carried out under both in vitro and in vivo conditions to compare dialysis sampling of VFA with direct sampling.

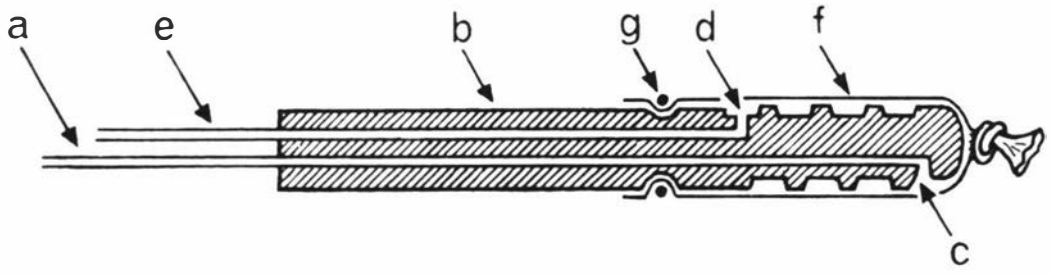


Plate II. Diagrammatic Representation of Dialysis Probe

a. Inlet tube
b. Teflon rod
c. Outlet duct
d. Inlet duct
e. Outlet tube
f. Dialysis tubing
g. Wire band

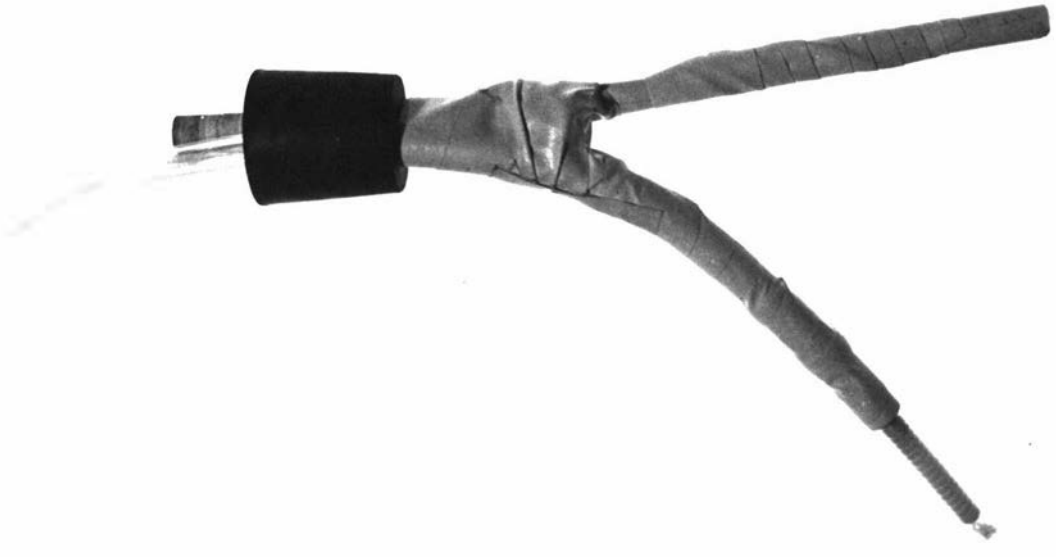


Plate III. Relative Positions of Dialysis Probe and Infusion Outlet

(1) In vitro

Solutions of VFA as the free acids or sodium salts were prepared at concentrations similar to those in the rumen of sheep fed pasture (approximately 15 mM/100ml), and placed in conical flasks in a shaking water bath at 38°C. The dialysis probes were set up in the flasks and dialysing medium run through them for periods of 6 or 12 hours.

Samples of the original solution and dialysate were steam distilled and titrated to neutrality with 0.05N NaOH using phenolphthalein as indicator. After evaporating to dryness in a forced-draught oven at 90°C, the VFA proportions present in each sample were determined by gas liquid chromatography (See 2.8.2).

(2) In vivo

(a) The degenerative effect of rumen micro-organism action, particularly that of cellulotytic bacteria, on the cellulose tubing was investigated by the suspension of rumen liquor filled lengths of tubing in the rumen of a pasture fed sheep.

(b) Investigation of dialysis sampling was extended to a number of trials in vivo using fistulated animals.

In experiment 1, two sampling sites in the rumen were compared.

Two probes were positioned in the rumen of a sheep; one "High", level with the cannula; and one "Low", approximately 4 inches below the cannula. Dialysis medium was run through

them for 12 hours while direct samples from the middle of the rumen (1.4) were taken by aspiration at hourly intervals over the 12 hour period. Rumen liquor samples were centrifuged at 2000 g. for 20 minutes and the supernatant stored at -10°C in glass bottles containing 10N H_2SO_4 saturated with $MgSO_4$.

In experiment 2, single probes in the "Low" position were operated in each of two sheep as in experiment 1, to compare sampling of rumen VFA by dialysis and by direct means from a fixed position in the rumen.

2.8. Analytical Methods

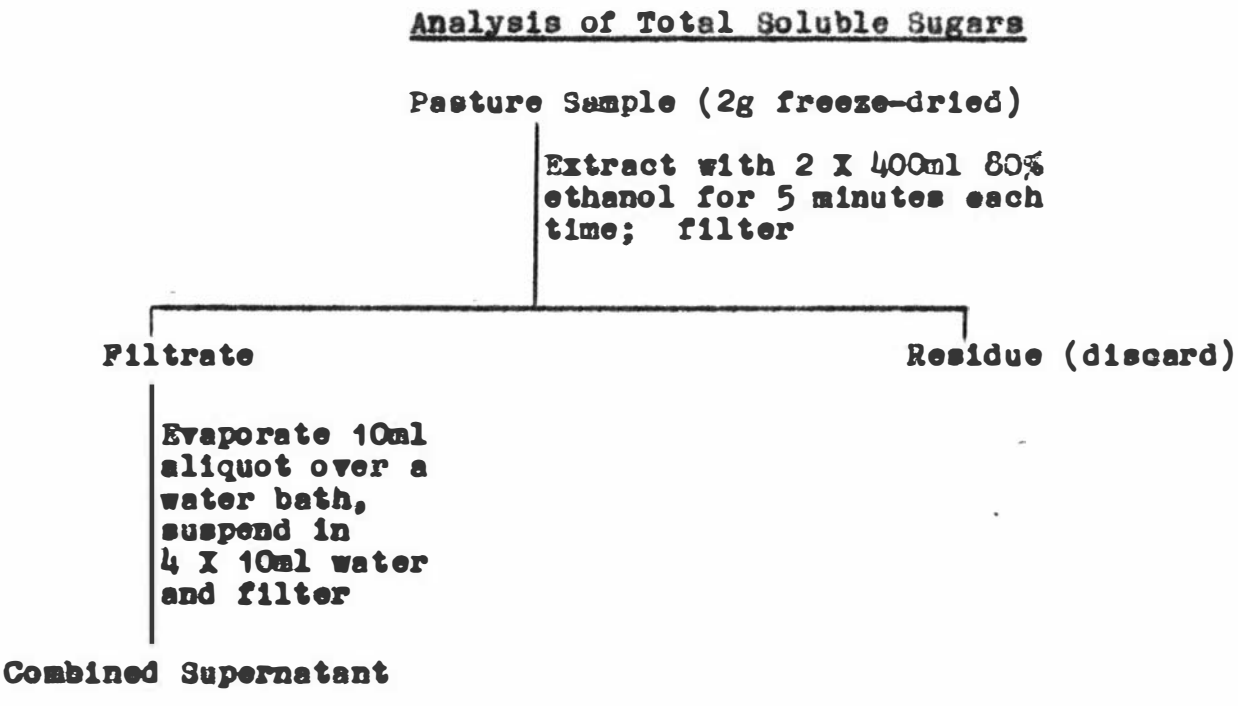
2.8.1. Chemical Analysis of Feeds

The total nitrogen content of the feeds was determined by a macro Kjeldahl technique (A.O.A.C., 1965) using mercuric sulphate calalyst.

