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VARIATIONS IN RYEGRASS VARIETIES
IN RESPECT TO MILK PRODUCTION

A thesis presented in partial fulfilment of the requirements for
the degree of Doctor of Philosophy in Animal Science at
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ABSTRACT

A study was made of some aspects of the nutritive value of ryegrasses with particular emphasis on the relationship between the chemical composition of the pasture and the quantity and quality of milk produced by grazing dairy cattle.

The fat content of the milk from Friesian cattle was shown to be depressed when grazing an annual variety of ryegrass during the winter. Depressions in the solids-not-fat content of the milk, on the other hand, were associated with the grazing of annual perennial ryegrass in experiments carried out in the winter and in the spring. The mechanisms involved in producing these differences in milk composition were investigated in a series of experiments in which various supplements were given to animals consuming pasture. Carbohydrate, protein, and lipid supplements were given to: lactating cows to measure effects on milk production, cattle and sheep with rumen fistulae to measure end-products of rumen fermentation, and dry sheep in metabolism crates to study the digestibility of pasture components.

It was concluded that the nature or levels of the carbohydrate, protein and lipid fractions of pasture may all influence milk composition and that optimum plant composition is likely to vary according to the type of milk product that is required to be produced.

INTRODUCTION

Efficient livestock production is dependant on the successful feeding, breeding and general management of livestock. In New Zealand a successful nutritional policy depends largely on manipulation of the pastures which provide the basic feedstuffs. Improved pastures consist of mixtures of different species and varieties, with ryegrasses the dominant grasses, and white clover the main legume to provide for nitrogen fixation in addition to its contribution to the food supply. The studies reported here were concerned with the nutritive value of different varieties of ryegrass because they probably make a greater contribution to the food supplies of cattle on higher producing farms than any other species.

Nutritive value is a general term and in requiring our pastures to be of good nutritive value we demand the presence of sufficient energy-producing and protein-forming constituents, together with essential minerals and vitamins necessary for animal health and high production. In addition a feedstuff should be free of potentially toxic substances or compounds that may be detrimental to the final product.

Although these attributes in a pasture plant are clearly desirable little progress has been made in assessing the deficiencies of our grasses in these terms, or of devising methods or indices which the plant breeders could use as a basis of selection. To date the breeder has continued to select mainly for agronomic characteristics which include total productivity, seasonal distribution of yield, and persistency.

While metabolic disorders and mineral and vitamin deficiencies are important factors affecting dairy cattle production, the amount of pasture eaten by the animal and the efficiency with which it obtains its energy and protein requirements from the material ingested will mainly determine the

productive output of the animal. Accurate methods are available for measuring the intake of animals and the efficiency of utilisation of pastures fed indoors, but techniques with the necessary accuracy have not been developed sufficiently to enable similar measurements to be applied to the lactating cow grazing pasture.

For this reason it seems best to use dairy cow production, under conditions where the supply of pasture is more than adequate, as a measure of the nutritive value of pasture and to accept that variation measured may be due to either differences in palatability or differences in composition of pasture, or to both. Although a direct measure of intake is not obtained some indirect evidence is provided by variations in milk yield. Any important fall in nutrient intake is very likely to cause a fall in milk yield.

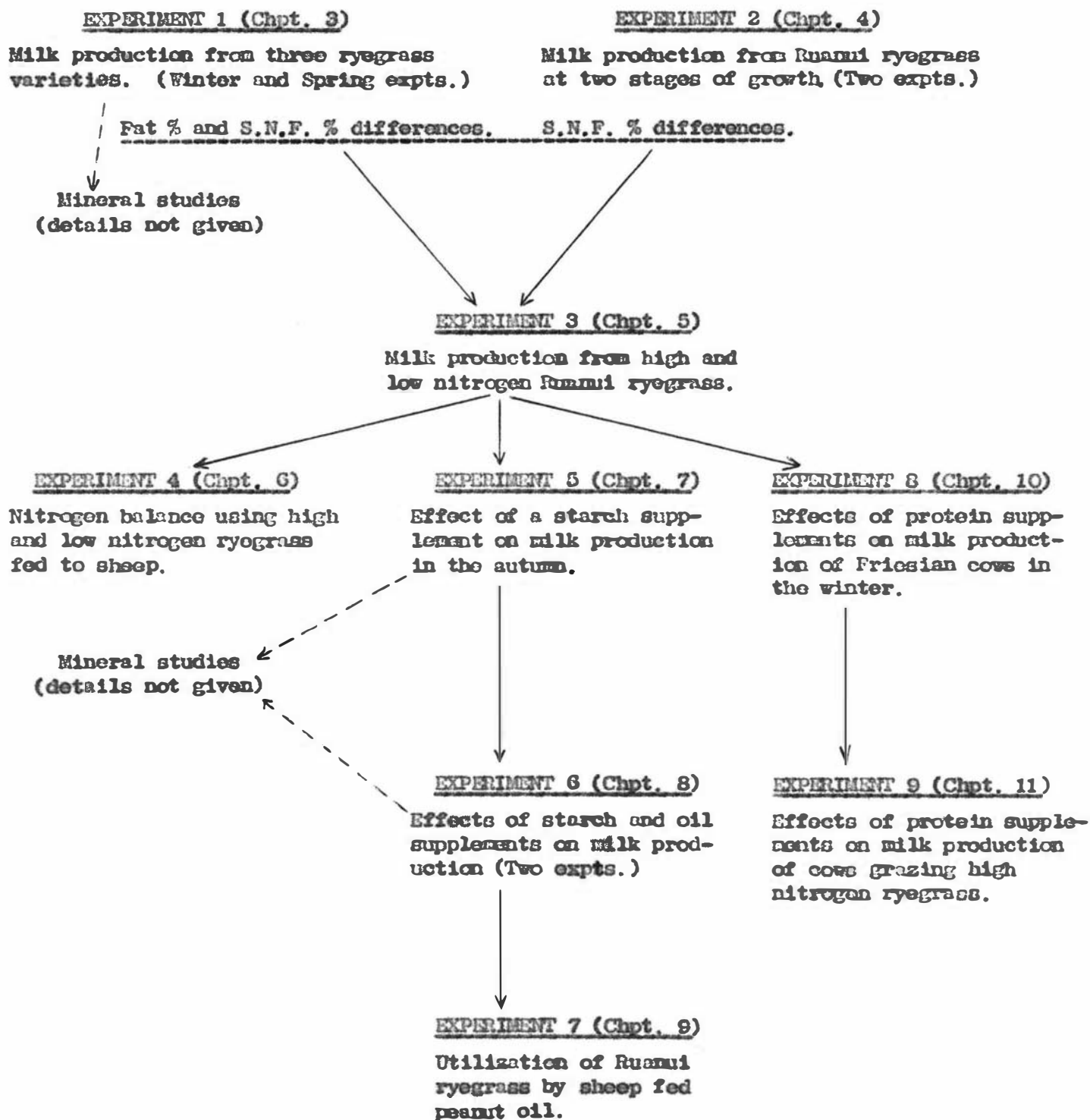
The different varieties of ryegrass used in this study* have different yield potentials, seasonal growth patterns, and physical and chemical characteristics (Barclay and Vartha, 1966) so that it is difficult to compare them on a common basis. However, all varieties make most growth during the spring months so that this seemed the most appropriate time to undertake the majority of the experiments.

* "Grasslands Ruanui" perennial ryegrass = Lolium perenne var. "Grasslands Ruanui"; "Grasslands Arika" ryegrass = (Lolium multiflorum x perenne) var. "Grasslands Arika"; "Grasslands Manawa" ryegrass (short rotation) = (Lolium multiflorum x perenne) var. "Grasslands Manawa"; "Grasslands Paroa" ryegrass (Italian) = (Lolium multiflorum) var. "Grasslands Paroa"; "Grasslands 4707" tetraploid Western Wolths ryegrass.

Throughout this Thesis these ryegrasses will be referred to as Ruanui, Arika, Manawa, Paroa and Western Wolths respectively.

Fig 1.1

Summary of experiments described in this Thesis.



Objectives of the Study

This study of some aspects of the nutritive value of ryegrasses had the following objectives.

- (1) To assess the extent to which milk yield and composition may be influenced by within and between variety differences in the ryegrasses. These results should assist in accounting for some of the variations in milk yield and composition observed in the field.
- (2) To attempt to discover the mechanisms involved in producing the observed differences in milk yield and composition. Such studies should add to existing knowledge pertaining to the formation of milk solids and factors affecting these processes.
- (3) To attempt to define possible meaningful general relationships between the chemical composition of ryegrasses and the yield and composition of milk produced by cows grazing the ryegrasses. Such general relationships could be of value to plant breeders in deciding selection objectives when endeavouring to improve the nutritive value of pasture plants.

Three varieties of ryegrass have been compared by the author before the thesis work started (see section 1.1), and the first two experiments (Chapters 3 and 4) to be described were designed to extend these previous investigations. The object of the first experiment (Chapter 3) was to extend the range of ryegrasses examined to two annual varieties, and the second (Chapter 4) to assess the importance of variations in Ruazani ryegrass with stage of growth.

Subsequent chapters each describe details of a separate experiment, or group of experiments, planned to test hypotheses made following the earlier experiments. To facilitate the inter-relating of the large number of experiments undertaken a flow diagram (Figure 1.1) is provided to indicate the logical progression of experiments.

PART I

REVIEW OF LITERATURE

CHAPTER 1

NUTRITIVE VALUE OF RYEGRASSES, AND NUTRITIONAL
FACTORS AFFECTING MILK YIELD AND COMPOSITION

1.1 RYEGRASS VARIETIES IN RELATION TO MILK YIELD AND COMPOSITION

Prior to the commencement of the present study the author in collaboration with Dr F.H. McDowall of the Dairy Research Institute compared three varieties of ryegrass (Manawa, Arika and Ruamui) as feeds for lactating dairy cattle (Wilson and McDowall, 1966). The three ryegrasses grown in pure swards were fed to groups of monozygous twin cattle in a series of experiments carried out during the spring and autumn growing periods. During the spring periods the mean milk yield produced by the different groups, in decreasing order were: Manawa > Arika > Ruamui ($P < 0.01$). The butterfat and solids-not-fat yields from these three groups were ranked in the same order as milk yields but did not differ significantly. In the autumn experiments the cows grazing Ruamui ryegrass produced higher milk and butterfat yields than those on the Arika ryegrass ($P < 0.01$). This difference was considered to be associated with a lack of palatability of the Arika ryegrass at this time of the year.

The major deficiency inherent in the use of animal output as a sole criterion for evaluating pastures, is that it seldom provides reasons for the differences observed between the pastures being compared. To facilitate the measurement of many of the variables which contribute to nutritive value the same ryegrasses were fed *in vitro* to six lactating animals, with rumen fistulae, in a further experiment (Wilson, 1966). In this experiment, the apparent digestibility of the Manawa ryegrass was two to three percentage units higher than that of the other two varieties. No significant differences were found between the total concentration of volatile fatty acids or the molar proportions of acetic,

propionic and butyric acids in the rumen liquor taken from the cows fed the different ryegrasses. It was concluded, therefore, that while minor differences in digestibility of the ryegrasses may have contributed to the observed differences in milk production in the grazing experiment, voluntary intake differences between groups were probably involved.

The only consistent chemical difference between the ryegrasses in the two previous studies was that the Manawa samples contained a lower content of crude fibre than either the Arika or Ruamui samples. This finding was in agreement with the results of Bailey (1964), who found that Manawa ryegrass had a consistently lower cellulose content than Ruamui ryegrass.

It may be concluded that information on the nutritive value of ryegrass varieties for milk production appears to be limited to that from one study carried out at Massey University. However, the New Zealand varieties of ryegrass have also been assessed as feeds for sheep (see 1.3), and the energy values of several English varieties determined (see 1.2).

1.2 THE NUTRITIVE VALUE OF RYEGRASS IN TERMS OF ENERGY

Because the nutritional significance of the organic matter of herbage lies largely in the extent to which it contributes to the energy requirements of the animal, previous experiments designed to assess the energy value of grasses and in particular of ryegrasses, are of interest.

The gross energy values of different pasture plants are normally very similar (Hutton, 1961), however, the extent of the loss of energy in the faeces, and to a lesser extent losses in the urine, methane, and as heat, are important factors which influence the net energy value of a herbage.

The differences between hedges in their net energy values are normally due to variable faecal losses, hence the usefulness of digestible energy as a measure of feeding value. Armstrong (1964) for example, studied the effect of stage of maturity on the energy value of two ryegrasses, cocksfoot, and timothy, as determined in calorimetric experiments. Stage of maturity within species was shown to have a far larger effect than species differences. For each species increasing maturity resulted in highly significant increases in the losses of energy in the faeces. Metabolizable energy expressed as a percentage of digested energy was almost constant, indicating low variability in the "urine plus methane" losses. The efficiency of utilisation of metabolizable energy (i.e. 100 - heat production) for maintenance did not differ significantly with stage of growth although values consistently declined as plants aged. For almost all species the efficiency with which metabolizable energy was used for fattening declined significantly with increasing maturity. Results from analyses of the rumen liquor indicated that the molar proportions of the volatile fatty acids were affected very little by level of feeding, and that real differences occurred in the proportions of acids found in the rumen when different species of grass were fed. The proportion of acetic acid in the rumen liquor increased as grass matured with small concurrent reductions in the proportions of propionic and butyric acids. These observations were therefore in general agreement with previous experiments, (Armstrong, Blaxter and Graham, 1957; Armstrong, Blaxter, Graham and Wainman, 1958) which indicated that the molar proportions of the individual volatile fatty acids metabolised affect the efficiency with which they are used for productive purposes. Fibrous diets which produce a high proportion of acetic acid to propionic acid in the rumen, are used less efficiently for fattening than those which produce a low proportion. It should be noted, however, that recent criticisms of the results of Armstrong (Grakov and Allen, 1966; Bull, Johnson and Reid, 1967) throw some doubt on the significance of small

differences in volatile fatty acid proportions. Additional factors also apply in the case of the lactating cow as individual volatile fatty acids have a direct effect on milk composition (Wilson, Dovey and Dolby, 1967).

The comparison between the energy values of the two ryegrasses (S24 and S23) in the experiment of Armstrong (1964) cannot be considered entirely satisfactory as the dried grasses were grown in two successive seasons rather than in the same season. However, the results indicated that differences in nutritive value between the ryegrass varieties on a given date in the spring would probably be accounted for by differences in their stage of maturity and hence digestibility. In this regard it is also worth noting that digestible energy values within varieties declined slowly with time up until ear emergence and thereafter declined rapidly.

Because of the difficulties involved few laboratories are able to measure energy retention and hence considerable attention has been directed towards establishing methods of predicting the energy value of forages from the chemical composition of the forage. The majority of results relate to the prediction of digestibility and it has been demonstrated that a high negative correlation normally exists between the content of crude fibre (and lignin) and the digestibility of organic matter. Armstrong, Blaxter and Waite (1964) took a further step by examining the relationship between the chemical constituents of forages and their digestible, metabolizable, and net energy values. They showed that the protein content of forages was positively, and crude fibre and lignin were negatively related to all the above measures of energy value. Provided that the application of regression equations based on such correlations is restricted to the types of herbage from which the equation was derived they may provide some indication of relative nutritive values (Van Soest, 1965).