The content of cell wall constituents (cellulose, hemicellulose and lignin) was determined by the method of Van Soest and Wine (1967), and lignin by the method of Van Soest (1963).

The soluble carbohydrate content of samples of the a.m. and p.m. feeds from Period II were compared by a method based on that of Bailey (1964). The method used is summarised in Fig. 2.2.

Fig. 2.2.



The total soluble sugars were estimated by the method of Bath (1958). One ml of supernatant was diluted to 100ml with water and 1ml of this solution added to 6ml of 98% H_2SO_4 in a test tube, chilled in a ice-water bath, and stirred. The test tube was then placed in boiling water for exactly 5 minutes and finally cooled to room temperature in a cold water bath. Optical density was measured on a Beckmann Spectrophotometer at 322 mμ against a water blank and compared with a set of standard glucose solutions.

2.8.2. Volatile Fatty Acid Analysis

Rumen liquor samples stored at -10°C were thawed and further centrifuged at 105,000 g. for 30 minutes.

All samples, i.e. clarified rumen liquor samples and dialysates, were steam distilled in a Markham (1942) Still. As the samples were already acidified with 10N H_2SO_4 saturated with $MgSO_4$ (McAnally, 1944; Olmsted, 1929) further acidification was unnecessary. Distillates of 70ml were collected and titrated against 0.05N NaOH using phenolphthalein as indicator. CO_2- free air was passed through the distillates for three minutes prior to titration and CO_2- free water used throughout for washing.

The NaOH was standardised against sodium potassium phthalate and a blank correction was made for organic acids which were slightly steam volatile, by collecting and titrating a second 70ml distillate every five samples. Determinations were repeated where the difference between duplicates was greater than one per cent (Davey, 1964). After titration an excess of 4ml of

NaOH was added.

Bulked duplicate distillates were then evaporated to dryness at 90°C, redissolved in 0.5ml of distilled water, and the proportions of individual VFA determined by gas-liquid chromatography using the method of James and Martin (1952). The column used was packed with 20% P.E.G.A. on Celite 545 (Storry and Millard, 1965), and run at 137°C with a carrier nitrogen flow rate of approximately 15 ml/min. One or two drops of sodium salts of VFA were added with a hypodermic syringe to a teflon boat containing a dry mixture of equal parts of celite and NaH_3O_4. This boat was then pushed quickly into the column and the nitrogen supply connected.

Repeat determinations were carried out where duplicates differed by more than one percentage unit in any of the three acids. The accuracy of the results was checked by using a number of standard solutions containing different proportions of VFA.

Some of the samples from the preliminary tests were analysed on a Varian Aerograph Hy-Fi III Gas Chromatograph equipped with a flame ionisation detector. Responses were recorded by a Leeds and Northrop recorder fitted with a Disc integrator (Disc Instruments Inc., California). The column used was of $\frac{1}{8}$ inch stainless steel packed with 20% FFAP on 60/80 mesh Chromosorb W (Hammarstrand, 1966; Bonelli, pers. comm.). The column temperature was 140°C and the flow rate of nitrogen, 20 cc/minute. Approximately 1 µl of phosphoric acid was drawn into a Hamilton syringe, followed by the same quantity of VFA salt, and the two injected into the mixing chamber.

Detector response factors were estimated from standard solutions of pure VFA using the following formula (Packett and McCune, 1965):-

$$\text{Relative response factor for VFAx} = \frac{\text{area of butyric acid peak X molar \% in sample}}{\text{area of VFA x peak X molar \% in same sample}}$$

Molar percentages of VFA samples were then determined from the corrected peak areas. The same conditions of acceptance of results were used as with the James and Martin apparatus.

2.8.3. Separation of VFA for Scintillation Counting

(1) Separation Technique

The titration cell of the James and Martin apparatus was fitted with a reflux condensor (Storry and Millard, 1965) to minimise possible acid losses through volatilisation.

After the VFA sample had been applied to the column and the gas flow connected, 20ml of phenol red. (0.01% w/v) (James and Martin, 1952) was added to the titration cell and the photocell sensitised. Immediately the separation of acetic acid was complete, the contents of the titration cell were aspirated into a beaker containing 1ml of 0.05N NaOH, followed by three washings of 20ml of distilled water.

Fresh indicator solution was then poured into the titration chamber and titration carried out to bring the colour to the previous sensitivity setting. This process was then repeated for propionic and butyric acids.

(2) Decolourisation

Phenol red indicator in the acid solution caused

considerable quenching of radioactivity in the scintillation mixture. A number of methods of decolourising the separated VFA samples were investigated.

(a) Paper chromatography

Because phenol red (phenolsulfonphthalein) is soluble in alcohol, (Merck Index, 1968) ascending paper chromatography was tried as a means of removing the indicator from the salt solutions.

A solution containing phenol red, ^{14}C-labelled acetic acid, and NaOH was applied, with drying, to one end of a piece of chromatography paper 2cm x 10cm. The paper was suspended in a chromatography bath containing acetone dried with anhydrous sodium sulphate, and ascending chromatography carried out for 24 hours. The piece of paper carrying the Na salts was then removed and placed in a vial containing a scintillation mixture for measurement of the activity present.

(b) Centrifugation with charcoal black (Rapkin, 1961)

Solutions of VFA salts containing indicator were spun at 1500 g. for 10-15 minutes with charcoal black.

(c) Boiling with Zinc in Aqueous Alkali or Carbonate (Merck Index, 1968)

To 20ml of indicator solution, a quantity of ^{14}C-acetate with excess NaOH was added and the solution evaporated to dryness. 0.1M Na_2CO_3 was then added and the solution boiled with zinc powder until it became colourless. The supernatant was then decanted off, and the zinc powder washed twice with further Na_2CO_3, followed by centrifugation at 1500 g. for 10-15 minutes.

The clear solution was evaporated to dryness, taken up with 3ml of distilled water and 5ml of Bray's (1960) counting solution, and counted in a Nuclear Enterprise scintillation counter.

(d) Steam Distillation (Leng and Leonard, 1965)

Solutions of individual VFA collected from the titration cell of the chromatogram were evaporated to dryness over a water bath. Each sample was then washed into a Markham still with 10ml of distilled water, acidified with 1ml of 10N H_2SO_4 saturated with $MgSO_4$, and 150ml of distillate collected into excess 0.005N NaOH. The amount of NaOH required for excess was calculated from the amount needed to neutralise each acid passing through the James and Martin detector. After evaporation the decoloured VFA salts were washed carefully into counting vials and again evaporated to dryness.

(3) Recovery of ^{14}C Label

To determine the recovery of labelled VFA after the separation treatment a standard solution of known activity was used.

2.8.4. Scintillation Counting

Bray's (1960) solution was used as the scintillation mixture in the early investigatory work because of its capacity to hold a small amount of water with little quenching. Higher counting efficiencies were subsequently achieved with a Triton-toluene mixture (Patterson and Green, 1965). The components of this mixture are cheap and do not deteriorate on storage. Furthermore it has the capacity to hold up to 10% water with very

little quenching.

VFA samples dried in the counting vials were taken up with 0.5ml of deionised water and 10ml of the Triton-toluene mixture. The activity of the samples was determined either in a Nuclear Enterprises counter or automatically in a Packard 3000 Tri-carb Liquid Scintillation Spectrometer. The effect of quenching caused by excess alkali, water or indicator was determined by plotting counting efficiency against Channels Ratio using standard ^{14}C-acetate solutions containing variable amounts of quenching agent (Baillie, 1960; Bruno and Christian, 1961). The use of the quaternary compound Hyamine (Rohin and Haas, Inc.) instead of NaOH, did not reduce quenching.

2.8.5. Determination of Specific Activity of Total VFA

The ^{14}C content of total VFA in the dialysed samples was also assayed, and compared with activity from the partitioned acids. This was an alternative method of the calculation of specific activity (Gray et al., 1967; Weston and Hogan, 1968; Weller et al., 1969).

Duplicate 5ml samples of dialysate were distilled and collected in an amount of alkali calculated from previous determinations of VFA concentration to provide a slight excess. These solutions were dried in a forced-draught oven at 90°C and washed carefully into counting vials. After drying in the vials the VFA salts were taken up with 0.5ml distilled water and 10ml of Triton-toluene scintillation mixture. The mean total activity per 5ml of sample was then combined with the previously determined VFA concentration in the dialysate, to determine the specific

activity (μc/mMole) of the total VFA present.

Percentage recovery of total labelled VFA was determined by the use of a standard solution of known activity.

2.9. Statistical Analysis of Data

Individual estimates of the rate of VFA production in the rumen were obtained for each of the 12 hour samples from:

$$\text{Production rate (mM/min)} = \frac{^{14}C \text{ infused } (\mu c/min)}{^{14}C \text{ in VFA of dialysate } (\mu c/mM)}$$

The elimination of a number of the estimates on the basis of faulty technique (See 3.7.), resulted in unequal sub-class numbers in each period. A test for disproportionate numbers (Snedecor, 1967) was applied to the remaining data to determine the significance of the differences between treatments, periods and sets of apparatus in the presence of unequal sub-class numbers.

CHAPTER THREE

RESULTS

3.1. Techniques

The method of measurement of VFA production decided upon, required considerable preliminary investigation before it was applied to the experimental animals. In particular three sections of the method needed preparatory work. These were; the accuracy of dialysis sampling, the subsequent treatment of the sample, and the measurement of the radioactivity it contained. Results and discussion of these investigations are given here.

3.1.1. Sampling of the Rumen VFA by Dialysis

(1) Results of *in vitro* Tests (See 2.7.(1))

The molar proportions of VFA in standard salt solutions were compared with those of samples taken from them by dialysis. Table 3.1. illustrates the results of regression analysis of the data obtained.

The dialysis membrane appeared to exhibit a slightly greater permeability to acetate than to propionate. This was of a similar order to that observed by Gray *et al.* (1962, 1965, 1967). However the correlations between the proportions in dialysates and standards were high (> 0.92). The regressions of dialysate upon standard were above one for acetate and propionate (Sy was slightly greater than Sx as might be expected) but the 95% fiducial limits in both cases included 1.0. For butyrate the regression coefficient was close to one. It was

Table 3.1.

Analysis of Regression of VFA Proportions in Dialysates on Proportions in Prepared Standards

VFA	Number of Samples	Mean Molar %		Correlation Coefficient	Regression Coefficient				
		Dialysate	Standard						
	n	$\bar{Y}$	$\bar{X}$	r_{xy}	b	S.E.	t	p	95% Confidence Limits
Acetic acid	11	68.91	67.81	0.97	1.11	0.09	12.33	<.01	$0.906 \leq \beta \leq 1.314$
Propionic acid	11	18.66	19.36	0.93	1.21	0.17	7.20	<.01	$0.803 \leq \beta \leq 1.59$
Butyric acid	11	12.60	12.82	0.92	0.92	0.13	6.87	<.01	$0.617 \leq \beta \leq 1.223$

concluded that adjustment of the dialysate results on the basis of the small differences in proportions was not warranted (cf. Gray *et al.*, *loc. cit.*). As the acetate produced in the rumen during infusion of ^{14}C-acetate would be more highly labelled than the other acids, a higher proportion of this acid in the dialysate could result in an underestimation of VFA production rate. However a difference of the order of 2 per cent in the molar acetate proportion would not affect the final estimate of the production rate by more than 3 per cent. This is considered to be well within the random error.

(2) Results of *in vivo* Tests (See 2.7.(2))

(a) Dialysis tubing suspended in the rumen of a sheep fed fresh pasture showed no visual indication of degeneration after 48 hours. It was concluded that the effect of cellulolytic microorganisms on the tubing would be unimportant during a 12 hour infusion period (cf. Weller *et al.*, 1969).

(b) The mean molar proportions of VFA from samples taken by dialysis at the "Low" and "High" positions in the rumen, and by direct sampling from approximately the middle of the rumen are shown in Table 3.2. It was concluded that a dialysis probe in the "Low" position, approximately 4 inches below the level of the rumen cannula, gave a more accurate sample (See 1.4.) than one situated level with the cannula ("High"). The lower position was used in all subsequent work (See Plate III).

The proportions of VFA in the dialysis samples taken from the "Low" position were compared with those taken by direct sampling by regression analysis (Table 3.3). The proportion of

Table 3.2.

Molar Proportions of VFA in Rumen Samples

VFA	"Low" Probe	"High" Probe	Direct Sample
Acetic acid	63.6	66.8	62.8
Propionic acid	24.4	23.0	25.0
Butyric acid	12.0	10.2	12.8

Table 3.3.

Analysis of Regression of VFA Proportions in Dialysates on Proportions in Direct Samples

VFA	n	Dialysate $\bar{Y}$	Direct Sample $\bar{X}$	r_{xy}	b	S.E.	t	p	95% Confidence Limits
Acetic acid	4	67.0	65.1	0.98	1.21	0.15	8.01	<0.05	$.56 \leq \beta \leq 1.86$
Propionic acid	4	22.2	23.3	0.95	1.08	0.24	4.58	<0.05	$.06 \leq \beta \leq 2.10$
Butyric acid	4	11.0	11.6	0.72	0.72	0.49	1.47	N.S.	

acetate was again consistently higher in the dialysate than in the direct samples. However, less of the variation in VFA proportions in the dialysate was accounted for by variation in VFA proportions in the direct samples. This could have been a result of variation within the rumen.

3.1.2. Preparation of Rumen VFA Samples for Determination of Specific Activity

A number of methods of decolourising solutions of VFA salts containing phenol red indicator after separation by G.L.C. were investigated (2.8.2. (2)). Only steam distillation completely removed the indicator without considerable loss in activity. The recovery of ^{14}C label after this process is given in Table 3.4.

Table 3.4.

Recovery of ^{14}C Label after Preparation for Counting

Process	Number of determinations	Mean Recovery (%)	S.E.
Separation of VFA by G.L.C.	7	92	1.4
Total VFA by steam distillation	7	95	0.9

Bergman et al. (1965) avoided the necessity of decolourising VFA solutions after separation by using phenolphthalein indicator which is colourless in the alkaline range of

pH. However this method entailed redesigning of the titration cell of the James and Martin chromatogram so that the gas bubbles were not be detected by the photocell. Leng and Leonard (1965) used a column chromatography technique involving two chromatographic separations and monitoring by G.L.C.

The availability of recently developed electronic G.L.C. equipment has enabled a number of techniques to be developed for the separation, measurement and collection of samples without the necessity of titration (Hammarstrand, 1966). These could prove satisfactory for VFA analysis.

The measurement of specific activity of the total VFA sample without separation of the individual acids was also inveatigated (See 2.8.5). Recovery of ^{14}C label by this process is given also in Table 3.4.

The error in determining the specific activity of VFA was reduced by the use of the total VFA rather than the separated acids, and the percentage recovery was higher.

3.1.3 Determination of Counting Efficiency

The observed quenching of radioactivity in the VFA samples analysed might have been due to the presence of any of several known quenching agents. Phenol red, NaOH, sodium salts of VFA, and water may all have caused a reduction in counting efficiency to some extent (Birks, 1969). However the type of quenching agent appears to have little or no effect on the validity of the calibration curves for a solvent system (Bruno and Christian, 1961). Even colour quenching need not be distinguished from other types of quenching unless the sample is highly quenched

(Baillie, 1960).