While calorimetric determinations of the net energy value of herbage are the absolute measure of energy values per unit weight, the energy available

to the animal will depend on the voluntary intake of the feedstuff as well as the efficiency of utilisation of energy in the feed. According to Blaxter, Wainman and Wilson (1961) and Blaxter and Wilson (1962), the intakes of dried forages are positively related to their digestibilities. If these results also apply to fresh forages, and accepting that the results may not apply to forages of very high digestibility (Hutton, 1962; Conrad, Pratt and Hibbs, 1964), Blaxter's results indicate that a difference in digestible energy intake between two forages will generally be greater than that expected from the digestible energy contents of the forages. In this regard it is relevant to note that in field assessments, intake and quality aspects of nutritive value are both measured together in terms of milk production, provided that pasture availability does not limit intake.

1.3 RYEGRASS IN RELATION TO THE PERFORMANCE OF SHEEP

There have been a number of grazing experiments carried out in which the performance of sheep consuming grasses, clovers, or grasses together with clover, have been compared (see Joyce, 1967; and Butler, Rao and Bailey, 1968). In all experiments liveweight gains have been much higher (up to 35%) from white clover than from pure ryegrass swards. These differences have been attributed partly to a higher digestible intake of clover and partly to a more efficient utilisation of the digestible energy content of clover (Johns et al., 1963; Joyce and Newth, 1967). Joyce and Newth suggested that this latter difference may have been associated with differences in the proportions of individual volatile fatty acids in the rumen liquor of the animals grazing the two types of pasture, and because he had no data of his own quoted the data of Johns et al. (1963) to support this contention. It must be pointed out, however, that the volatile fatty acid data of Johns et al. (1963) were obtained at the time of slaughter and they were used to represent the situation which applied over the

whole of the treatment period from 16 July 1959 to 7 December 1960. There is in fact little direct evidence available at present to show that volatile fatty acid proportions present in the rumen of animals grazing clover and ryegrass differ significantly.

Liveweight gains resulting from sheep grazing a number of New Zealand ryegrass varieties have also been measured in a number of experiments (Butler et al., 1968). In many cases the differences were not significant but in most trials conducted between November and June, weight gains have been in the following order: Paroa > Manawa > Arika > Ruamui. That is, the less persistent ryegrasses gave greater rates of gain than the more perennial varieties. Bailey (1964) has focussed attention on the differences in carbohydrate composition between ryegrasses and considers that gains are inversely related to cellulose content because less fibrous pastures will be more quickly broken down in the rumen. This, he suggested allows for a greater consumption of food and more efficient utilisation of volatile fatty acids because of a reduction in the ratio of acetic to propionic acids in the rumen liquor. Bailey (1964) unfortunately, gave no data on intake or on the proportions of volatile fatty acids present to support his suggestions.

Milford and Minson (1966) have compared the energy retentions of lambs grazing cocksfoot (S37) and two varieties of ryegrasses (S23 and S24) at low grazing pressure, under 'worm free' conditions. Live weight gains were in the order S23 > S24 > S37. That is, the late flowering S23 which also had the higher digestibility (difference of 1.5% units) and the lowest acetic to propionic acid ratio in the rumen liquor, was superior in meat-producing ability.

It is clear from the above experiments that the nutritive value of ryegrass varieties in terms of ability to support liveweight gain does vary, at least to some extent, and that ideally it is necessary when interpreting animal production results to take into account intake, digestibility, and rumen volatile fatty acid differences.

1.4 THE INFLUENCE OF VARIATIONS IN THE LEVEL OF FEEDING AND THE COMPOSITION OF THE RATION ON MILK YIELD.

As with other productive processes milk yield depends primarily on the net energy value of the ration. Feeding standards which state the requirements per gallon of milk in terms of total digestible nutrients, starch equivalent or digestible organic matter, imply that the relationship between energy intake and milk production is linear at all levels of feeding. While it is true that increases in food intake are normally associated with increases in the fat-corrected milk yield, there are many experimental results which suggest that the increments are subject to the law of diminishing returns (Jensen et al., 1942; Hansson et al., 1954). That is, the increases in milk yield become progressively smaller with each successive increment of food consumed.

The fall in the efficiency with which additional increments of food energy are used for production of milk is probably due to an increased proportion of the dietary energy being diverted to body fat synthesis, and to a fall in the digestible energy content of the foodstuff with increasing intake (Lever, Campbell and Holmes, 1969). While it is also possible that an increased proportion of absorbed energy may be lost as heat with increased intake, recent evidence reviewed by Reid, Moe and Tyrrell (1966) suggests that this factor may be disregarded. The basis for this conclusion was the linear relationship observed between the metabolizable energy input, at body-energy equilibrium,

and the milk energy output of cows, at feeding levels up to four times that required for maintenance.

Whereas level of feeding and fat-corrected milk production are normally correlated it does not follow automatically that when the fat-corrected milk yield from a group of cows on one ration is higher than a similar group on another, the digestible energy intakes are similarly related. This is because the chemical or physical characteristics of some rations may lead to a decrease in the acetic to propionic acid ratio in the rumen liquor which in turn may cause a marked depression in milk fat content (see also section 1.52). Rations which lead to the production of milk containing a lowered fat content therefore result in a reduction in the efficiency with which metabolizable energy is used for milk production.

There is now some evidence that the efficiency of utilisation of metabolizable energy for milk production also varies with food quality under feeding conditions where milk fat content is not altered. The experiments of Elliot and Loosli (1959), Coppock, Flatt, Moore and Stewart (1964), Hinders and Owen (1963), and Flatt et al. (1963) support the contention originally made by Blaxter (1962) that when the molar proportion of acetic acid in the rumen liquor is about 60% the efficiency of conversion of metabolizable energy to milk energy is at its maximum (70%) and that either higher or lower proportions of acetic are associated with lower efficiencies.

The influence of a given change in the level of feeding on milk yield also depends on many factors including previous plane of nutrition (Wallace, 1957; Flux, 1949; Patchell, 1957), length and severity of the change, the stage of lactation (Riddet et al., 1941) and factors such as milking technique, health and climate which may limit the degree to which an

animal can respond to changing levels of nutrition. Variation between individual animals is another important factor which is well illustrated by a series of experiments carried out by Blaxter (1962) in which milking cows were given different amounts of food for their whole lactation. The results showed that the higher the level of secretion, the greater is the effect of a given change in the nutrition on that secretion.

Rock (1961) in an excellent review dealing with variations in the chemical composition of milk considered that increases in the protein content of the diet of a cow above normal (Woodman, 1957) invariably have no effect on the yield of milk but moderate reductions below Woodman's standards (85%) have given small decreases. More recent experiments (Rock and Line, 1962; Reid, Tyrrell and Moe, 1967), however, have indicated that milk yield may vary in the same direction as the protein content of the ration, over a very wide range of protein intakes. Factors responsible for the variable results probably include the duration of the experiments, the level of milk production, and the protein reserves available.

A certain amount of dietary fat (3 - 4%) is required in the concentrate part of the diet for maximum milk and fat yields (Rock, 1961). However, the addition of large amounts of edible fat (e.g. 2 lb.) causes digestive disturbances and loss of production (Smith, 1959).

For pastures such as the ryegrasses used in this study the protein and fat content is normally high enough to preclude the possibility of a deficiency affecting milk production so that the level of feeding remains the crucial factor.

From the data discussed in this section, it is probably reasonable to conclude that milk production from the ryegrasses will be positively correlated to digestible energy intake, provided that the characteristics of the pastures

are not such that the ratio of acetic to propionic acid in the rumen liquor is altered to such an extent that the efficiency of utilisation of metabolizable energy is affected. The other factor which may confound the general relationship between digestible energy intake and milk yield is the possibility of interactions between the nature of the diet and the proportion of the metabolizable energy diverted to body weight gain, or alternatively, the amount of body tissue mobilized to maintain milk production. With the above reservations, and in the absence of intake data, differences in milk production are used in this study to indicate parallel differences in the digestible energy intake of groups of cows.

1.5 NUTRITIONAL FACTORS AFFECTING THE CHEMICAL COMPOSITION OF MILK

Throughout this review emphasis will be given to the major milk constituents, fat and solids-not-fat (protein and lactose), and no reference will be made to minor constituents such as trace elements and vitamins. The effects of level and type of feeding on milk composition are discussed with most attention being devoted to factors which may be of importance when using pasture as the basic feedstuff.

1.51 Fat content and level of feeding

Adequate quantities of protein and energy produce a fat yield corresponding with the animals' inherited potential. With underfeeding both the quantity of milk and fat produced will normally drop, but the effect on the fat content of the milk may be variable. Hence Møllgaard (1929), Swan and Jamieson (1936) and Wilson, Davey and Dolby (1967) have recorded reductions whilst Flux and Patchell (1954) and Robertson et al. (1960) have recorded an increase. It is probable that an increase in the fat content of milk is more likely to occur following a short term sudden reduction in food intake which leads to a loss of

body weight. While the mobilisation of body fat may account for the increase in fat content (Flux and Patchell, 1957) and the change in butterfat properties observed following underfeeding (Manford et al., 1964) other workers (Williams and Christian, 1956; Shaw, 1961) have noted that the relative proportions of butyric acid in the rumen rises while that of acetic acid diminishes. It is possible, therefore, that the direction of the change in fat content of the milk following underfeeding, may be influenced by feed quality which in turn affects the amount and proportions of the various volatile fatty acids formed in the rumen.

If protein and energy are fed in excess of requirements there is normally little change in the fat content of milk (see review of Burt, 1957), that is, except when a highly concentrated ration is fed which alters the rumen fermentation products (see below).

1.52 Dietary carbohydrates and milk fat content

It is now well established that the type and concentration of carbohydrate present in feedstuffs can have a considerable effect on the proportions of volatile fatty acids formed in the rumen of cows. On 'normal' diets, acetic acid accounts, on a molar basis, for 60-70% of the total volatile fatty acids, propionic acid 15-22%, and butyric acid 8-15%. On diets rich in readily soluble carbohydrates and low in crude fibre the percentage of acetic acid may be as low as 35% and propionic acid as high as 40%. Such diets cause a large reduction (up to 60% of normal fat content) in the fat content of milk (Balch et al., 1954, 1955; Shaw et al., 1959) accompanied by little or no change in milk yield.

In an excellent review Van Soest (1963) has discussed the possible physiological and biochemical mechanisms which may lead to the relationship between the molar proportions of volatile fatty acids and milk fat content.

Reductions in milk fat content involving similar changes in volatile fatty acids as those described above have also been obtained when cows were given diets in which the fibre has been finely ground (Powell, 1941), or large amounts of concentrates relative to hay have been fed (Balch et al., 1952). Also of particular interest are the experiments of McClymont (1950) in which he obtained depressions in milk fat percentage with cows grazing green oats and immature pasture.

Taparia (1936) has summarised many of the experiments in which additional concentrate supplements have been given to cows grazing pasture, and in these milk fat content was generally depressed by 0.1 to 0.3%.

The relationship between rumen fermentation products and the fat content of milk is further illustrated by experiments carried out by Rook and Balch (1959 and 1961) and later by many others, in which certain fatty acids were administered directly into the rumen. Acetic acid increased the fat percentage while propionic acid reduced it and increased the protein content of milk.

1.33 Protein levels in the diet and milk fat content

The fat content of milk is generally little affected by variations in the protein level of the feed where sufficient energy is present (Rook and Line, 1962; Logan et al., 1959), however, in a few cases level of protein feeding has been shown to be positively related to fat content in the milk (Byers et al., 1949; Haacker, 1914).

1.54 Dietary fat and milk fat content

A certain amount of fat (3-4%) in the ration is necessary for maximum milk and fat yields (Rook, 1961). However, there are many conflicting results as to the effect of the amount and type of fat in the diet of cows on the butterfat content of milk. The feeding of highly unsaturated fish oils (e.g. codliver oil) has generally reduced the fat content of milk (McDowell, Reid and Patchell, 1957; Shaw and Ensor, 1959). The feeding of oils of plant origin has had variable effects on fat content. McDowell *et al.* (1957) obtained no effect from added peanut oil, linseed oil, soya bean oil (and tallow) whereas Storry, Rook and Hall (1967) using coconut and red palm oils obtained a significant increase in the fat content of milk (see also Van Soest, 1963). In most experiments the constituent fatty acids of the milk fat are altered towards that of the dietary fat, particularly when the fat contains mainly fatty acids which are not subject to hydrogenation.

It is quite probable, therefore, that the effect of added fats and oils will depend on factors such as the basic ration, level of feeding, form in which it is administered, chemical composition of the fat and length of treatment.

1.55 Solids-not-fat content (S.N.F.) and level of feeding

When the level of feeding is reduced the protein and solids-not-fat contents of the milk diminish (Riddet *et al.*, 1941; Rook, 1961; Wilson *et al.*, 1967). The effect of additional feed is generally more marked at low than at high planes of nutrition. Thus increases in the feeding level above normal ad libitum grass feeding results in only a small increase in S.N.F.% (up to 0.2) whereas reductions in feeding level have given

decreases of up to 0.5% S.N.F. Changes in S.N.F. with feeding level are normally due to a change in the protein content of the milk but with underfeeding small decreases in the lactose content also occur (Rock and Line, 1961). It is now well established (Rock, 1961) that the changes in S.N.F. that occur with changes in the level of feeding are related to the energy content of the food rather than to variation in any other component of the food. The extent of the change in S.N.F. content with a change in plane of nutrition varies with level of feeding before calving (Patchell, 1957; Campbell and Flux, 1948), and with type of diet (Rock, 1953; Rock and Line, 1961) (also see section 1.56). It also should be noted that while cows giving a good milk yield (say above 1.5 gal. per day) will show the changes in fat and protein content described above, at low milk yields drying off processes intervene. In the latter case a low level of feeding will generally cause lactose content to fall, and protein content to plateau or even rise (Riddet et al., 1941).

1.56 Dietary carbohydrates and S.N.F. content

In interpreting the results of many experiments it is difficult to separate the effect of energy level per se, and the proportion or concentration of various carbohydrates in the ration because any change in one normally affects the other. However, additions of flaked maize to a hay and concentrate diet sometimes have had a particularly marked effect on the S.N.F. content of milk that cannot be accounted for by the additional energy (Balch et al., 1955; Rock and Line, 1961). These results and those of Castle et al. (1959) suggest that the concentration of energy in the feed as well as feeding level may affect S.N.F.%. Feeds containing high concentrations of energy may affect S.N.F.% by causing a reduction in the ratio of acetic to propionic acid produced in the rumen, as in some

experiments in which the fat content of the milk was depressed there was also a concurrent elevation in S.N.F.% (Balch et al., 1955; Ensor and Shaw, 1961; Shaw, 1961; Castle et al., 1963). The observations that cereal grains containing a high level of crude fibre have given only poor responses in S.N.F.% when used to improve plane of nutrition (Durt, 1957b; Brown et al. 1962) and pelleting of the ration may increase the S.N.F. content of milk (King and Henken, 1962) are in general agreement with the possibility that the ratio of individual fatty acids produced may be of importance in affecting the S.N.F. content of milk.

In most of the experiments in which additional concentrates have been fed to cows grazing grass an increase in the S.N.F.% (up to 0.2) of milk has been noted (Taparia, 1966) but whether the increase resulted from a change in energy intake or a change in the proportion of volatile fatty acids produced cannot be judged from the reported results.

It is now well known (see Wilson et al., 1967) that additions of propionic acid to the rumen of lactating cows leads to an increased protein and hence S.N.F. content in the milk. Lactic acid, which is present in rumen contents only when diets containing large amounts of readily available carbohydrates (e.g. flaked maize) are fed, also increases S.N.F.% but does not at the same time depress fat content (Rook, 1961). These findings are clearly in harmony with most of the experimental work reviewed in this section.

1.57 Dietary protein and S.N.F. content

Increases in the protein content of the diet above the recommended levels (Woodman, 1957) and also moderate reductions below these standards have little influence on milk protein or S.N.F. percentages (Holmes, 1956;

Rook and Line, 1962; Frans and Djikstra, 1959). Huber (1966) examined the possibility of interactions between protein and energy levels but found that protein level had no effect on S.N.F.% at any energy level. With an extreme reduction in the protein content of the diet to 60% of Woodman's standard, Rowland (1946) observed a reduction of 0.18 in the S.N.F.% of milk and Waite (1956), Rook and Line (1962) and Isachsen, Ulevasli and Breirem (1956) obtained essentially similar results.

1.58 Non-protein nitrogen intake and milk composition

A review of the important aspects of urea supplementation and the production of milk from non-organic compounds will not be undertaken, but the possibility of non-protein nitrogenous substances affecting milk production must be considered.

The toxic effects of some nitrogenous substances such as nitrate in pasture are well known, the safe limit for ruminants being generally considered to be 0.20 g. of nitrate nitrogen/100 g. of dry feed (Garner, 1963). The effects of nitrate on milk production on the other hand vary from no effect (Jones et al., 1966) to a marked depression in milk yield (Muhrer, Garner, Pfander and O'Dell, 1966) and it appears that the availability of the carbohydrate in the diet has a major effect in determining the effects, and in particular the toxicity of nitrate (Barnett and Bowman, 1957).

1.59 Dietary fat and S.N.F. content

Additional fat to the ration of dairy cows has had a variable effect on the S.N.F. content of the milk. For example Peters, Harris, Hulay and Pinkerton (1961) who added tallow depressed S.N.F.% with Friesian but not

Jersey cows whereas McDowall et al. (1957) found no change in S.N.F.% using a number of different oils and fats including tallow.

The finding that dietary additions of oils (particularly codliver oil) may modify the ruminal production of volatile fatty acids, causing an increase in propionic acid relative to that of acetic acid (Shaw and Ensor, 1959; Robertson and Hawke, 1964) might suggest that increases in S.N.F. could be expected following the feeding of fats and oils. However this effect has not been demonstrated in practice.

1.510 Variations in the lactose content of milk

Compared with the fat and protein contents, the lactose content of milk is only very slightly affected by nutrition. Thus no increase in lactose content was recorded when the energy level of the ration was raised to 135% of requirements (Rook et al., 1960; Holmes et al., 1956 and 1960), and only a slight increase when it was raised to 250% (Rook and Line, 1961). In cases of energy deficiency the alterations are generally also small (Flux and Patchell, 1954; Rook et al., 1960) although gross underfeeding (Burt, 1957b; Flux and Patchell, 1957), or the starving of cows for several days caused a sharp fall in lactose content (Robertson et al., 1960).

The lactose content of milk is also influenced little by the infusion of short chain fatty acids into the rumen (Storry and Rook, 1961).

P A R T I I

EXPERIMENTAL METHODS, RESULTS
AND DISCUSSION

CHAPTER 2

MATERIALS AND METHODS

Because many of the grazing experiments in this study involved the use of similar methods, cows, sampling procedures and analyses, these will be described and discussed together in this chapter.

2.1 FACILITIES

The majority of the experiments were carried out using the facilities of the Massey University No. 2 Dairy Unit which consists of a milking herd of about 30 pairs of monozygous twins, together with replacement young stock. The majority of the pastures on the farm consist of mixed grass and clover swards and it was these that were used to feed the experimental cows during the preliminary periods of experiments in order to obtain milk production data for use in covariance analyses. Approximately 21 acres of relatively uniform land (for subdivision see Figure 2.1) were used to grow the pure swards of ryegrasses for the experimental comparisons. For the experimental period of an experiment twin pair mates were allocated to the two treatments at random. In experiments in which there were three treatments the experimental layout used was the incomplete balanced block design described by Cochran and Cox (1950). The twin cows were milked in the No. 2 Dairy Shed, a three-bail "doubled up" milking system fitted with milk recorders which enable the milk yields from individual cows to be measured and representative milk samples to be obtained for subsequent analysis.

In one experiment (Experiment 1), Friesian cattle from the No. 1 Herd were milked in the No. 2 Dairy Unit shed, and in another (Experiment 8)

Friesians in the No. 1 Herd were used. These cattle were used in order to obtain cows which had calved in the autumn rather than the spring. In two other experiments (Experiments 4 and 7) sheep were fed indoors using the facilities at the Animal Physiology Unit.

2.2 EXPERIMENTAL DESIGN

The covariance design was utilized in all of the grazing experiments. The milk yield and composition data were analysed by covariance analysis. Tests of significance were made between the adjusted treatment means using the values obtained from individual cows during the preliminary period as independent variables (Snedecor, 1955).

Discussion: The covariance design was particularly suited to this study for a number of reasons. The area of land available for the growth of the ryegrasses was limited so that the choice had to be made between grazing a small number of cows using a change-over experimental design (or Latin Square) and the covariance design with a short experimental period, but using a greater number of cows. Disadvantages of the former design included the problems of treatment carry-over effects on milk production, the changing chemical composition of the pastures within a longer experiment, and the limited number of cows with different genotypes which could be included in a single experiment. The covariance design did not suffer from any of these disadvantages, and because of the high correlation between successive measurements of milk yield and milk components in the short term, the covariance adjustments were highly effective in reducing error variance.

In the experiments involving three treatment groups, the data were also analysed by covariance, but the twin animals were allocated to treatment

TABLE 2.1

Dates of sowing experimental pastures.

Paddock No.	Prior to April 1964	April 1964	April 1966	April 1967
2	Mixed pasture	-	Tetraploid Western Wolths	Mixed pasture
3	Mixed pasture	-	Paroa	Mixed pasture
4	Mixed pasture	-	Tetraploid Western Wolths	Mixed pasture
5	Mixed pasture	Ruanui	-	-
6	Manawa	-	Paroa	Mixed pasture
7	Ariki	Ruanui	-	-
8	Ariki	Ruanui	-	-
9	Ruanui	-	-	-
10	Manawa	Ruanui + Clover	-	-
11	Ruanui	-	-	-
12	Ariki	Ariki + Clover	-	-
13	Ruanui	Ariki + Clover	-	-
14	Manawa	Ariki + Clover	-	-
15	Mixed pasture	Ruanui + Clover	-	-

groups as for an incomplete balanced block design. This procedure was followed in order to minimize preliminary period differences between treatment groups and also to take some advantage of the fact that the yields and compositions of the milks from twin mates are highly correlated during the course of a lactation (McDowall and Patchell, 1958).

2.3 PASTURE ESTABLISHMENT AND MAINTENANCE

The soils in which the pastures were grown consisted of Manawatu sandy loam overlying gravel. The pasture species growing in the 14 experimental paddocks immediately prior to April 1964 are given in the first column of Table 2.1 and the subsequent changes made are also shown in this table under the appropriate sowing dates.

All pastures were sown in the autumn along with 2 cwt. of super-phosphate. The pure swards of Ruanui ryegrass were maintained with sulphate of ammonia (for details see individual experiments). About once a year, normally in the late autumn, it was necessary to spray the pastures with 'Tordon' (Ivan Watkins-Dow) to eliminate the volunteer clover plants.

The pastures were irrigated as required during the summer periods to maintain adequate growth and soil cover to help prevent weed infestation.

A botanical analysis was undertaken on the pastures at regular intervals and the ryegrass content was found to be above 94% at all times.

The mixed pasture used during the preliminary periods consisted of various combinations of Arika, Ruanui, and Manawa ryegrasses, white clover (Trifolium repens L.) var. "Grasslands Buia", cocksfoot (Dactylis glomerata L.) var. "Grasslands Apanui", and prairie grass (Bromus unioloides Kunth.).

2.4 PASTURE SAMPLING AND STORAGE

Representative pasture samples were taken at regular intervals from each type of pasture used in an experiment. A large sample (approx. 1 kg.) collected at random from within each paddock was obtained on the morning of the first day that the cows were given a break of grass in a new paddock. Sampling was always carried out at about 9 a.m. to eliminate possible diurnal variation in composition (see soluble sugars) as a source of variation between samples. The samples were obtained with shears and the grass was cut to the level at which the cows were grazing. The break feeding system used facilitated the assessment of what the animals were eating and hence the cut material was probably representative of what was consumed.

The pasture samples were frozen within 15 minutes of sampling, freeze-dried over 24 hours, and subsequently ground to pass a 1 mm. sieve and stored in air tight containers at room temperature. Freeze drying was used in preference to oven drying as this is less likely to alter the composition of the herbage. Losses of dry matter (particularly from the carbohydrate fractions) following heating have been attributed to respiratory losses, and chemical breakdown by excessive heat (Ragase and Smith, 1965).

2.5 PASTURE ANALYSES

Because there was limited data available on the chemical composition of the New Zealand ryegrasses it was considered that the analyses undertaken should include fat, protein and carbohydrate determinations.

The classical methods (Henneberg system) of analysing feedstuffs have often been criticised as empirical (Van Soest, 1965) and improvements have been suggested by many workers (Crampton and Whiting, 1943; Waite and Gorrod, 1959), but with the exception of the recently established detergent methods of Van Soest (1965), which divide the dry matter into cell-wall constituents and cellular contents, they have produced little additional information of biological significance. The Henneberg system was used in this study because of its simplicity and because of the large amount of data which are available in these terms for comparative purposes. Because the system of analysis includes the major deficiency that the nitrogen-free extract is inaccurately calculated, and may be a poor indicator of the readily available carbohydrates in the pasture, the total soluble sugar content of the grasses was also determined.

2.51 Henneberg analysis

The freeze-dried grass was analysed for crude protein (Nitrogen x 6.25), crude fibre, and ether extract by standard methods (A.O.A.C., 1965). The content of nitrogen-free extract was determined by difference $\sqrt{100 - (\text{cr. protein} + \text{cr. fibre} + \text{ether extract})}$. All samples were analysed in duplicate.

2.52 Total soluble sugars

Total soluble sugar contents of the freeze-dried samples of pasture were determined in duplicate using the procedure of Bailey (1964). In this method the dried grass is extracted with 80% ethanol after which the total soluble sugar concentration in aqueous solution is measured using Bath's sulphuric acid method and a glucose standard.

Discussion: As it is well known that the content of sugars in grasses decline rapidly after cutting due to respiration (Raguse et al., 1965), it was

considered necessary to check that values obtained using freeze-dried grass were similar to values obtained using freshly cut grass. The comparison was undertaken using samples of Ruamui ryegrass pastures at two stages of growth and sampled twice daily (9 a.m. and 3 p.m.) on three consecutive days to provide a range of soluble sugar concentrations. The results from this experiment (Appendix 2.1) indicated that freeze-drying does not alter the total soluble sugar content of grass samples. Furthermore, the method was more satisfactory as the variation between duplicate samples was much smaller for the freeze-dried samples.

The time of the day that pastures are sampled has a considerable effect on the sugar content (Kingsbury, 1965; and Appendix 2.1), so that care was taken to sample all experimental pastures being compared on the same day and at the same time. However, as day to day variations in peak soluble sugar levels are apparently related to the number of sunshine hours preceding the sampling (MacKenzie and Wylan, 1957) it is clear that comparisons between days and especially experiments, are subject to considerable error.

2.6 MILK SAMPLING AND ANALYSIS

Milk yields for individual cows were measured at all milkings during the preliminary and experimental periods of each experiment. For all the grazing experiments, except 8 and 9, two-day composite samples were taken from individual cows twice each week, for the duration of the experiment (Sunday p.m. to Tuesday a.m., Tuesday p.m. to Thursday a.m.). For Experiments 8 and 9, daily composites were obtained from individual cows on six days of each week of the experiment. Milk samples were taken to the Dairy Research Institute where they were analysed for fat by the Gerber method (British Standards Institution, 1955), total solids by the gravimetric method (heating a 2 g. sample at 100 - 102°C for 5 hr.), and the solids-not-fat content calculated

by difference. Total nitrogen was determined by the Kjeldahl method and the protein content calculated (protein = N x 6.38). The lactose (monohydrate) content of the milk was determined in some experiments and the procedure used was the Lane and Synnott volumetric copper reduction method. All determinations were made in duplicate and if these were not in close agreement they were repeated.

For Experiments 8 and 9 the daily composites were analysed for fat, protein and lactose contents, using an Infra-red milk-analyser (I.R.M.A.; for description see Biggs, 1967, and Mumford, 1967), which was purchased by the Dairy Husbandry Department in August 1967. The instrument was initially calibrated using a range of milk samples analysed by the standard chemical methods, and day to day calibration was achieved by use of the internal optical filter values. Final calibration will not be completed until more chemical data are available. Mumford (1967) determined the error for a single determination (standard deviation) on a single sample using I.R.M.A. as 0.044% for fat, 0.030% for protein and 0.019% for lactose, values which are similar to the errors for the chemical methods.

Discussion: Early in this study it became apparent that, individual cow, solids-not-fat percentages calculated from results obtained by the chemical methods (i.e. protein % + lactose % + ash %), or from I.R.M.A. results, were 0.1 to 0.6% (average of about 0.28%) higher than those from the gravimetric oven method and fat test (i.e. S.N.F. = T.S. - fat %).

Mr A.K.R. McDowell of the Institute has investigated the possible reasons for this discrepancy and his conclusions (McDowell, 1968) are summarized as follows.

In the protein method the factor 6.38 to convert N to total protein is too high for the non-protein nitrogen compounds (5% of total N in milk) such as urea. This may result in an error of about + 0.1% in the protein value. In the ashing of milk, citrate is lost but it is included in the total solids value. This means the ash content of milk which is normally considered to be about 0.70 is probably 0.2% low. The phosphorus content of milk is counted twice, once in casein and once in ash. Casein contains 0.80% phosphorus but in ash it would occur as phosphate (P_2O_5) and amount to 0.05% of the milk. This results in an error of + 0.05. These errors collectively mean that the addition of protein lactose and ash is low by about 0.05.