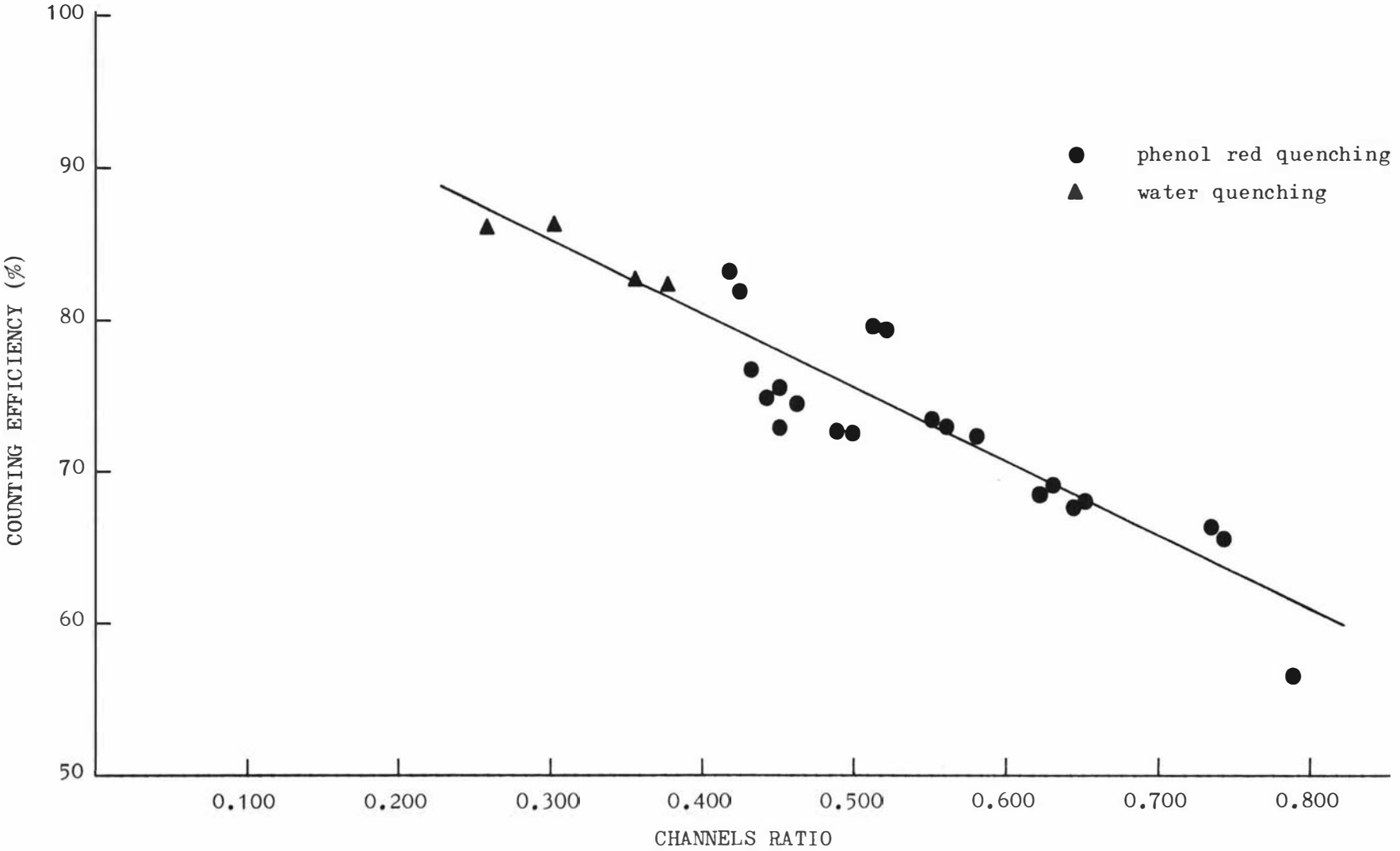
A plot of channels ratio versus counting efficiency was derived from a series of serially quenched samples of known activity. Using a series of samples containing phenol red as the quenching agent and a few quenched with water (Fig. 3.1), a regression line fitted to the data demonstrated a significant relationship ($p < .01$) for :-

$$\underset{\text{(Counting efficiency)}}{Y} = 99.99 - 48.77\underset{\text{(Channels ratio)}}{X} \quad ; \quad Sb = 3.21$$

If this line is extrapolated towards the Y axis it can be seen that when quenching is zero the counting efficiency is close to 100 per cent, as would be expected in an ideal system.

Samples containing sodium hydroxide or sodium acetate at low concentrations ($<$ 0.2ml 1N NaOH or 1M Na acetate) as quenching agents gave results consistent with this relationship. However at concentrations higher than these the relationship did not hold and a marked opaqueness of the solutions was noted. A.C.Taylor (pers. comm.) has suggested that large amounts of alkaline substances might affect the micelle-forming ability of the detergent (Triton X-100; (Rohin and Haas, Inc.)) and relatively large micelles of the aqueous phase could be formed. Contact between the radioactive source in the aqueous phase and the scintillators in the toluene phase would then be disrupted and counting efficiency lowered. High and low energy emissions would be equally affected and hence there would be little or no change in the channels ratio.

Figure 3.1. Relationship between Channels Ratio and Counting Efficiency



The amounts of sodium acetate and sodium salts of VFA used during experimental analysis were well below the limiting values mentioned and the established relationship was used to compute the counting efficiency of all samples measured.

3.2. Animals

3.2.1. Health

Occasional slight infection occurred around the cannula and was treated with Streptopen injection (Glaxo Laboratories; 250,000 units/ml procaine benzyl penicillin, .25g/ml dehydrostreptomycin sulphate).

A low intake of ryegrass, particularly the fibrous P, during the preliminary period, was attributed to teething troubles as the gums of these sheep appeared swollen and inflamed. Where a serious decline in intake occurred, Vitamin B_{12} (Cytamen, Glaxo Laboratories) was administered intramuscularly.

3.2.2. Live Weights

Table 3.5.1. illustrates the live weights and live weight changes of the sheep over the two week experimental period. The mean live weight change of the sheep fed P was greater ($p < 0.05$) than that of the sheep fed C (Appendix I). However, as all sheep were of similar live weight prior to fistulation, this result probably reflects a recovery in condition lost during the preliminary period when the intake of P appeared to be depressed by teething troubles.

Table 3.5.1.

Live Weights and Live Weight Changes over the Experimental Period (lb)

Treatment	Sheep Number	Live Weight 5th Dec.	Live Weight 31st Dec.	Live Weight Change	Mean Change and S.E.
C	7	120.0	118.0	-2.0	
	25	87.0	89.0	+2.0	
	88	109.0	108.0	-1.0	
					-.33 ± 0.92
S	79	96.0	98.0	+2.0	
	94	93.5	95.5	+2.0	
	29	81.5	82.5	+1.0	
					1.67 ± 0.92
P	3	86.5	91.5	+5.0	
	113	81.0	86.0	+5.0	
	122	85.0	87.0	+2.0	
					4.0 ± 0.92

3.3. Pastures

The botanical composition of the pasture swards used throughout the experiment is given in Table 3.5.2. and shows that the pastures were reasonably pure. The greatest contamination was the 10 per cent of poa species in the S.

Table 3.5.2.

Botanical Composition of Pastures
(% DM)

	C	S	P
Clover Species	91.7	0.25	0
Ryegrass Species	} 8.1	89.2	93.6
Poa Species		10.3	4.5
Other Species	0.2	0.25	1.9

Data on the chemical composition and gross energy (GE) content of the pastures are given in Table 3.6.

The estimates of the structural carbohydrate content of the pastures appeared to be slightly high when compared with previous estimates using similar feeds (e.g. Ulyatt, 1969). However the low levels of soluble carbohydrate present in these samples (see below) would have inflated the proportions of the other components. The ryegrasses were considerably higher in structural carbohydrate content than was C.

The results of the soluble carbohydrate analysis of the a.m. and p.m. feeds are given in Table 3.7.