One possible source of error in the gravimetric oven method of estimation of total solids arises because a certain variable percentage of the lactose may be present in the anhydrous form rather than the monohydrate. The difference in weight between the two forms could alter the total solids by up to - 0.25% so that even a small difference in the degree of hydration would be significant. In addition the browning reaction which always occurs in oven drying of milk solids due to a reaction between lactose and protein results in the formation of a number of products, including water, which are lost. Experiments comparing freeze-drying with oven-drying have shown that the browning reaction could result in S.N.F. figures which are low by about 0.17%.

These results, while they do not completely explain the original discrepancy, amply demonstrate that the chemical methods for the determination of the constituents of milk can not be considered "absolute" and emphasize the importance of making all comparisons "within experiments" as has been done in this thesis.

2.7 RUMEN FISTULATED COWS

A number of fistulated milking cows were available for use from the commencement of this study. These included two Pedigree Jersey cows (M45 and M48) and one pair of monozygous twins (15 and 16) also of Jersey breeding. Because it was considered necessary to have at least six animals available for Experiment 1, a further pair of twins (Jerseys 121 and 122) were fistulated with the assistance of Mr A.W.F. Davey of the Dairy Husbandry Department, using the technique described by Balch and Cowie (1962). These six animals, together with a further three pairs of dry monozygous twin animals borrowed from time to time from Plant Chemistry Division, D.S.I.R., were used throughout this study to examine the influence of the ryegrasses on the end-products of rumen fermentation. It was hoped that these measurements would help in explaining the observed changes in milk composition.

2.8 RUMEN LIQUOR SAMPLING AND ANALYSIS

In the experiments in which samples of rumen liquor were obtained, two fistulated cows were allowed to graze with each of the treatment groups for at least one week before rumen sampling commenced to ensure that the microbial population was fully adapted to the new type of pasture.

The milking cows were kept off pasture between morning milking (6 a.m.) and 9 a.m., at which time the first rumen samples for the day were obtained. The cows were then returned to the experimental pastures and a further three samples were taken at two hourly intervals. Samples of rumen liquor (200-300 ml.) were taken by hand from the middle of the rumen (Davey, 1964) on two consecutive days. The rumen liquor was obtained by forcing samples through muslin and the pH of each sample was measured to the nearest 0.05 pH unit within a few minutes of removing the sample from the rumen.

Volatile fatty acid concentration: All rumen samples were stored at 0°C. until required for analysis, which was completed on the day of sampling. The concentration of volatile fatty acids (V.F.A.) were determined by the titration of the steam distillate obtained from a Markham (1942) still. A 5 ml. aliquot of rumen liquor was placed in the still with 1 ml. of 10 N-H₂SO₄, saturated with MgSO₄ (McAnally, 1944). One 50 ml. portion of distillate was collected and titrated with approximately 0.05-N NaOH under CO₂-free conditions. A second distillate of 50 ml. was collected on a number of occasions and used as a blank correction for the "slightly steam volatile" organic acids. Potassium hydrogen phthalate was used to standardise the NaOH. Determinations were carried out in duplicate and if they differed by more than 2% of the mean value, they were repeated. The standard deviation of a single duplicated analysis was 0.02 mM/100 ml. and the percentage recovery obtained, using the method on samples to which known quantities of volatile fatty acids were added, was 100.5%, with a range on the basis of 10 samples of 99.8 to 101.3%.

Separation of individual acids: Following titration of the V.F.A. as described above an excess of NaOH was added and the salts of the fatty acids were concentrated to a suitable volume (0.5 - 1.0 ml.) for gas-liquid chromatographic analysis. The relative proportions of acetic, propionic, and butyric acids were estimated by gas-liquid chromatography (James and Martin, 1952) using the column packing described by Hawke (1957). Columns were run at 137°C with a nitrogen flow rate of approximately 15 ml. per minute. Approximately 0.025 ml. of sample was added, using a hypodermic syringe, to a platinum boat containing equal parts of celite and NaHSO₄ as a dry mixture. The boat was quickly pushed into the column and the nitrogen supply connected.

The reliability of the separation was checked by using a standard solution containing acetic, propionic and butyric acids in the molar proportions of 6.5 : 2.0 : 1.5, at least once in every eight samples analysed. The standard deviations for a single analysis were 1.03, 0.65 and 1.03 for the molar proportions of acetic, propionic and butyric acids respectively. These values were obtained by repeating one sample 10 times during the course of analysing some 50 samples. Because of this rather high error, duplicate determinations were undertaken on all samples.

Ammonia concentration: Ammonia was determined in 1 ml. of rumen liquor by the boric acid - hydrochloric acid procedure of Conway and O'Malley (1942) using potassium carbonate in the outer compartment. The error of the method when using the mean value from duplicate samples was shown to be 0.02 mg./100 ml. on the basis of 10 mean figures obtained from the same sample of rumen liquor.

Statistical analysis of rumen liquor data: The basic objective in measuring the end-products of rumen fermentation was to obtain representative daily data which might help explain changes in milk composition. For this reason, mean values for the day (i.e. mean of 4 sampling times) were used in the statistical analyses although the possible existence of a large treatment X time interaction was checked for by the examination of diurnal graphs. The daily means for individual cows were analysed by analysis of variance using the method described by Henderson (1959) for designs in which there are two observations (days) nested within animals. Assuming animals and observations are random classifications the expectations of mean squares for a two treatment comparison are as follows:-

	df.	Expectation of mean square.
Treatment (T)	1	$\sigma_w^2 + \sigma_o^2 : at + 2\sigma_a^2 : t + 4\sigma_t^2$
Animals (A) : T	2	$\sigma_w^2 + \sigma_o^2 : at + 2\sigma_a^2 : t$
Observations (O):AT	4	$\sigma_w^2 + \sigma_o^2 : at$

This provides a test for treatments (i.e. $\sigma_t^2 = 0$) of T/A:T and also for differences among animals within treatments of A:T/O:AT

Discussion: The basic errors in the methods of determining total V.F.A. and ammonia concentrations were extremely small and are therefore unlikely to interfere with the experimental comparisons made in this study. The errors in determination of the molar proportions of V.F.A. were, however, somewhat larger and indicated the use of mean data from duplicates.

The sources of variation in volatile fatty acid concentration and molar proportions of individual acids have been determined previously (Wilson, 1966), and it was shown that time of sampling accounted for from 50 to 70% of the variation in these measures of rumen fermentation. Much of the remaining variance was due to day to day variation and there were no statistically significant differences between cows on the same ration. This information implies that differences in cow grazing behaviour between treatments would be expected to confound the true treatment effects. However, the procedure of keeping the cows off pasture between milking and first sampling at 9 a.m., probably helped to induce all cows to graze for a reasonably long period thus ensuring that the diurnal changes (see Figure 3.3) in volatile fatty acids were similar for all cows.

The statistical analysis described was not very efficient for testing treatment effects where treatment differences were small, owing to the small number of degrees of freedom (1 and 2 df.). However, it proved adequate (see Chapter 3) in that large differences are required before volatile fatty acid proportions are of any significance from an "animal performance" point of view.

The use of ammonia, total volatile fatty acid and individual volatile fatty acid data expressed as a concentration in a given quantity of rumen liquor, has serious interpretation limitations in that the total amounts of rumen liquor in the experimental animals were not measured and differences in rumen liquor volume and or rate of passage associated with treatments would be expected to complicate the treatment comparisons. It must also be remembered that the amount of these substances absorbed is the vital factor and while authors have found a correlation between the uptake of volatile fatty acids and their concentration in rumen liquor (Stewart, Stewart and Schultz, 1958; Leng, Corbett and Brett, 1968) this may not apply under all circumstances or with all rations. Rumen concentrations of total volatile fatty acids and ammonia together with the molar proportions of individual volatile fatty acids must, therefore, only be regarded as providing a qualitative picture of rumen metabolism.

CHAPTER 3

EXPERIMENT 1 : MILK PRODUCTION FROM THREE RYEGRASSES

3.1 INTRODUCTION

The object of Experiment 1 was to extend the range of ryegrasses that have already been compared for nutritive value (see section 1.1) by examining two annual varieties, Tetraploid Western Wolths and Paroa ryegrass. Tetraploid Western Wolths was developed recently by Grasslands Division of D.S.I.R. (Barclay ^{et al.} 1966) and because this variety produces a high dry matter yield per acre it is a possible alternative to Paroa ryegrass, from an agronomic point of view.

Paroa ryegrass was chosen because it was known to have a lower proportion of structural carbohydrates (Bailey, 1964) than the three ryegrasses already examined and Western Wolths was expected to have even lower values. Ruamui ryegrass was included as a third treatment, first, so that results could be related to previous experiments and secondly, to give, within the same experiment, the widest possible range in chemical composition that is obtainable from existing ryegrass varieties at similar stages of growth.

The ryegrasses were compared in two short-term grazing experiments carried out in June (Winter, W) and October (Spring, S) 1966. In addition to the lactating cows three pairs of non-lactating cows with rumen fistulae were used to assess the influence of the ryegrasses on the end-products of rumen fermentation.

3.2 EXPERIMENTAL METHODS

3.21 Animals and design of experiments

Eighteen Friesian cows which had calved about two months previously were transferred from a ration of pasture, hay and silage, to full pasture feeding on 7 June 1966. The cows were blocked on the basis of milk yield, and, on 17 June 1966, cows within blocks were allocated at random to the three ryegrass groups. The duration of the experiment was two weeks, during which the cows in the three groups were always given sufficient pasture to ensure full feeding. This was achieved by providing the cows with a fresh break of grass twice daily by using an electric fence.

Twelve pairs of monozygous twins were used in the spring experiment in a balanced incomplete block design (Cochran and Cox, 1950). The majority of the twins were Jerseys or Jersey cross animals, and all had calved in July or August 1966. The preliminary period with all cows grazing mixed pasture commenced on 18 September and the experimental ryegrass feeding period of three weeks commenced on 3 October.

3.22 Pastures

The Paroa and Western Wotiths ryegrasses were sown in paddocks 2,3,4 and 6 as pure swards (Table 2.1). Sowings were made in April, and because the paddocks used had previously carried a permanent mixed pasture sward for a number of years and fertility was high, no nitrogen top-dressing was applied during the experiments. The pure swards of Ruamui ryegrass used (Table 2.1) had been maintained with sulphate of ammonia for a number of years and 1 cwt. of sulphate of ammonia per acre was applied two to four weeks prior to the commencement of each experiment.

3.23 Sampling and analytical methods

Herbage: Sampling and analytical methods were as described in 2.4 and 2.5 except that in vitro determinations of organic matter digestibility were also made.

In vitro digestibility: The ground samples of herbage used for chemical analyses were also used to estimate digestibility. The method used was that of Tilley and Terry (1961) where centrifuging was replaced by filtration and organic matter was used instead of dry matter for digestibility calculations. A separate in vitro fermentation experiment was carried out for each of the grazing experiments. Within each experiment four 0.5 g. samples of each grass were digested together in the water bath. In addition four replicates of a standard grass sample with a known in vivo digestibility (89.2%) and four "blanks" containing rumen liquor only, were included to provide "correction factors" for the unknowns. Rumen liquor was obtained from a fistulated cow which had been grazing mixed pasture and had been starved for 12 hours in order to reduce the plant residue in the rumen liquor blanks. All mean results were "corrected" in an additive manner using as a basis the difference between the mean data obtained on the standard grass sample, and its known in vivo value (see Appendix 3.3).

Milk: Milk yields were measured at all milkings and two-day composite samples were taken from individual cows twice each week and analysed for fat, solids-not-fat, and protein contents as described in 2.6.

Rumen liquor: Samples of rumen liquor were taken from two cows on each of the three treatments on two consecutive days during the second week of both experiments. Samples were obtained and analysed as described in 2.8.

TABLE 3.1 **Chemical analysis (on a dry matter basis) and dry matter content of three ryegrasses used in Experiment 1 (Standard error of mean).**

	Winter experiment			Spring experiment		
	Western Wolths	Paroa	Ruanui	Western Wolths	Paroa	Ruanui
No. of samples	2	2	2	4	4	4
Cr. protein (N x 6.25)	27.6	28.4	28.8	15.3(1.4)	17.4(1.0)	17.8(0.6)
Cr. fibre	12.9	13.5	15.3	18.6(0.3)	19.6(0.5)	22.1(0.4)
Ether extract	5.9	6.4	5.7	5.8(0.9)	5.8(0.5)	6.4(0.6)
Ash	9.9	10.4	9.1	9.9(0.8)	10.5(0.7)	8.5(0.4)
Nitrogen-free extract	43.7	41.3	41.1	50.4(2.7)	46.7(1.7)	45.2(1.4)
Soluble sugars	23.5	20.6	18.0	24.8(1.3)	20.7(0.7)	18.3(0.5)
Dry matter content	14.1	13.7	18.2	16.6(1.9)	16.6(2.0)	20.8(2.1)

Cow body weights: Individual cows were weighed each Friday at 9 a.m. during the preliminary and experimental periods of the spring experiment. Weights were recorded to the nearest 1 lb. on the scale.

3.24 Statistical analyses

The milk yield and composition data were analysed by covariance analyses (Appendix 3.1). The rumen liquor data were analysed by analyses of variance, an example of which is given in Appendix 3.2. The in vitro digestibility data given in Appendix 3.3 were also analysed by analyses of variance. The significance of the differences between the mean values for the three ryegrasses was determined using Duncan's (1955) multiple range test.

3.3 RESULTS

3.31 Description of the pastures

At the commencement of the experiments the Western Wolths and Paroa ryegrasses were between 10in. and 14in. high and the Ruanui Gin. to 8in. The chemical compositions of the ryegrasses are given in Table 3.1. In the winter the crude fibre content was lowest in Western Wolths, intermediate in Paroa and highest in Ruanui. The total soluble sugar content of the ryegrasses varied in the reverse order to that of fibre (WW > Paroa > Ruanui). The only other difference of any great magnitude, between the ryegrasses, was the higher dry matter content of Ruanui ryegrass compared with the other two.

During the spring experiment the protein levels were lower than in the winter experiment, and varied among the varieties (WW < Paroa < Ruanui).

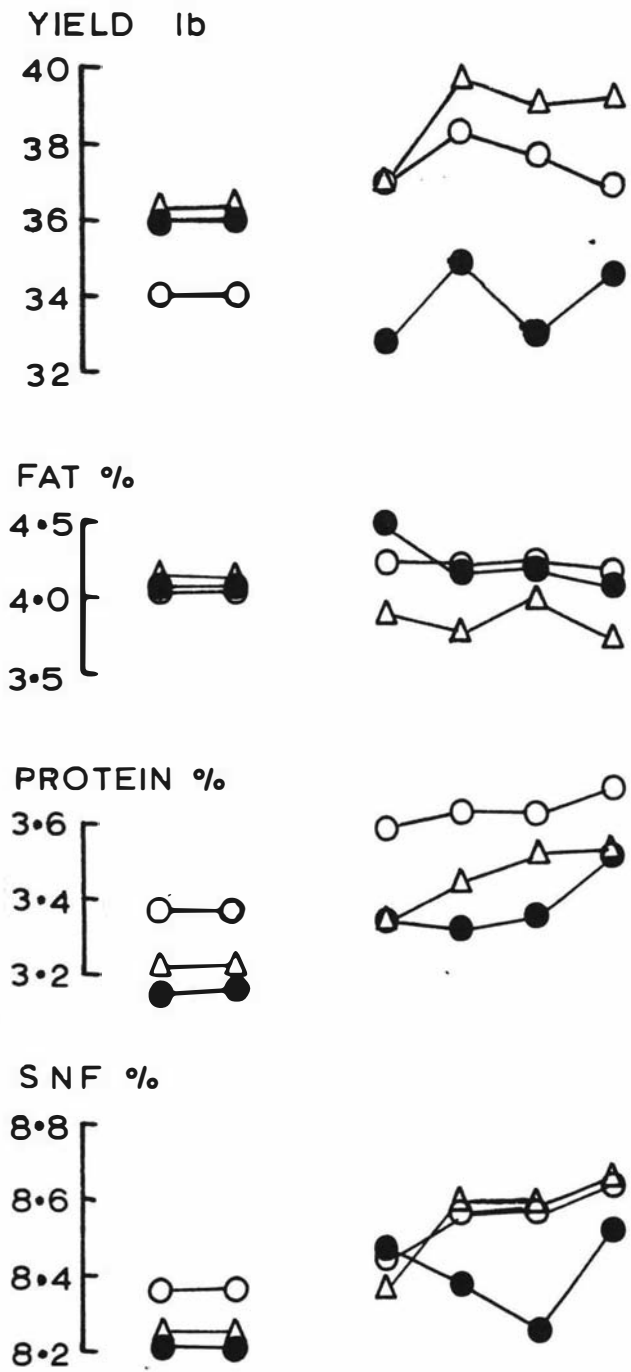


Fig. 3.1 Average daily milk yields and milk compositions from groups of Friesian cattle grazing Western Wolths (Δ), Paroa (\circ) and Ruanui (\bullet) ryegrasses during June. (The first two samples, mixed pasture feeding).

TABLE 3.2 The yield and composition of milk from cows grazing three ryegrasses: adjusted mean values for experimental period.

	Western Woltha (W)	Paroa (P)	Ruamui (R)	S.E. of means	Significant differences	
					P < 0.01	P < 0.05
<u>Winter experiment</u>						
Milk (lb./cow/day)	37.7	37.9	32.6	1.97	-	-
Fat %	3.84	4.18	4.19	0.09	-	W < P W < R
Fat (lb./cow/day)	1.43	1.60	1.35	0.07	-	-
Solids-not-fat %	8.54	8.55	8.39	0.06	-	-
Solids-not-fat (lb./cow/day)	3.23	3.23	2.77	0.17	-	-
Protein %	3.42	3.51	3.48	0.07	-	-
Protein (lb./cow/day)	1.27	1.34	1.13	0.09	-	-
<u>Spring experiment</u>						
Milk (lb./cow/day)	30.5	30.8	29.2	0.45	-	-
Fat %	4.67	4.77	4.72	0.10	-	-
Fat (lb./cow/day)	1.39	1.40	1.34	0.08	-	-
Solids-not-fat %	9.16	9.03	8.74	0.07	W > R	P > R
Solids-not-fat (lb./cow/day)	2.76	2.75	2.54	0.08	-	-
Protein %	3.66	3.61	3.49	0.04	-	P > R W > R
Protein (lb./cow/day)	1.09	1.09	1.01	0.02	W > R P > R	-

TABLE 3.3 In vitro organic matter digestibilities of the three ryegrasses used in Experiment 1.

	Winter experiment			Spring experiment		
	Western Wolths(WW)	Paroa(P)	Ruanui(R)	Western Wolths	Paroa	Ruanui
Mean	82.52	80.18	80.54	71.53	70.47	68.97
S.E. of means		0.33			0.82	
Significant differences						
P < 0.05	-				-	
P < 0.01	WW > R	WW > P			-	

The crude fibre levels were higher in the spring than in the winter but the ryegrasses ranked in the same order. The soluble sugar levels were similar in the two experiments and varied in the same order in both cases (WW > Paroa > Ruanui).

The mean in vitro organic matter digestibilities for the three ryegrasses, in both experiments, are given in Table 3.3. In the winter experiment the value for Western Wolths was significantly ($P < 0.01$) higher than the values for the other two varieties. In the spring experiment the digestibilities were about 10 units lower than those in the winter experiment and although they were ranked (WW > P > R) in the reverse order to the crude fibre values the differences between them were not significant ($P > 0.05$).

3.32 Milk yield and composition

Winter experiment: The mean data for milk yield and composition, sample day by sample day, are presented in Figure 3.1, and the mean values for treatment groups, after adjustment for preliminary period differences, are given in Table 3.2. One cow suffered from "grass staggers" (hypomagnesaemia) while grazing Western Wolths ryegrass and subsequently died, so that the results for this group are the mean values for the remaining five cows.

The milk yields from the Western Wolths and Paroa groups were similar, and both higher than that from the Ruanui group although the differences were not significant ($P > 0.05$). The butterfat percentage of the milk from the Western Wolths group was significantly lower ($P < 0.05$) than those from the other two groups. Butterfat yield was hence greatest from the Paroa group, intermediate from the Western Wolths group and lowest from the Ruanui group.

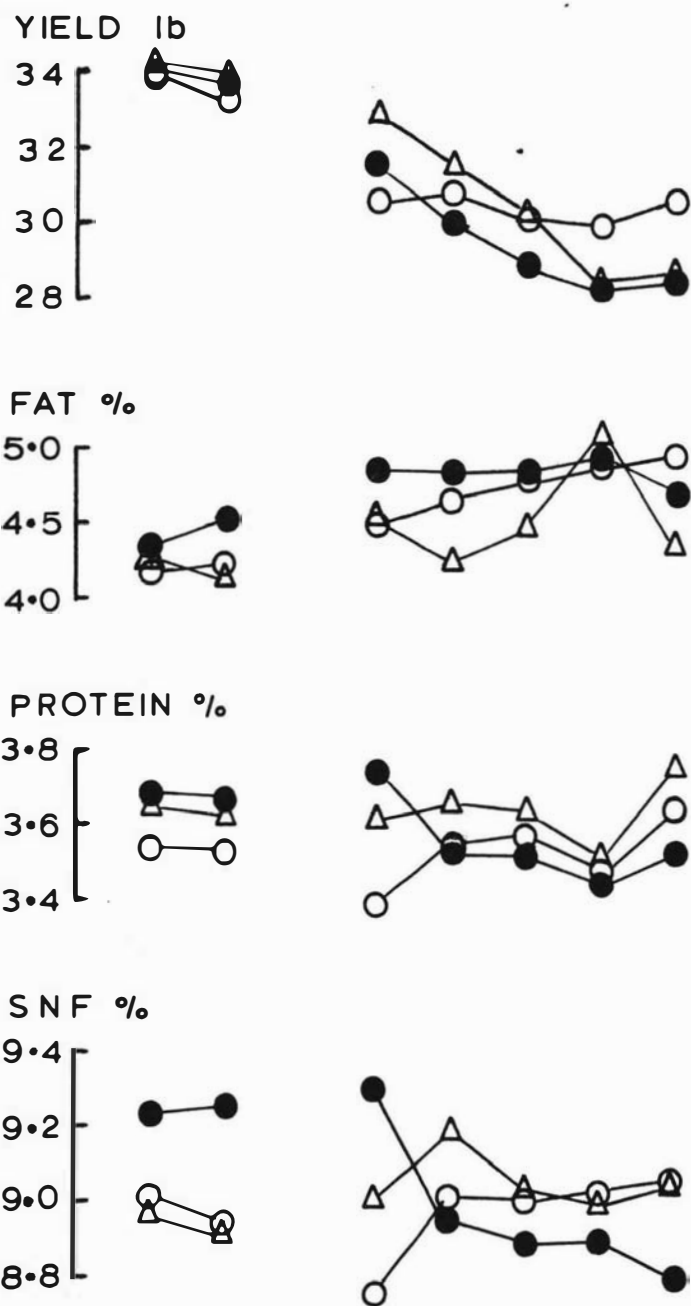


Fig. 3.2 Average daily milk yields and milk compositions from groups of twins grazing Western Wolths (Δ), Paroa (\circ) and Ruanui (\bullet) ryegrasses during October. (The first two samples, mixed pasture feeding).

TABLE 3.4 Analyses on the rumen liquor from three pairs of twins fed three ryegrasses.

Treatment	Cow	pH ₃ conc. (mg. N/100ml.)	pH	Total V.F.A. conc. (mM/100 ml.)	Molar proportions of V.F.A.		
					Acetic	Propionic	Butyric
<u>Winter experiment</u>							
West. Wollths (WV)	15	29.14	6.25	9.88	59.13	33.84	17.03
Ruanui (R)	16	43.57	6.34	10.80	63.45	18.81	17.74
West. Wollths	50	44.04	6.34	11.38	59.88	21.90	18.22
Paroa (P)	60	53.99	6.20	12.78	64.35	20.00	15.65
Paroa	121	50.34	6.27	11.50	64.35	20.51	15.14
Ruanui	122	51.05	6.30	11.81	66.03	17.76	16.21
Treatment diff. signif. at 5% level		WV < R almost signif.	-	-	-	WV > P	-
1% level		-	-	-	WV < R WV < P	WV > R	-
<u>Spring experiment</u>							
West. Wollths	1	30.0	6.30	10.90	67.2	20.2	12.6
Ruanui	2	37.0	6.48	10.49	68.9	17.9	13.2
Paroa	29	38.9	6.40	11.24	68.8	18.6	12.6
West. Wollths	30	24.3	6.35	9.67	66.7	19.2	14.1
Paroa	119	37.9	6.44	10.68	70.1	18.3	11.6
Ruanui	120	36.8	6.50	10.67	69.1	18.9	12.0
Treatment diff. signif. at 5% level		-	-	-	WV < R WV < P	-	-
1% level		WV < P WV < R	-	-	-	-	-

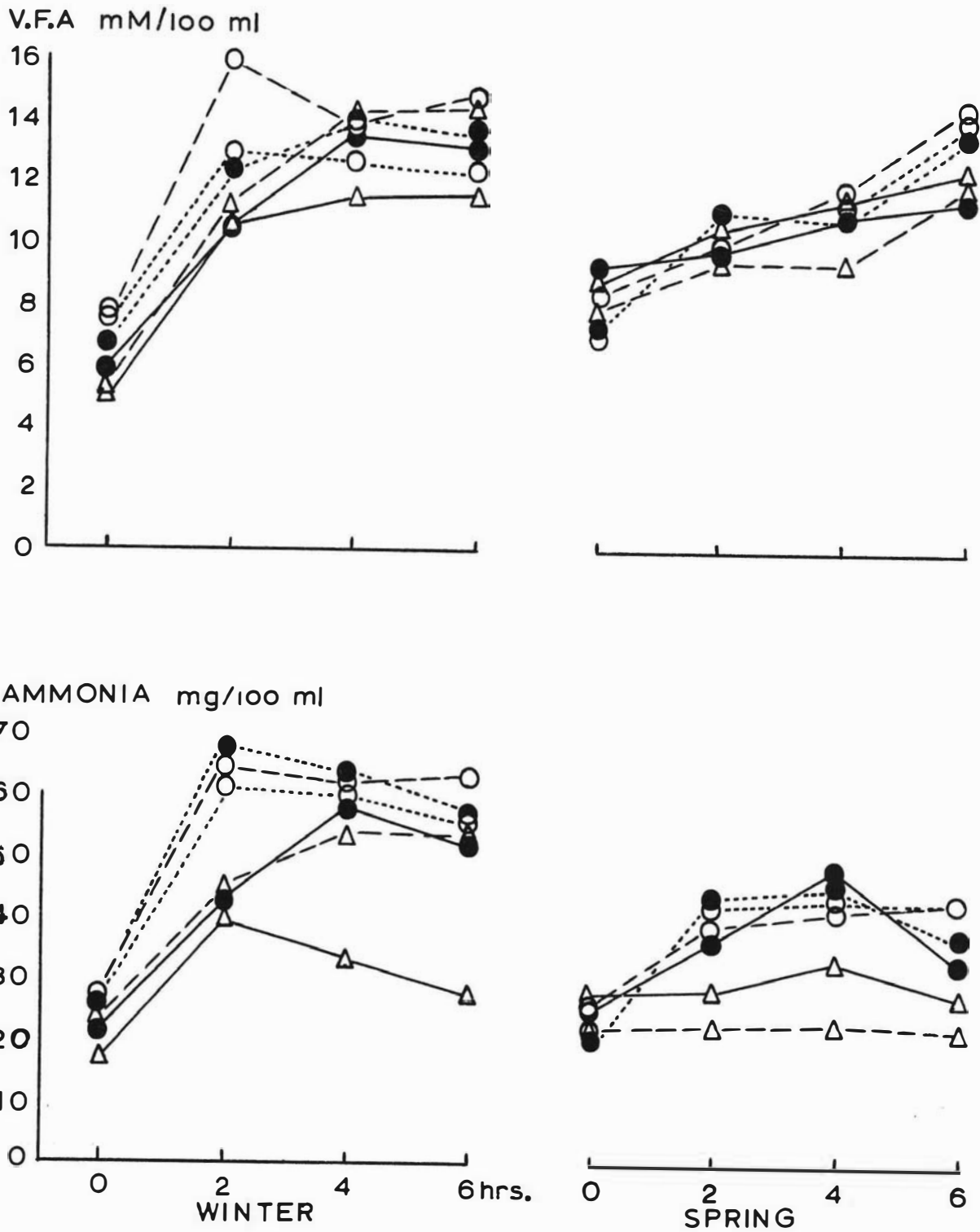


Fig.3.3 Diurnal variations in volatile fatty acid and ammonia concentrations of rumen liquor, from individual cows grazing three ryegrasses during the winter and spring experiments (Western Wolths Δ; Paroa ○; Ruanui ●. Connecting lines similar for twin pair mates).