Table 3.6

Chemical Composition and Energy Content of Herbage

(DM basis)

Period	Pasture	Structural Carbohydrate (Neutral-detergent fibre) (%)	Lignin (%)	Nitrogen (%)	Ash (%)	GE Content (cal/g)
I	C	26.12	3.75	4.70	9.38	4526
	S	43.58	2.73	4.83	9.92	4424
	P	43.81	5.43	4.65	10.32	4556
II	C	25.12	3.38	4.87	8.15	4671
	S	40.16	2.89	5.06	7.75	4579
	P	47.68	4.05	4.31	8.86	4616

Table 3.7.

Soluble Carbohydrate Content of Cut Feeds
(% DM)

Sample	C	S	P
a.m. Feed	10.74	6.67	5.58
p.m. Feed	7.72	4.36	4.82

All results were low (cf. Bailey, 1964) but this may have been a result of cutting in the early morning (Waite and Boyd, 1953; Kingsbury, 1965). The storage of cut pasture for twelve hours resulted in a lower soluble carbohydrate content, particularly in C. Raguse and Smith (1965) have shown a rapid decline in the sugar content of grasses after cutting, through respiration losses. However, this material was not used during the measurement of VFA production.

Table 3.8.

Mean Daily Intakes and Digestibility of Pastures

Period I

Treatment	Sheep No.	DM Intake (g)	Digest. of OM (%)	DOM Intake (g)	DE Intake (kcal)
C	88	980	81.9	727	3245
	7	994	81.9	737	3291
	25	978	81.3	721	3215
	Mean	984	81.7	728	3250
S	79	937	78.3	661	2884
	29	855	78.6	605	2652
	94	960	80.7	698	3041
	Mean	917	79.2	655	2859
P	113	628	82.2	463	2065
	3	958	80.9	696	3106
	122	796	79.5	567	2562
	Mean	794	80.9	575	2578

Table 3.9.

Mean Daily Intakes and Digestibility of Pastures

Period II

Treatment	Sheep No.	DM Intake (g)	Digest. of OM (%)	DOM Intake (g)	DE Intake (kcal)
C	88	987	81.7	741	3451
	7	973	81.9	732	3394
	25	970	81.6	727	3374
	Mean	977	81.7	733	3406
S	79	994	76.2	699	3184
	29	920	77.5	658	2996
	94	1003	78.0	722	3277
	Mean	972	77.2	693	3152
P	113	964	76.0	678	3025
	3	996	78.7	714	3224
	122	803	77.1	564	2560
	Mean	921	77.3	652	2936

3.4. Digestible Energy Intake

Data on individual intakes of dry matter (DM), digestible organic matter (DOM), and digestible energy (DE), and apparent digestibility of organic matter (OM) are presented in Tables 3.8. and 3.9. The aim of feeding each sheep approximately one kg of DM per day was achieved when all sheep were eating to appetite.

Mean DE intakes of the three pasture types over both experimental periods are given in Table 3.10.

Table 3.10. Digestible Energy Intakes (kcal/day).

Means and Standard Errors of Differences Between Means

		Treatment			
		C	S	P	Week Means
Week	I	3250 ± 210.5	2859 ± 210.5	2578 ± 210.5	2896 ± 59.7
	II	3406 ± 210.5	3152 ± 210.5	2936 ± 210.5	3165 ± 59.7
Treatment Means		3328 ± 185.3	3006 ± 185.3	2758 ± 185.3	

Differences between treatments were close to significance ($p < 0.10$)

and differences between periods were significant ($p < 0.05$). There were no significant interaction effects (Appendix II).

The higher intakes overall during Period II appear to be primarily due to the recovery of appetite of the sheep fed P, and secondarily to the slight increase in energy content of the feeds (See 3.3).

3.5. VFA Infusion

The weight of the infusates before and after the 12 hour infusion period was used to calculate the rate of infusion of acid. Specific activity of the infusates was determined from duplicate samples of 0.5ml. Over the short range of means of specific activity of infusates there was no apparent relationship between the variance between duplicates and the random count of activity of the sample.

The rates of infusion of ^{14}C-labelled acetate were calculated as:

^{14}C infused (μc/min) = rate of infusion (g/min) x specific activity of infusate (μc/g)

and are given in Appendix III.

Errors in the determination of specific activity did not affect the differences between infusates (Appendix IV).

3.6. VFA Sampling and Analysis

3.6.1. Operation of Dialysis Probe

On a number of occasions during the operation of the sampling probes, the dialysis tubing was holed, presumably by the abrasive action of the rumen contents. This was noted from the cessation of flow of dialysate from the probe and the probe immediately withdrawn for replacement of the cellulose tubing. Thus there was only minimal time lost in sampling. The concentration of VFA in the dialysates was consistent over the whole experiment, indicating the consistent operation of the dialysis probes.

3.6.2. Analysis of Dialysates

The molar proportions of VFA in the dialysates are summarised in Table 3.11. Molar percentage of acetic acid was greatest for P and least for C while for butyric the reverse was true. These results suggest that the diets used were comparable with those in previous work with the same species (Johns et al., 1963; Ulyatt, 1969).

The labelled carbon in the VFA analysed was predominantly in the form of the acid infused (Table 3.12). These results agree well with values obtained using ^{14}C-labelled acetate to measure production of VFA from dry feeds (Table 3.13).

Table 3.11.

Molar Proportions of VFA in Dialysates (%)

Treatment	Sheep No.	Period I			Period II		
		Acetic acid	Propionic acid	Butyric acid	Acetic acid	Propionic acid	Butyric acid
C	7	69	19	12	69	20	11
	25	65	21	14	65	22	13
	88	67	21	12	68	20	12
	Mean	67	20	13	67	21	12
S	79	71	19	10	70	20	10
	94	72	18	10	71	18	11
	29	70	20	10	66	21	13
	Mean	71	19	10	69	20	11
P	3	73	18	9	71	19	10
	113	70	20	10	73	18	9
	122	69	20	11	74	17	8
	Mean	71	19	10	73	18	9

Table 3.12.

Percentage of Total ^{14}C in Each Rumen VFA

(Results for two sets of apparatus only)

Treatment	Period	Sheep No.	Acetic acid	Propionic acid	Butyric acid
C	I	25	80.3	8.6	11.1
	I	88	83.1	6.9	10.0
	II	88	75.1	12.2	12.7
	II	7	89.0	4.2	6.8
		Mean	81.9	8.0	10.1
S	I	29	84.4	5.4	10.2
	I	94	86.1	6.6	7.3
	I	79	84.6	6.9	8.5
	II	79	88.4	6.7	4.9
		Mean	85.9	6.4	7.7
P	I	113	74.6	13.4	12.0
	II	113	84.7	7.5	7.8
	II	3	83.9	8.6	7.5
	II	122	87.6	5.0	7.4
		Mean	82.7	8.6	8.7
		Overall Mean	83.5	7.7	8.8

Table 3.13.

Recovery of Radioactivity in Rumen VFA during Intraruminal Infusion of ^{14}C-labelled Acetic Acid

^{14}C-labelled Acid used	% of ^{14}C Recovered			Reference
	Acetic Acid	Propionic Acid	Butyric Acid	
1-^{14}C Acetate	77	4	19	Bergman et al. (1965)
1-^{14}C Acetate	80	6	14	Weller et al. (1967)
1-^{14}C Acetate	84	8	8	Weston and Hogan (1968)

Mean estimates of the percentage of VFA arising from acetate (Table 3.14.) were consistent over the three treatments although there was considerable variation within treatment results.

The value of 35 per cent for the proportion of butyrate carbon apparently produced by interconversion from acetic acid is in agreement with reported estimates (Table 1.5.). The proportion of propionate arising in the same way (approx. 20%) was higher than most reported estimates except that of Bergman et al. (1965).

3.6.3. Measurement of Specific Activity of Rumen VFA

During Period II samples of rumen liquor were taken

Table 3.14.

Interconversion of VFA in the Rumen

Treatment	Period	Sheep No.	% Propionic Acid Produced from Acetic Acid	% Butyric Acid Produced from Acetic Acid
C	I	25	21.5	33.7
	I	88	17.8	32.2
	II	88	32.7	45.8
	II	7	11.1	23.7
		Mean	20.8	33.9
S	I	29	20.6	40.1
	I	94	20.2	29.8
	I	79	15.8	39.9
	II	79	17.3	19.2
		Mean	18.5	32.3
P	I	113	42.8	52.3
	II	113	26.0	32.3
	II	3	16.2	37.5
	II	122	23.5	37.4
		Mean	27.1	39.9
		Overall Mean	22.1	35.3

prior to infusion and analysed for a possible carryover of labelled acids from the previous infusion. There was no carryover from Period I to Period II.

(1) Specific Activity of Individual VFA

The specific activity of the separated acids was calculated from the activity of duplicate samples after chromatography (Appendix V). Errors in determination had no effect on the differences between samples (Appendix VI).

(2) Specific Activity of Total VFA

The specific activity of the total VFA in the dialysates was calculated from the activity of duplicate 5ml samples and the VFA concentration (Appendix V). Differences between duplicates had little effect on the accuracy of the results (Appendix VII).

Specific activities of total VFA determined in this manner were consistently lower than those calculated from summing the individual values for the separated acids. The differences between the two sets of results varied considerably (2-40%) suggesting that systematic errors were not involved. However there was a considerable time lag between the two operations (12 months) and it is considered that the lower results from total VFA resulted from losses by volatilisation during that period.

Results from the separated acids were used in all further calculations.

3.7. VFA Production Rates

Several of the values for specific activity of VFA samples were much lower than the remainder and resulted in extremely high estimates of VFA production rate. When four of these values were compared with the rest of the data for each treatment they were found to lie between 10 and 25 standard deviation units beyond the mean of the remainder (Appendix VIII). The probability of a value lying 3.9 standard deviation units from the mean is .02% (Fisher and Yates, 1949). Hence it is unlikely that these estimates were from the same population as the remainder and they can be discarded on this basis (Flux; pers.comm.). Furthermore, estimates of the energy contributed by these particular VFA production rates were near to or in excess of 100% of the DE intake. All four results were from one set of infusion and sampling apparatus, and as a consequence a faulty technique was suspected. All results from this set of apparatus were disregarded in later calculations.

The remaining estimates of VFA production rates and their treatment means are given in Table 3.15. as both moles and kilocalories produced per day. The total calorific values of the VFA produced were calculated using the molar proportions of VFA and calorific values of the individual acids. An example calculation is shown in Appendix IX.

Because of the discarded data, the subclass numbers of VFA production rate estimates within treatments in a period were unequal. Analysis of variance using the variance

Table 3.15.

VFA Production Rates

Treatment	Period	Sheep Number	Moles/day	Mean and SE.	kcal/day	Mean and SE.
C	I	25	2.54		728	
	I	88	3.05		855	
	II	88	3.38		942	
	II	7	2.98		821	
				2.99 ± 0.22		837 ± 106
S	I	29	1.49		406	
	I	94	2.00		539	
	I	79	2.28		618	
	II	79	1.33		362	
				1.78 ± 0.22		481 ± 106
P	I	113	3.29		896	
	II	113	3.09		822	
	II	3	2.93		794	
	II	122	4.00		1037	
				3.33 ± 0.22		887 ±106

due to differences between treatments, periods, sets of apparatus and their interactions was not possible. Instead the effects of treatments versus periods, apparatus versus periods and treatments versus apparatus, with their respective interactions, were analysed separately using in the first case the sums of squares adjusted for unequal subclass numbers (Snedecor, 1967), (Appendix X).

Differences between treatments were significant in all analyses ($p < 0.01$); mean VFA production rates from C and P being higher than that from S. There were no significant differences between periods, between sets of apparatus, nor significant interactions, in any of the analyses.

3.8. The Contribution of VFA Production to Digestible Energy Intake

The percentage of the mean daily DE intake accounted for by the calorific value of the VFA produced is given in Table 3.16. for each of the treatments. An example calculation is shown in Appendix IX.

Analysis of variance of the main effects, treatments and periods, using the correction for disproportionate sub-class numbers (Appendix XI), demonstrated a highly significant difference between S and P ($p < 0.01$). The difference between C and P was close to significance ($p < 0.1$).

Analysis using the effects of treatments and apparatus gave a similar result for the differences between treatments ($p < 0.05$). There were no significant differences between periods and between sets of apparatus, and no significant interaction effects (Appendix XI).

Table 3.16

Contribution of VFA Production to DE Intake

Treatment	Period	Sheep Number	% DE Intake	Mean and S.E.
C	I	25	22.6	
	I	88	26.3	
	II	88	27.3	
	II	7	24.2	
				25.1 ± 2.7
S	I	29	15.3	
	I	94	17.7	
	I	79	21.4	
	II	79	11.4	
				16.5 ± 2.7
P	I	113	43.4	
	II	113	27.2	
	II	3	24.6	
	II	122	40.5	
				33.9 ± 2.7

CHAPTER FOUR

DISCUSSION

4.1. The Production of VFA on Different Pasture Types

Two major discussion points arise from the results of this study:

(1) Total rumen production of VFA from the diets used appeared to be generally lower than that reported in the literature for a variety of feeds.

(2) There appeared to be considerable differences in VFA production among the pasture species used.

As the accuracy of the technique used to measure VFA production in the rumen could not be verified under the conditions of the experiment, the possibility that the final results were spurious must be considered. For example, if the mixing of labelled substrate with rumen contents was incomplete, unrepresentative dialysis sampling of rumen VFA might have resulted in erroneous measurements of VFA production rate. However there is no available evidence to suggest that this did in fact occur. Furthermore, the estimates of VFA production were consistent within treatments and this must add support to the results obtained.

Of the published estimates of VFA production in sheep, all but one have been made using dry or poor quality feeds (see Table 1.3). Corbett (1968) has given an estimate of VFA production in sheep grazing a pure sward of young, growing white clover. VFA produced in the rumen contributed 57 per cent of the estimated DOM intake, while the comparable figure for penned sheep

fed lucerne chaff was 53 per cent. No details of the actual levels of intake, nor of the method of estimating these are given however and further comment is not possible.

Faichney (1968a), using an *in vitro* technique, found differences in VFA production between lucerne, straw and concentrate diets, and suggested these might have been caused by different retention times of organic matter in the rumen. The rationale behind this argument is that the longer food particles are retained in the rumen, the longer they are subjected to microbial degradation with subsequent greater VFA production. In later work Faichney (1968b) noted that VFA energy accounted for a lower proportion of the energy intake with increasing intake. He suggested that with higher intakes food residues would remain in the rumen for a shorter time (Balch and Campling, 1965) and so the extent of breakdown would be reduced. On the basis of this reasoning he concluded that variation in the level of intake of food, and in protein and soluble carbohydrate content, would be expected to change the ratio of VFA produced to DE intake.

There are other reports of a decreased contribution of rumen VFA to DE intake with an increase in the level of intake (Gray *et al.*, 1967b, 1968; Nicholson and Sutton, 1969), and Gray and his colleagues supported the hypothesis that this is caused by changes in rumen retention times.

Ørskov *et al.* (1969) noted that an increased level of feeding decreased the extent of fermentation in the rumen, and Ulyatt (1969) reported lower retention times of organic matter in the rumen for fresh pasture species than those given by

Minson (1966) for dry feeds. The turnover times for the chaff diets used by Hogan (1964) were shown by Hungate (1966) to be longer than those for fresh pastures.

On the basis of this evidence it is suggested that the low contribution of VFA to the intake of DE demonstrated for fresh pasture species in the present work, could be due to their low retention times in the rumen.