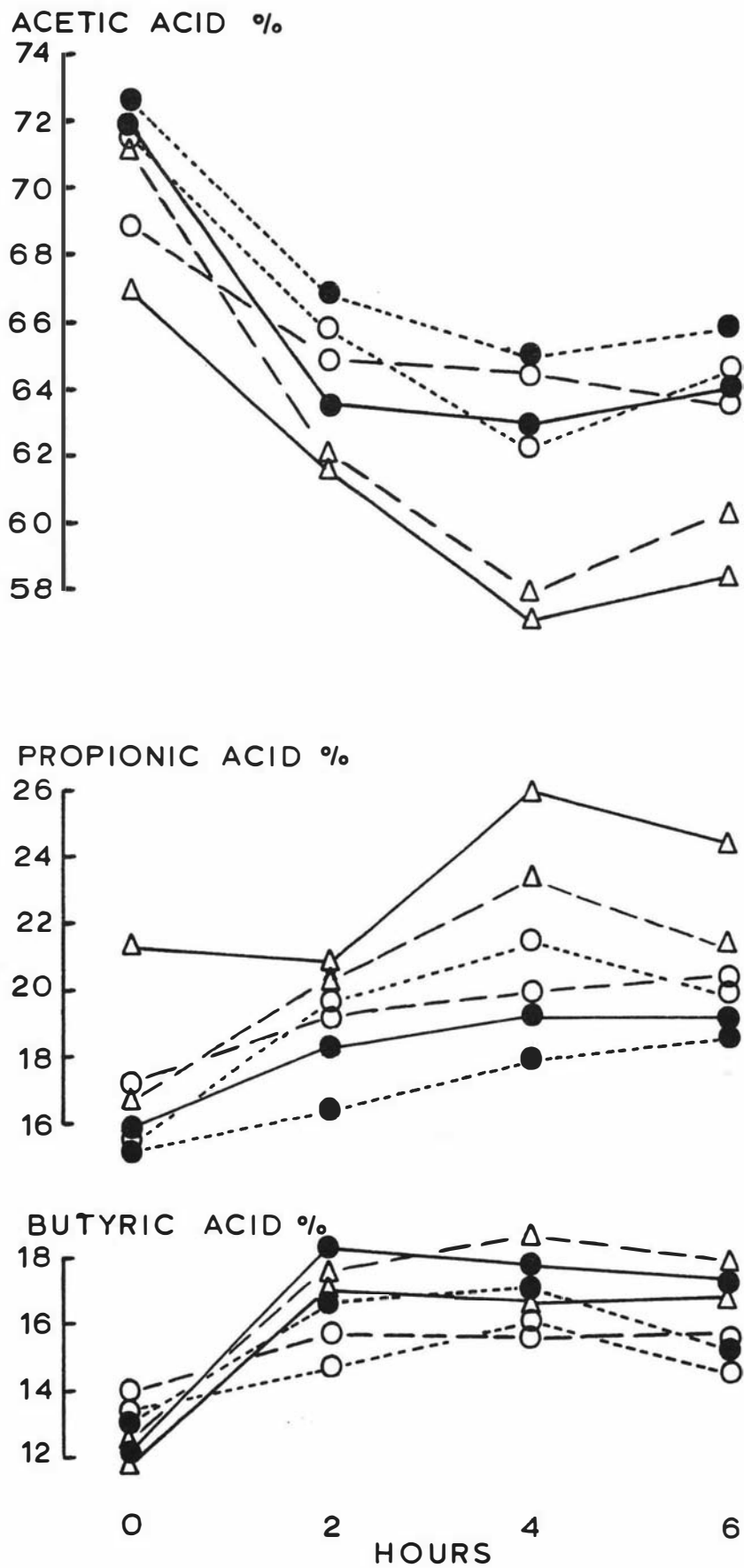


Fig.3.4 Diurnal variations in the molar proportions of individual V.F.A. of rumen liquor from individual cows grazing Western Wolth (Δ), Paroa (\circ), and Puanui (\bullet) ryegrasses during the winter experiment. (Connecting lines similar for twin pair mates).

The solids-not-fat percentage in the milk and the solids-not-fat yield produced by the Ruamui group was lower than the corresponding values for the other two groups but the differences were not significant. The protein yield was lowest from the Ruamui group but the differences between groups were not significant.

Spring experiment: The milk yield and composition data for the spring grazing experiment are presented in Figure 3.2 and the adjusted data in Table 3.1. The milk yields, fat percentages and fat yields for the three groups were very similar. However, the solids-not-fat and protein percentages were highest for the Western Woliths group, intermediate for the Paroa group and lowest for the Ruamui group. For both these variables the differences between the values for the Ruamui group and the other two varieties were statistically significant ($P < 0.05$). In addition the protein yield from the Ruamui group was reduced significantly ($P < 0.01$).

3.33 Characteristics of rumen liquor

The mean pH, volatile fatty acid concentrations, ammonia concentrations and molar proportions of individual fatty acids in the rumen liquor taken from the three pairs of twins grazing the ryegrasses in the two experiments are given in Table 3.4. The significance of the differences between mean values obtained for ryegrass treatments is also indicated. In order to illustrate the variation present within the mean data, diurnal variations in volatile fatty acid and ammonia concentrations for individual cows grazing the three ryegrasses during the winter and spring experiments have been plotted in Figure 3.3, and the diurnal variations in the molar proportions of individual volatile fatty acids in the winter experiment in Figure 3.4.

In the winter experiment the ammonia concentrations in the rumen liquors from the cows grazing Western Wolths ryegrass following the commencement of grazing, were considerably lower than the corresponding concentrations obtained from the cows grazing the other ryegrasses. There were no consistent differences between treatments for pH or total volatile fatty acid concentration. The molar proportions of individual fatty acids in the rumen liquor produced from the three ryegrasses, however, differed markedly. The mean figures for the two cows on each of the ryegrasses were: Western Wolths 59.2, Paroa 64.3, Ruamui 64.8 for acetic acid and Western Wolths 22.9, Paroa 20.2 and Ruamui 18.3 for propionic acid.

The results for the spring experiment followed the same pattern as those for the winter experiment except that all the ammonia levels were lower, and the molar proportions of acetic acid higher and propionic acid lower, than in the previous experiment. The only treatment differences to reach significance were the differences between Western Wolths and the other two ryegrasses for ammonia concentration and for the molar proportions of acetic acid ($P < 0.05$).

3.34 Body weight

Following the transference of the cows from mixed pasture feeding to the ryegrass treatments, the cows grazing Western Wolths ryegrass apparently gained 4 lb. body weight (mean of three, weekly weighings). The corresponding gains made by the cows grazing Paroa and Ruamui were 11 lb. and 20 lb. respectively. The weight changes made by the three groups of cows following the change back to the mixed pasture at the completion of the experiment were in the reverse relative order (Western Wolths + 14 lb., Paroa + 12 lb., Ruamui + 4 lb.).

3.4 DISCUSSION

The ryegrasses examined gave a range of values for crude fibre, protein, and total soluble sugars which was greater than that obtained in previous experiments, (Wilson and McDowall, 1966), so that the original objective of using additional ryegrass varieties to provide greater extremes in chemical composition was achieved. These variations led to various statistically significant variations in milk composition which were also of a greater magnitude than those obtained previously.

Winter experiment: During the winter experiment the three groups of cows produced either different yields of milk, or milk with a different composition, when offered unrestricted access to the ryegrasses. The milk yields from the Western Wolds and Paroa groups were similar, but the butterfat percentage ($P < 0.05$), and hence the butterfat yield, was lower from the Western Wolds group.

Western Wolds differed from Paroa in that the crude fibre content of the dry matter was lower and the total soluble sugar content three percentage units higher (Table 3.2). This difference in the ratio of readily available carbohydrates to the "fibrous fraction" probably accounted for the higher proportion of propionic acid and lower proportion of acetic acid in the rumen liquor (Table 3.4), and is in agreement with the results of Tilley, Deriaz and Terry (1960) who found a positive correlation between the soluble carbohydrate content of pasture and the proportion of propionic acid. The relatively high level of propionic acid and low level of acetic acid in the rumen liquor of the cows grazing Western Wolds was probably responsible for the depressed butterfat percentage in the milk produced by these cows. This conclusion is suggested by a number of experiments in which depressions in the butterfat

content of milk have been obtained when cows are fed diets low in roughage and high in starchy concentrates which also lead to low acetic and high propionic acid proportions in the rumen (e.g. Balch, Balch, Bartlett, Cox and Rowland, 1952). In addition, intra-ruminal infusions of propionic acid have led to reductions in the butterfat percentage of milk (Wilson, Davey and Dolby, 1967). There is, however, as far as the author is aware, only one previous report of reduced fat percentages in milk which may be related to composition changes in forages (McClymont 1950).

The low milk yield obtained from the cows grazing Ruazui ryegrass during the winter experiment is difficult to explain from the variables measured, but it is in line with results from a previous experiment in which Ruazui was compared with Manawa ryegrass (Wilson and McDowell, 1966). In view of the small differences in organic matter digestibility (Table 3.3) the most probable explanation is a lowered voluntary intake of Ruazui ryegrass. It is well established that a restriction in the level of feeding leads to lowered solids-not-fat percentages in milk, and this occurred in the present experiment (see also 1.55).

Spring experiment: The butterfat content of the milk produced by the Western Folds group in the spring experiment was not reduced. The crude fibre content of the ryegrass was approximately 3% higher during the spring experiment, which could account for the higher acetic acid and lower propionic acid proportions in the rumen liquor and hence the lack of a reduction in fat percentage.

Milk yields and butterfat percentages were very similar during the spring experiment. However, the solids-not-fat content of the milk produced by the Ruazui group was significantly reduced ($P < 0.01$). Part of this reduction was accounted for by a reduction in the protein percentage but lactose was probably also reduced.

A difference of 0.42 percentage units in solids-not-fat percentage cannot be explained entirely by a change in the plane of energy nutrition, as the milk yield of the Ruamui group was only slightly lower than that produced by the Western Wolths group. The body weight changes observed in the spring experiment cannot be used to contribute towards the evidence for or against underfeeding of the cows grazing Ruamui ryegrass as the experiment was very short and therefore the weight changes were probably due to differences in digestive tract "fill" rather than to true differences in body weight. Hence the high apparent body weight gain made by the group of cows grazing Ruamui ryegrass was probably due to a slower rate of passage of digesta brought about by the higher crude fibre content and lower digestibility of this variety of ryegrass. Experimental evidence supporting this suggestion has been obtained using sheep by Johns et al. (1963).

While there is evidence from infusion experiments that variations in the proportion of propionic acid in the rumen liquor are associated with variations in the solids-not-fat percentage in the milk (Wilson et al., 1967), it seems unlikely that the 1% to 2% difference in the proportions of propionic acid produced by the cows on Ruamui and Western Wolths could produce the observed reductions in solid-not-fat percentage. The possibility that the relatively low levels of propionic acid produced by the Ruamui cows were at least partly responsible for the reduced solids-not-fat cannot, however, be ruled out.

The relatively high level of soluble carbohydrate in the Western Wolths was probably responsible for the lower ammonia concentration in the rumen liquor (Fig.3.3) although in the spring experiment the lower level of protein in the ryegrass probably also contributed to the lower ammonia levels obtained. The differences in the rumen ammonia concentration suggest that interactions between the level of readily available carbohydrate and the level of protein

could affect the utilisation of protein and hence the protein content of the milk. Chalmers and Marshall (1964), for example, have demonstrated an inverse relationship between rumen ammonia levels and nitrogen retention, and Head and Rook (1957) have shown that added starch is effective in controlling the urinary wastage of nitrogen.

The separate effects of a slightly lower plane of energy nutrition and a higher ratio of acetic to propionic acid in the rumen liquor seem unlikely to explain the lowered solids-not-fat levels obtained in the milk from the Ruamui group, and the importance of the ratio of soluble carbohydrate to protein contents in the pasture in possibly affecting solids-not-fat contents clearly awaits further experimentation. However, one possibility, that must be borne in mind, is that two or more of these factors acting synergistically may have produced the observed effect.

3.5 SUMMARY AND CONCLUSIONS

Tetraploid Western Wolths, Paroa, and Ruamui ryegrasses were compared in terms of their effects on milk yield and composition. A winter and a spring experiment were carried out.

During the winter experiment, when groups of Friesian cattle were used, the cows on Western Wolths and Paroa ryegrasses produced similar yields of milk, but the milk from the Western Wolths group was significantly lower ($P < 0.05$) in butterfat percentage than that from the other groups. This reduction was associated with a decreased proportion of acetic acid and an increased proportion of propionic acid in the rumen liquor. It was considered that these changes in rumen liquor, brought about by a high ratio of soluble carbohydrates to crude fibre in the Western Wolths ryegrass, adequately explained the reduction in fat percentage obtained.

In the spring experiment, when lactating monozygous twin cattle were used, milk yields and butterfat percentages were similar for the three groups, but the protein and "solids-not-fat-protein" contents of the milk produced by the cows grazing Ruamui ryegrass were significantly depressed. No adequate explanation for the reductions could be suggested and it was decided to design further experiments to examine the possibility that interactions between the level of available carbohydrate and the level of protein in pastures may affect the utilization of protein and hence influence the protein content of milk (see Experiments 3, 5 and 6).

The data from these experiments have been published (Wilson and Dolby, 1967). Samples of blood were also obtained at regular intervals from the individual cows used in these experiments and analysed for mineral composition by D.S.I.R. staff. These data, which do not form part of the present study, have also been published (Butler and Metson, 1967). It is relevant, however, that the cows grazing Ruamui ryegrass had lower blood magnesium levels than the cows grazing the annual varieties. The possibility that hypomagnesaemia may induce changes in milk yield or composition, therefore exists. However, as it is probable that other factors such as level of feeding which affect milk composition will also affect blood magnesium levels, the association between the two variables is unlikely to be a casual relationship.

CHAPTER 4

EXPERIMENT 2 : MILK PRODUCTION FROM RUANUI

RYEGRASS AT TWO STAGES OF GROWTH.

4.1 INTRODUCTION

Previous experiments (see 1.2) suggest that variations in stage of growth within ryegrass varieties have a considerable effect on the nutritive value of pastures. In fact there is considerable evidence (e.g. Armstrong, 1964) that differences in stage of growth are more important as a source of variation in nutritive value than differences between species.

This experiment was undertaken to examine the effect on milk yield and composition of cows grazing Ruanui ryegrass at two stages of growth. Ruanui ryegrass was chosen rather than any other variety for a number of reasons. First, it was considered that this ryegrass probably differed to a greater extent chemically, with stage growth, than the less persistent varieties. Secondly, it provided the opportunity to examine further the possibility that the production differences obtained between Manawa or Ariri and Ruanui ryegrasses (Wilson, and McDowell, 1966) may have been associated with the grazing of the Ruanui ryegrass pasture at an above-optimum grazing height.

A short preliminary experiment (A) was carried out in November, 1964, and the cows grazing the short (less mature) ryegrass produced milk which contained a much lower solids-not-fat content than that from the cows grazing the long mature ryegrass. A second experiment (B) was therefore undertaken, in the following November, in which the number of cows used was increased, the milk was analysed more completely and rumen-fistulated animals were included in the experiment so that the importance of possible variations in the end-products of rumen fermentation could be measured.

TABLE 4.1 Experimental details for Experiment 2.

Experiment A:

Pastures

Treatments	<u>Short Ruanui ryegrass</u>	<u>Long Ruanui ryegrass</u>
Description	6 - 10 inches long, some seeding	8 - 12 inches long, considerable seeding
Date pasture closed	27 Oct.	24 Sept.
(NH ₄) ₂ SO ₄ applied/acre	18 Aug. (1 cwt.) 14 Sept. (1 cwt.) 27 Oct. (1 cwt.)	14 Aug. (1 cwt.) 14 Sept. (1 cwt.)

Experimental Dates

Pre-experimental period (mixed pasture) 25 Oct. - 15 Nov.

Experimental period (short and long Ruanui ryegrass) 15 Nov. - 8 Dec.

Animals

Number of cows used. Five pairs of monozygous twins.
Breed of cows. 3 pairs Jersey, 1 pair Jersey x Friesian,
1 pair Jersey x Ayrshire.