Armstrong (1969) has presented estimates for several diets of the probable proportion of the total digested energy absorbed by the animal as VFA from the reticulo-rumen. These were calculated from the disappearance of total digestible energy prior to the small intestine, losses of energy as methane, and losses as heat of fermentation. For a hay and cereal ration where 60-70 per cent of the digested energy disappeared prior to the small intestine, the proportion of DE absorbed as VFA was calculated to be 48-52 per cent. This figure is comparable with most published estimates (see Table 1.3.). However, with ground and pelleted lucerne diets the disappearance of digested energy from the reticulo-rumen was low (23%) and the estimate of the contribution of absorbed VFA was as low as 13-15 per cent. Hence for some diets VFA may contribute relatively little to the energy utilised by the animal.

The differences observed in VFA production in the present work may also be accounted for by differences in rumen retention times of food particles. P, which produced the greatest amount of VFA in the rumen, has been shown to be retained longer in the rumen than S or C (Hogan, 1964; Ulyatt, 1969).

This explanation implies a change in the distribution

of digestion between the rumen and post-rumen sites, influenced by the type of food. With the lucerne diets of Armstrong (1969) mentioned above, 51 per cent of the digested energy disappeared in the small intestine. The small intestine has been demonstrated to be an important site of digestion for the soluble constituents of fresh plant material; soluble carbohydrate (Armstrong and Beever, 1969) and protein (Hogan, 1965). Blaxter (1962) demonstrated that glucose and protein are utilised 31 and 44 per cent more efficiently by sheep when digested in the small intestine by the host's enzymes, than by the microorganisms in the rumen. Thus in a diet digested largely distal to the rumen, food substrates would be available for the more efficient process of intestinal digestion. This might explain in part the superiority of C and S over P in pasture quality.

Finally there is the possibility of digestion and subsequent VFA production in the colon and caecum. There appears to be no published evidence of the importance of this factor with fresh high quality diets (See 1.2.2). However the diets which underwent little ruminal digestion in the work of Thomson et al. (1969), underwent extensive digestion of cellulose in the lower tract (MacRae and Armstrong, 1969).

To summarise, the reported results suggest that the ruminal production of VFA from high quality pasture species is less important to the total utilisation of digested food by the ruminant than has been previously supposed. This might be particularly true of pasture species high in protein and soluble carbohydrate where post-ruminal digestion could play a significant role.

4.2. The Relationship Between VFA Concentration and VFA Production in the Rumen

Prior to the development of accurate methods of measuring VFA production, there existed considerable controversy as to whether the rates of production of rumen VFA were reflected by the concentration of VFA in the rumen contents (Rook, 1964). However, with the recent use of isotope dilution methods of measuring VFA production, relationships between VFA concentration and production in the rumen have been established (Leng and Brett, 1966; Leng et al., 1968; Weston and Hogan, 1968; Weller et al., 1969) some applicable to a wide range of diets (e.g. Leng et al., 1968; Weston and Hogan, 1968).

It was considered undesirable in the present experiment to collect separate samples of rumen liquor in order to measure VFA concentrations, because of the risk of disrupting the dialysis sampling system. Weller et al. (1969) correlated the concentration of VFA in the dialysate with that in the rumen, and used this value in their published relationship of VFA concentration and production. In the present study VFA concentration in the dialysate was considerably below that in the rumen and consequently was not suitable for predictive purposes.

A consideration of previous data on the pasture species used (see Table 1.2) and the present results, suggests that there would be little possibility of establishing a relationship for VFA concentration and production applicable to these diets. The two ryegrass species, which have in previous work given similar ruminal VFA concentrations, were shown in the present study to have very different VFA production rates.

Without data on VFA concentrations and production rates for the same pasture material little can be said of this apparent lack of relationship. However it may be pertinent that differences in weight of rumen contents and rumen volume have been demonstrated with these feeds (see Table 1.2). Weston and Hogan (1968) found with a variety of dry feeds that rates of rumen VFA production and absorption were more closely correlated with rumen VFA pool than with VFA concentration. It is possible then that a similar VFA concentration in sheep with large and small rumen volumes might be associated with a greater VFA production and hence VFA pool, in the larger rumen. Gray et al. (1967b) showed that the correlation between VFA production and concentration was most precise within sheep and within diets.

None of the reported relationships have included widely different pasture species of the types used here. Although the existence of a fixed relationship between VFA concentration and VFA production in the rumen would offer a simple and hence desirable approach to field studies, unless it can be applied to pasture plants within a species, for example the ryegrasses, it will provide little information on the importance of VFA production in determining pasture quality.

4.3. The Measurement of VFA Production in the Rumen of Grazing Sheep

A recent review of the nutrition of grazing ruminants (McDonald, 1968) has drawn attention to the differences between pen feeding and grazing conditions. The hazards in equating knowledge of the nutritive value of a forage determined with penned animals fed cut pasture, with the situation under grazing conditions was pointed out.

Thus the logical approach to the investigation of the role of VFA in determining the quality of pasture species should be to extend production measurement studies to animals actually grazing these pastures. This procedure has been successfully carried out by Leng *et al.* (1968) and by Weller *et al.* (1969).

The infusion pump used in the present study would obviously not be of use with grazing animals. However Leng *et al.* (1968) and Weller *et al.* (1969) have developed satisfactory portable infusion pumps.

The dialysis sampling technique has been successfully applied to grazing sheep (Weller *et al.*, *loc. cit.*). Experience in the present trial was not as promising however. Weller's group replaced the complete probe assembly every two days although tests showed that it could remain effective in the rumen for longer periods. In the reported work the cellulose tubing had often to be replaced during the 12 hour sampling period because of perforation. This was presumably caused by the friction of plant particles in the rumen contents against the tubing. Under grazing conditions this would mean the

wastage of a large number of samples. The slightly greater permeability of the dialysis membrane to acetic acid than to the other VFA, while having little effect on the final estimates (3.1.1.), also casts some doubt on the suitability of the dialysis method for VFA sampling.

Some form of direct sampling device, operating continuously or near continuously, which could not be blocked by feed particles in the rumen would be preferable. A sample produced by such a device would be more representative of rumen contents than a dialysis sample and would also provide information on VFA concentrations in the rumen (see 4.2.).

If the proportions of VFA produced in the rumen have little or no effect on the efficiency of utilisation of energy for growth, as recent work suggests (see 1.2.5.), then there appears to be little purpose in computing the contribution of individual VFA to the DE intake. Thus the simple measurement of total specific activity in a sample of rumen VFA, following the infusion of a single, labelled acid, would be all that is required to determine VFA production rate. The recovery of ^{14}C label by this method is high and the errors in determination low (see Table 3.4.).

With grazing animals the major limitation of an evaluation of pasture quality by the methods described, is likely to be the determination of DE intake. There are technical difficulties in accurately estimating the feed to faeces ratio in the field (Moule, 1965; Streeter, 1969).

If this problem can be satisfactorily resolved the modified technique described above should enable the ready

estimation of the contribution of VFA production to the DE intake of sheep grazing pasture. The continuing measurement of VFA production on a pasture would provide useful information on the changing value of pasture as the seasons progress.

The differences in VFA production among the pasture species studied in this work must be closely checked both under pen feeding conditions in comparison with dry feeds, and under grazing conditions, before they can be considered conclusive. However, if these results are verified, then VFA production in the rumen may not be the useful indicator of pasture value it has been thought to be. Determination of the importance of post-ruminal digestion would then be the logical procedure for continuing investigation of pasture quality. The use of sheep fitted with re-entrant cannulae into the proximal duodenum and terminal ileum (Brown, Armstrong and MacRae, 1968), offers a suitable technique for such studies (Topps, Kay and Goodall, 1968; Armstrong and Beever, 1969).

SUMMARY

A study was made of the production of volatile fatty acids (VFA) in the rumen of sheep fed different pasture species.

Nine wether sheep were caged indoors and three fed on each of, Grasslands 4700 white clover (C), Grasslands Manawa ryegrass (S), and Grasslands Ruanui perennial ryegrass (P), freshly cut. Ruminal VFA production was measured by isotope dilution using a single acid infusion technique, and rumen acids were sampled continuously by means of a dialysis probe. Digestibility determinations were carried out concurrently.

There were no significant differences in intake of digestible energy between treatments, but the VFA production rates from P and C were significantly higher than from S. The proportion of digestible energy intake accounted for by VFA production in the rumen was significantly higher for P than for S.

These results were discussed in relation to published estimates of VFA production in sheep and possible explanations of the results put forward.

The relationship between VFA production and VFA concentration in the rumen was discussed in relation to its possible use in determining pasture value.

Consideration was given to the possible application of measuring VFA production in the evaluation of pasture quality under grazing conditions.

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APPENDIX I

Live Weight Changes (lb)

Analysis of Variance

Source	d.f.	S.S.	M.S.	F.	Result
Between Treatments	2	28.22	14.11	5.51	<0.05
Within Treatments	6	15.34	2.56		
Total	8	43.56			

APPENDIX II

Digestible Energy Intake (kcal/day)

Analysis of Variance

Source	d.f.	S.S.	M.S.	F.	Result
Treatments	2	984741	492371	4.78	N.S.(5%)
Sheep within Treatments	6	617746	102958		
Weeks	1	326432	326432	20.34	< 0.05
Weeks X Treatments	2	32102	16051	0.34	N.S.(5%)
Weeks X Sheep within Treatments	6	280409	46735		
Total	17	2241430			

APPENDIX III

Rate of Infusion of ^{14}C-labelled VFA

Period	Treatment	Sheep No.	Rate of Infusion (10^{-2} μc/min)
I	C	25	0.5909
		88	0.8225
		7	0.9984
	S	79	0.5606
		94	0.6587
		29	0.8369
	P	3	0.6238
		122	0.7617
		113	0.7072
II	C	25	0.9009
		88	0.5286
		7	0.7604
	S	79	0.6577
		94	0.8722
		29	0.7454
	P	3	0.5275
		122	1.6439
		113	1.0536

APPENDIX IV

Measurement of the Specific Activity of Infusion Solutions

Analysis of Variance (DPM/g)

Source	d.f.	S.S.	M.S.	F.	Result
Between Solutions	17	1543606535	90800384	349.17	<0.01
Between duplicates within solutions	18	4680792	260044		
Total	35				

APPENDIX V

Specific Activities of Rumen VFA Samples

Period	Treatment	Sheep No.	Specific activity (10^{-2} μc/mM)	
			Partitioned Acids	Total VFA
I	C	25	.3346	.2998
		88	.3886	.3449
		7	.2184	.1725
	S	79	.3540	.3482
		94	.4754	.4293
		29	.8094	.6674
	P	3	.3386	.2849
		122	.2362	.1865
		113	.3094	.1827
II	C	25	.1229	.1143
		88	.2254	.1919
		7	.3698	.3619
	S	79	.7102	.5919
		94	.0964	.0674
		29	.1350	.0914
	P	3	.2592	.2242
		122	.5917	.5531
		113	.4902	.4233

APPENDIX VI

Measurement of the Specific Activity of Rumen VFA from Separated Acids (10^{-2} μc./mM)

Analysis of Variance

Source	d.f.	S.S.	M.S.	F.	Result
Between samples	17	1.3023	0.0766	174.10	<0.01
Between Duplicates within samples	18	0.0079	0.00044		
Total	35	1.3102			

APPENDIX VII

Measurement of the Specific Activity of Total Rumen VFA (DPM/5ml Solution).

Analysis of Variance

Source	d.f.	S.S.	M.S.	F.	Result
Between Dialysates	17	13993264	823133	708.38	<0.01
Between Duplicates within dialysates	18	20908	1162		
Total	35	14014172			

APPENDIX VIII

Elimination of Spurious Results

VFA Production Rates (mM/min)

Treatment	Results from Two Sets of Apparatus	Mean and Standard Deviation (s)	Results from Third Set	Distance from Mean of other Two Sets
C	1.766	2.075 ± 0.238	4.571	10.5s
	2.117		7.330	22.1s
	2.345			
	2.072			
S	1.034	1.233 ± 0.305	9.048	25.6s
	1.386		5.521	14.1s
	1.584			
	0.926			
P	2.286	2.312 ± 0.327	1.482	1.4s
	2.149		3.255	2.8s
	2.035			
	2.778			

At 3.0s from mean, p = 0.44%

At 3.9s from mean, p = 0.02%

APPENDIX IX

Example Calculation of VFA Production Rate and Contribution to DE Intake

Infusion : 12 hours; 625 ml containing 0.0085 µc/ml

^{14}C infused, µc/min = 0.007378

Dialysate Sample :

VFA molar proportions: Acetic acid 70%
Propionic acid 20%
Butyric acid 10%

Total specific activity of all VFA 0.00309 µc/mM VFA

Production of Total VFA $= \frac{0.007378}{0.00309}$ mM/min

$= 2.39$ mM/min

$= 3.44$ moles/day

Calorific Value of Total VFA

Acetic acid : $3.44 \times \frac{70}{100}$ moles/day x 207.4 kcal/mole = 499 kcal/day

Propionic acid : $3.44 \times \frac{20}{100}$ moles/day x 367.2 kcal/mole = 253 kcal/day

Butyric acid : $3.44 \times \frac{10}{100}$ moles/day x 524.3 kcal/mole = 180 kcal/day

Total 932 kcal/day

Mean daily Intake of DE : 3000 kcal

Percentage contribution of VFA to DE intake: $\frac{932}{3000} \times \frac{100}{1} = 31\%$

APPENDIX X

Production Rates of VFA (mM/min)

Analysis of Variance

(a) Treatments x Periods (Disproportionate numbers)

Source	df	SS	MS	F	Result
Periods	1	0	0	0	NS. (5%)
Treatments	2	2.193	1.097	11.43	< 0.01
Interaction	2	0.198	0.099	1.03	NS. (5%)
Error	6	0.577	0.096		
Total	11				

(b) Treatments x Apparatus

Source	df	SS	MS	F	Result
Apparatus	1	0.073	0.073	1.24	NS. (5%)
Treatments	2	2.575	1.288	21.83	< 0.01
Interaction	2	0.345	0.173	2.93	NS. (5%)
Error	6	0.354	0.059		
Total	11	3.347			

(c) Periods x Apparatus

Source	df	SS	MS	F	Result
Apparatus	1	0.073	0.073	<1	NS. (5%)
Periods	1	0.379	0.379	1.05	NS. (5%)
Interaction	1	0.006	0.006	<1	NS. (5%)
Error	8	2.889	0.361		
Total	11	3.347			

APPENDIX XI

Contribution of VFA Production to DE Intake (%)

Analysis of Variance

(a) Treatments x Periods (Disproportionate Numbers)

Source	df	SS	MS	F	Result
Periods	1	70.01	70.01	2.39	NS. (5%)
Treatments	2	674.76	337.38	11.50	< 0.01
Interaction	2	85.39	42.70	1.45	NS. (5%)
Error	6	176.02	29.34		
Total	11				

(b) Treatments x Apparatus

Source	df	SS	MS	F	Result
Apparatus	1	19.55	19.55	< 1	NS. (5%)
Treatments	2	610.77	305.39	6.29	< 0.05
Interaction	2	20.60	10.30	< 1	NS. (5%)
Error	6	291.27	48.55		
Total	11	942.19			

(c) Periods x Apparatus

Source	df	SS	MS	F	Result
Apparatus	1	19.55	19.55	< 1	NS. (5%)
Periods	1	6.02	6.02	< 1	NS. (5%)
Interaction	1	37.73	37.73	< 1	NS. (5%)
Error	8	878.89	109.86		
Total	11	942.19			