Experiment B:

Pastures

Treatments	<u>Short Ruanui ryegrass</u>	<u>Long Ruanui ryegrass</u>
Description	6 - 10 inches long, some seeding	10-15 inches long, very heavy seeding.
Date pasture closed	26 Oct.	28 Sept.
(NH ₄) ₂ SO ₄ applied/acre	7 Aug. (1 cwt.) 20 Sept. (1 cwt.) 23 Oct. (1 cwt.)	7 Aug. (1.5 cwt.) 20 Sept. (1.5 cwt.)

Experimental Dates

Pre-experimental period (mixed pasture) 27 Oct. - 12 Nov.

Experimental period (short and long Ruanui ryegrass) 12 Nov. - 30 Nov.

Animals

Number of cows used. Seven pairs of monozygous twins.
Breed of cows. 5 pairs Jersey, 1 pair Jersey x Friesian,
1 pair Jersey x Ayrshire.

4.2 EXPERIMENTAL METHODS

4.21 Animals and design of experiments.

Five pairs of monozygous twins were used in Experiment A and seven pairs in B. All of the cows, which had calved in July or August, were grazed together for a preliminary period on mixed pasture. Following this period, the cows within twin sets were allocated at random to the two treatment groups (long and short Ruamui ryegrass). The experimental dates are given in Table 4.1 together with a general description of the cows and pastures used. The objective of management was to ensure that, throughout each experiment, cows in the two groups were offered sufficient pasture to ensure maximum voluntary intakes.

Pastures: Five paddocks containing pure swards of Ruamui ryegrass (see Table 2.1) were used in each of the two experiments. Two paddocks were used for obtaining "long" pasture and the remaining three for "short" pasture. The details of fertilizer rates and dates of application together with the dates on which paddocks were closed are given in Table 4.1 for the first paddocks to be used in each experiment. In cases where two or more paddocks were used, the fertilizer rates were similar within treatments, but application dates were spaced at approximately weekly intervals. Pastures were topped just prior to the closing up of a paddock in order to provide a sward of reasonably uniform height.

4.22 Sampling and analytical methods.

Herbage and Milk: Sampling procedures and analytical methods were as described in Sections 2.4, 2.5, 2.6.

TABLE 4.2 Chemical analysis (on a dry matter basis) of the short and long Ruamui ryegrass used in experiments 2A and 2B (Standard error of mean).

	Experiment A		Experiment B	
	Short	Long	Short	Long
Number of samples	3	3	2	2
Crude protein (Nx 6.25)	21.9 (0.6)	13.0 (0.3)	18.0 (0.1)	15.5 (0.8)
Crude fibre	23.9 (1.8)	24.7 (2.1)	23.3 (1.1)	28.2 (0.1)
Ether extract	5.4 (0.5)	3.8 (0.1)	3.3 (0.3)	2.5 (0.3)
Ash	10.3 (0.3)	7.6 (0.3)	9.0 (0.1)	7.1 (0.8)
Nitrogen-free extract	38.5 (2.7)	50.9 (1.8)	46.4 (0.4)	46.7 (2.5)
Soluble sugars	-	-	15.4 (0.7)	13.9 (0.6)
Dry matter content	18.1 (0.3)	20.4 (0.2)	19.7 (0.6)	24.8 (0.8)

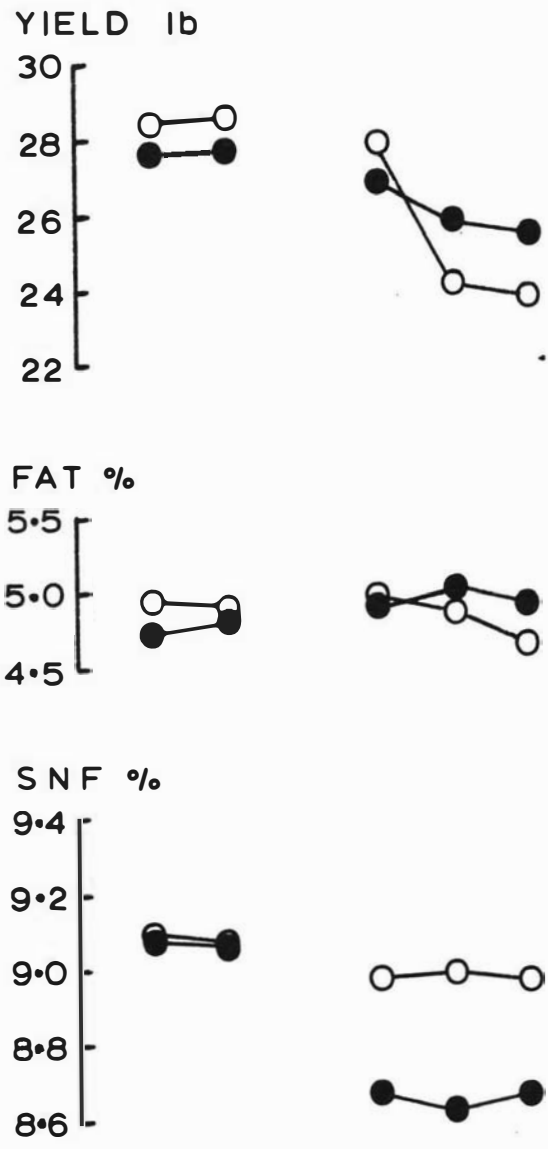


Fig.4.1 Average daily milk yields and milk compositions from two groups of twins grazing long (○) and short (●) Ruanui ryegrass in the 1964 experiment. (The first two samples, mixed pasture feeding).

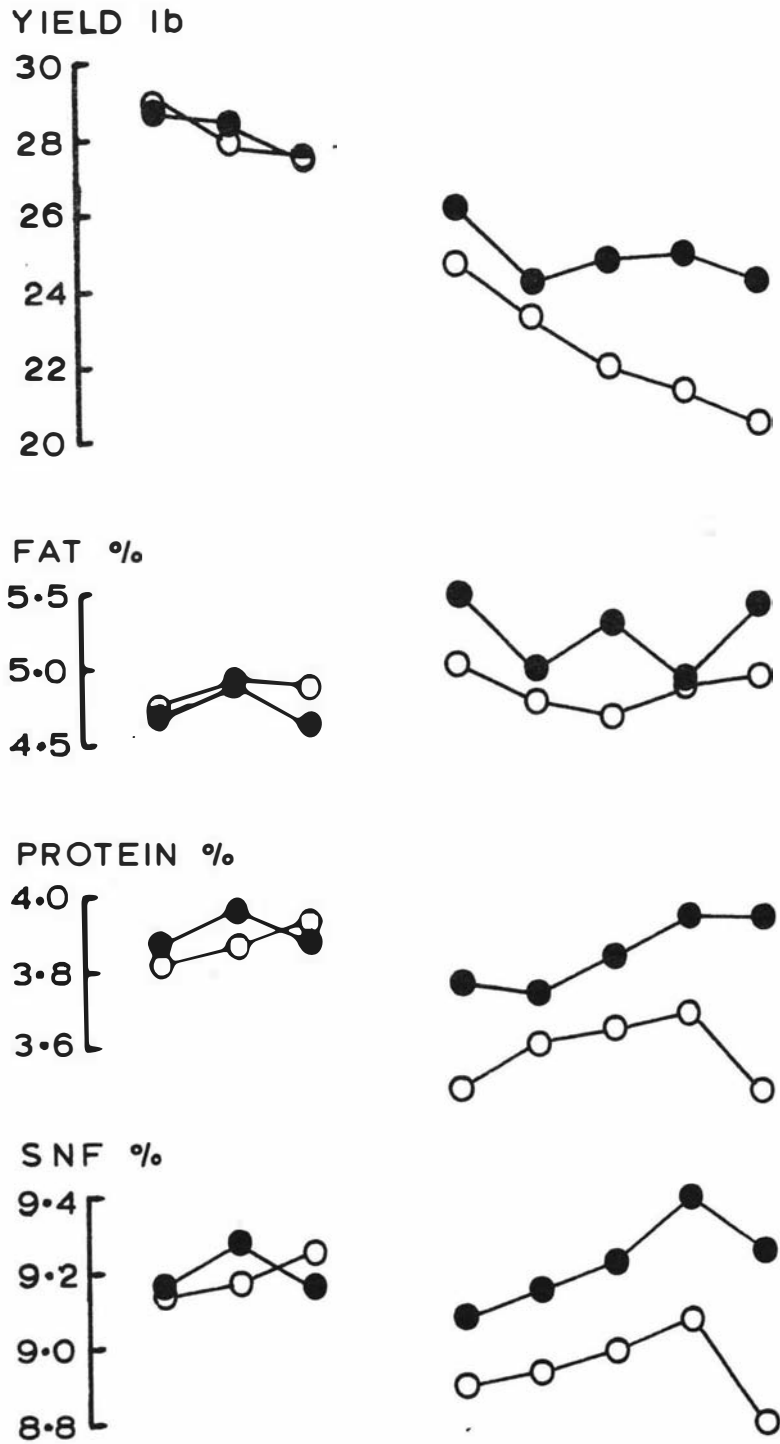


Fig.4.2 Average daily milk yields and milk compositions from two groups of twins grazing long (○) and short (●) Ruanui ryegrass in the 1965 experiment. (The first three samples, mixed pasture feeding).

TABLE 4.3 The yield and composition of milk from cows grazing Ruamui ryegrass at two stages of growth: adjusted mean values for experimental period.

Experiment A: 15/11 - 8/12/64.

	Short growth	Long growth	S.E. of means	Signif. of difference
Milk yield (lb./cow/day)	26.8	25.2	0.27	P < 0.01
Fat %	5.08	4.75	0.11	N.S.
Fat (lb./cow/day)	1.33	1.17	0.04	P < 0.05
Solids-not-fat %	8.68	8.98	0.05	P < 0.01
Solids-not-fat (lb./cow/day)	2.32	2.27	0.02	N.S.

Experiment B: 12/11 - 30/11/65.

Milk yield (lb./cow/day)	24.9	22.4	0.28	P < 0.01
Fat %	5.26	4.84	0.15	N.S.
Fat (lb./cow/day)	1.30	1.06	0.04	P < 0.01
Protein %	3.82	3.63	0.03	P < 0.01
Protein (lb./cow/day)	0.94	0.81	0.02	P < 0.01
Solids-not-fat %	9.22	8.98	0.03	N.S.
Solids-not-fat (lb./cow/day)	2.30	2.06	0.04	P < 0.01

Rumen liquor: Samples of rumen liquor were taken from two pairs of fistulated twins on two consecutive days during the second week of Experiment B. Samples were obtained and analysed as described in 2.8.

Statistical methods: These were as described for Experiment 1 (3.24).

4.3 RESULTS

4.31 Chemical composition of the pastures.

The results from the analyses carried out on the pasture samples are given in Table 4.2. Although it was intended that Experiment B should be an exact replicate of A, the pastures used in Experiment B were longer and more mature than those used originally.

In both experiments the long (and more mature) pasture was lower than the short in crude protein, ether extract, and ash content, and higher in crude fibre content, but the extent of the differences between treatments differed in the two experiments. The difference in the crude protein content between treatments in Experiment B was smaller (2.5% compared with 8.9%), and that for crude fibre larger (4.9% compared with 0.8%), than the corresponding differences in Experiment A.

4.32 Milk yield and composition.

The adjusted mean data for milk yield and composition are given in Table 4.3, and the original data are plotted in Figures 4.1 and 4.2 for Experiments A and B respectively.

In Experiment A the cows grazing the short pasture produced more milk ($P < 0.01$) which was slightly higher in butterfat percentage and very much lower in solids-not-fat percentage ($P < 0.01$). The difference between the

TABLE 4.4 Analyses on the rumen liquor from two pairs of twins grazing short and long Ruani ryegrass in Experiment 2B.

Treatment	Cow	NH ₃ conc. mg.N/100 ml.	pH	Total V.F.A. mM/100 ml.	Molar proportions of V.F.A.		
					Acetic	Propionic	Butyric
Long (L)	15	14.4	6.72	9.23	71.25	16.25	12.50
Short (S)	16	27.6	6.42	11.25	67.80	17.00	14.60
Long	121	20.3	6.70	8.78	70.45	17.05	12.50
Short	122	29.4	6.39	11.41	71.10	15.50	13.40
Treatment differences signif. at:							
10% level		S > L	-	-	-	-	-
5% level			-	S > L			
1%			L > S				

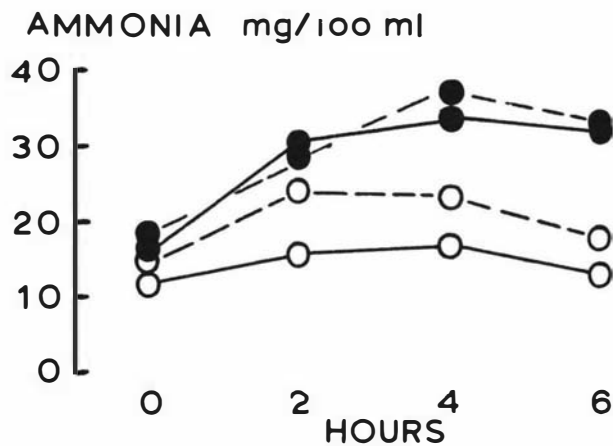
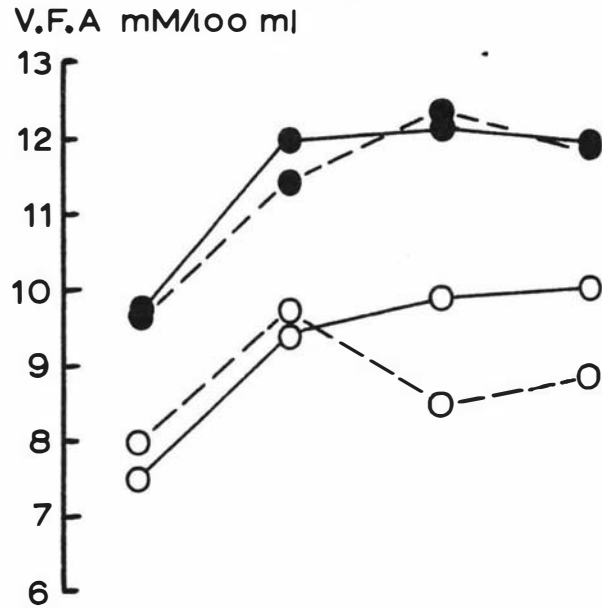


Fig.4.3 Diurnal variations in V.F.A. and NH_3 concentrations of rumen liquor from two pairs of twin cows grazing short (●) and long (○) Ruanui ryegrass in experiment 2B (1965). (Connecting lines similar for twin pair mates).

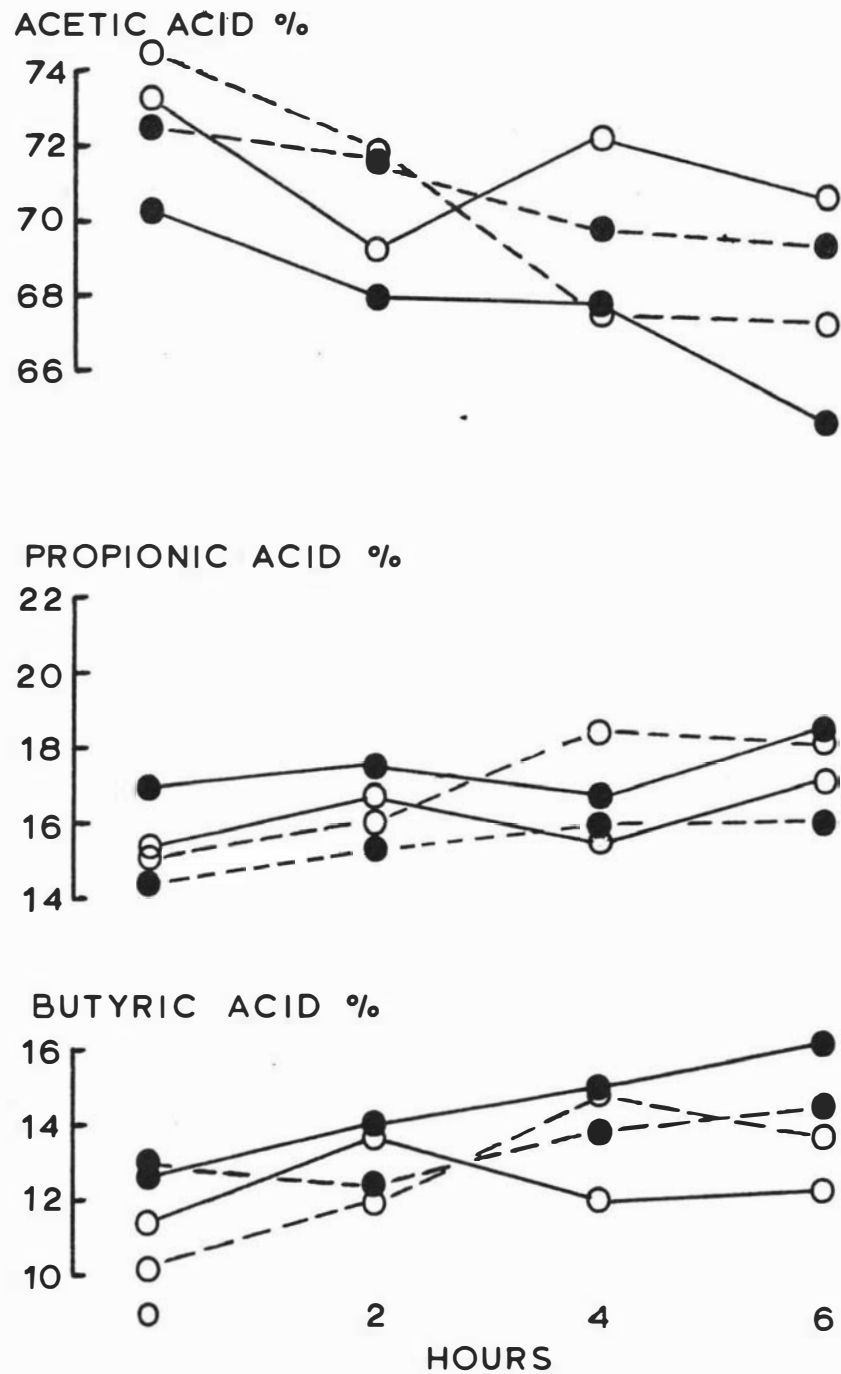


Fig.4.4 Diurnal variations in the molar proportions of individual V.F.A. of rumen liquor from two pairs of twin cows grazing short (●) and long (○) Ruanui ryegrass in experiment 2B (1965), (Connecting lines similar for twin pair mates),

adjusted treatment means for solids-not-fat percentage was 0.30%. The butterfat yield was higher ($P < 0.05$) from the group grazing the short pasture and the solids-not-fat yields did not differ significantly.

In Experiment B the cows in both groups produced considerably less milk following the change to pure Ruamui ryegrass feeding and again the cows grazing the short pasture produced a higher yield of milk ($P < 0.01$) than those grazing long pasture. The butterfat percentage in the milk from the cows grazing the short pasture was slightly higher and the solids-not-fat and protein percentages together with the yields of all components, were considerably higher than the corresponding values from the cows grazing the long pasture.

4.33 Rumen fermentation.

The mean pH, volatile fatty acid concentration and ammonia concentrations in the rumen liquor samples from the two pairs of fistulated twins which grazed the two types of ryegrass in Experiment B are given in Table 4.4. Those differences between treatment means which reached significance are also indicated. The diurnal variations in volatile fatty acids, ammonia concentration and molar proportions of acetic, propionic and butyric acids are illustrated in Figures 4.3 and 4.4.

The samples of rumen fluid from the two cows grazing short pasture had a lower pH ($P < 0.01$) and contained higher concentrations of ammonia ($P < 0.10$) and total volatile fatty acids ($P < 0.05$) than the corresponding values obtained from their twin mates grazing long pasture. The mean molar proportions of acetic, propionic and butyric acids in the rumen liquor from individual cows were all similar.

4.4 DISCUSSION

In both experiments the cows grazing the short pasture produced more milk than those on the long pasture. The milk yield differences obtained between ryegrass varieties in previous experiments (Wilson and McDowall, 1966) might therefore have been smaller if the Ruamui ryegrass had been grazed at a shorter stage of growth than the other two varieties.

The outstanding feature of the first experiment (A) was the significantly ($P < 0.01$) lower solids-not-fat percentage in the milk produced by the cows grazing the short Ruamui ryegrass. Unfortunately, protein and/or lactose percentages in milk were not determined in this experiment so that the fraction of solids-not-fat involved in the change could not be ascertained. Changes in solids-not-fat percentage of the order obtained in this experiment (0.30%) have previously been obtained only after reductions in the feeding level of cows or by using feeds containing very low concentrations of protein (see review 1.55). Although the change from mixed pasture to Ruamui ryegrass did involve a reduction in the plane of nutrition, as indicated by the change in milk yield (Fig. 4.1), the fact that the cows grazing short ryegrass produced significantly ($P < 0.01$) more milk than those grazing the long pasture indicated that an additional factor, apart from feeding level, was responsible for the low solids-not-fat percentage obtained. This factor was presumably related to the chemical composition of the short Ruamui ryegrass. Of the characteristics measured, the contents of crude protein and ether extract were higher in the short ryegrass, and the levels of nitrogen-free extract were considerably lower than the corresponding values for the long ryegrass.

In the second experiment (B) the milk yields for treatment groups were in the same order as for the first experiment, but the depressions in the milk yields were much greater following the change to pure ryegrass feeding. In

contrast to Experiment A, the solids-not-fat percentage in the milk in this experiment was higher from the short pasture than from the long. The pastures in B were longer and more mature than those used in Experiment A. This was reflected in the lower content of crude protein in both ryegrasses and the much higher level of crude fibre in the long ryegrass in Experiment B. The long ryegrass would be expected to be of low digestibility in view of the high crude fibre content of this ryegrass, and also to lead to lower levels of intake (Baxter, Wainman, and Wilson, 1961, and section 1.2), both of which could have been responsible for the rapid depression in milk yield and the low solids-not-fat percentages obtained in the milk. The finding of a lower concentration of volatile fatty acids in the rumen liquor of the two cows grazing long ryegrass, compared with the values from their twin pair mates grazing short ryegrass, supported the suggestion that the depression in milk yield and solids-not-fat percentage in Experiment B was due to a reduced level of feeding. Several workers have shown that a positive relationship exists between level of feeding and the volatile fatty acid concentration in the rumen liquor (Davey, 1964; Williams and Christian, 1956).

The differences in ruminal ammonia concentrations between treatments would be expected because of the different nitrogen contents of the two ryegrasses, but probably intake differences between treatments may also have contributed.

The short pastures in the two experiments which resulted in different changes in solids-not-fat percentage differed in chemical composition. These differences included higher levels of crude protein and ether extract and a lower level of nitrogen-free extract in the short pasture used in Experiment A. These same differences in pasture composition also occurred

between the short and long pasture used within Experiment A so that the need for further studies of pastures high in crude protein and ether extract, and low in nitrogen-free extract was indicated. It is also relevant to note that the Ruamui ryegrass used in Experiment 1 (see section 3.3), which resulted in a low solids-not-fat content in the milk: compared with values from cows grazing the annual varieties of ryegrass, was high in crude protein and low in nitrogen-free extract relative to the other ryegrasses.

4.5 SUMMARY AND CONCLUSIONS

The effects of cows grazing short and long pastures of Ruamui ryegrass on the yield and composition of the milk were compared in two experiments. The cows grazing short pasture produced significantly ($P < 0.01$) more milk in both experiments than those grazing long pasture. The butterfat percentage in the milk from the two groups did not differ, while the solids-not-fat percentage was depressed by the short pasture in the first experiment ($P < 0.01$) and by the long pasture in the second. It was suggested that the depression in solids-not-fat caused by the short pasture, in Experiment A, was associated with the chemical composition of the pasture which was high in crude protein and ether extract and low in nitrogen-free extract but the depression caused by the long pasture was probably associated with a lowered intake of digestible energy.

CHAPTER 5

EXPERIMENT 3 : MILK PRODUCTION FROM HIGH AND
LOW NITROGEN RUANUI RYEGRASS.

5.1 INTRODUCTION

This experiment was designed specifically to examine further the production of milk containing a low solids-not-fat percentage when cows graze Ruanui ryegrass. In both the previous experiments (1 and 2A) the Ruanui ryegrass which depressed the solids-not-fat percentage contained a higher content of crude protein and a relatively low content of nitrogen-free extract compared with the other grasses used in the experiments.

In the present experiment a comparison was made of the milk productions from two groups of cows grazing Ruanui ryegrass pastures differing in nitrogen content, but at similar stages of growth. These pastures were obtained by the application of two different levels of nitrogenous fertilizer. The high nitrogen treatment was also expected to produce ryegrass with a low soluble sugar content (Bryant and Ulyatt, 1965), and probably a low content of nitrogen-free extract (see 2.5).

Because a low soluble sugar content in pastures can be associated with the production of low molar proportions of propionic acid in the rumen liquor (Tilley, Deriaz and Terry, 1960) and propionic acid infusions increase the production of milk protein (see 1.56), it was considered necessary to include fistulated animals in this experiment so that variations in the end-products of fermentation could be measured.

The experiments of Bryant and Ulyatt (1965) and Bryant (1965) suggest that not only do pastures which have received high quantities of nitrogen fertilizer have a lowered soluble sugar content they also have a high non-

TABLE 5.1 Experimental details for Experiment 3.

Pastures

Treatments	<u>High N Ruamui ryegrass</u>	<u>Low N Ruamui ryegrass</u>
Description	8-12 inches long, some seeding.	8-12 inches long, some seeding.
Date pasture closed	25 Oct.	15 Oct.
2 cwt. $(\text{NH}_4)_2\text{SO}_4$ /acre	15 Aug., 15 Sept., 18 Oct., 20 Oct.	20 Sept., 15 Oct.

Experimental dates

Pre-experimental period (mixed pasture): 25 Oct. - 10 Nov.

Experimental period (high N v low N): 10 Nov. - 24 Nov.

Nitrate administered to cows grazing low N: 24 Nov. - 30 Nov.

Animals

Number of cows used - Eight pairs of monozygous twins

Breed of cows - 6 pairs Jersey, 1 pair Friesian,
1 pair Jersey x Ayrshire.

protein nitrogen content (60 - 100% of which is nitrate) which may be responsible for the production of high acetic to propionic acid ratios in the rumen liquor. Because of the possible importance of these findings in the present experiment, the non-protein nitrogen content of the two pastures was determined. In addition the effect on milk yield and composition of administering 100 g. potassium nitrate per day to two fistulated cows grazing low nitrogen pastures was examined.

The alkaloid content (mainly perfolino) of Ruauui ryegrass is known to vary considerably (Johns, 1963) and although there are no reports of alkaloids causing harmful effects in animals, the report of Cunningham and Clare (1943) that low concentrations caused the death of paramoecia (in vitro) suggested that rumen protozoa may be similarly affected. As a possible change in rumen microflora may explain the changes in milk composition obtained, the alkaloid content of the two types of ryegrass was determined.

5.2 EXPERIMENTAL METHODS

5.21 Animals and design of experiments.

Eight pairs of monozygous twins which had calved in July or August were grazed together for a preliminary period on mixed pasture. Following this period cows within twin sets were allocated at random to the two treatment groups and then allowed to graze the high or low nitrogen pastures for a further two weeks (see Table 5.1).

After the end of the experimental period the two fistulated cows (18 and 121) which had been grazing the low nitrogen pastures, were kept on the same pasture for a further week and potassium nitrate (50 g. twice/day) was administered to the rumen of each cow during milking (6.30 a.m. and 3.30 p.m.).

The objective of management throughout the experiment was to see that all cows were offered sufficient pasture to ensure maximum intakes.

Pastures: Two paddocks of Ruamui ryegrass were used for each of the treatments. During the spring period prior to the experiment 8 cwt./acre of ammonia sulphate was applied to produce the high nitrogen pastures and 4 cwt./acre for the low nitrogen pastures. Details of the dates of application of fertilizer are given in Table 5.1. In all cases the dates shown are those applicable to the first paddock to be grazed, and the second was treated similarly but about a week later.

5.22 Sampling and analytical methods.

Herbage and milk: Sampling procedures and analytical methods were as described in sections 2.4, 2.5, and 2.6, except that the non-protein nitrogen and total alkaloid contents of pasture samples were also determined. The non-protein nitrogen content was determined on the water soluble fraction of the alcohol extract used for soluble sugar determinations, the nitrogen being determined after a Kjeldahl digestion following a pre-reduction of nitrate (Humphries, 1956). For the determination of the total alkaloid content of the ryegrasses the method of Bothurst and Reifer (1943) was used.

Rumen liquor: Samples of rumen liquor were taken from two pairs of fistulated twins on two consecutive days during the second week of the experimental period. Samples were obtained and analysed as described in 2.8.

Statistical methods: These were as described for Experiment 1 (3.24).

5.3 RESULTS

5.31 Chemical composition of the pastures.

The results from the analyses carried out on the samples obtained from

TABLE 5.2 Chemical analysis (on a dry matter basis) of the high and low nitrogen Ruamui ryegrass used in Experiment 3 (Standard error of mean).

	High N.	Low N.
No. of samples	4	4
Crude protein (N x 6.25)	20.1 (0.95)	16.4 (0.30)
Non-protein nitrogen	0.31 (0.05)	0.24 (0.03)
Total alkaloids	0.056	0.043
Crude fibre	21.1 (0.98)	22.5 (0.86)
Ether extract	4.9 (0.12)	4.5 (0.27)
Ash	8.1 (0.16)	7.7 (0.11)
Nitrogen-free extract	45.8 (0.08)	48.9 (0.11)
Soluble Sugars	12.2 (0.50)	13.9 (0.91)
Dry matter content	20.0 (0.20)	21.6 (0.21)

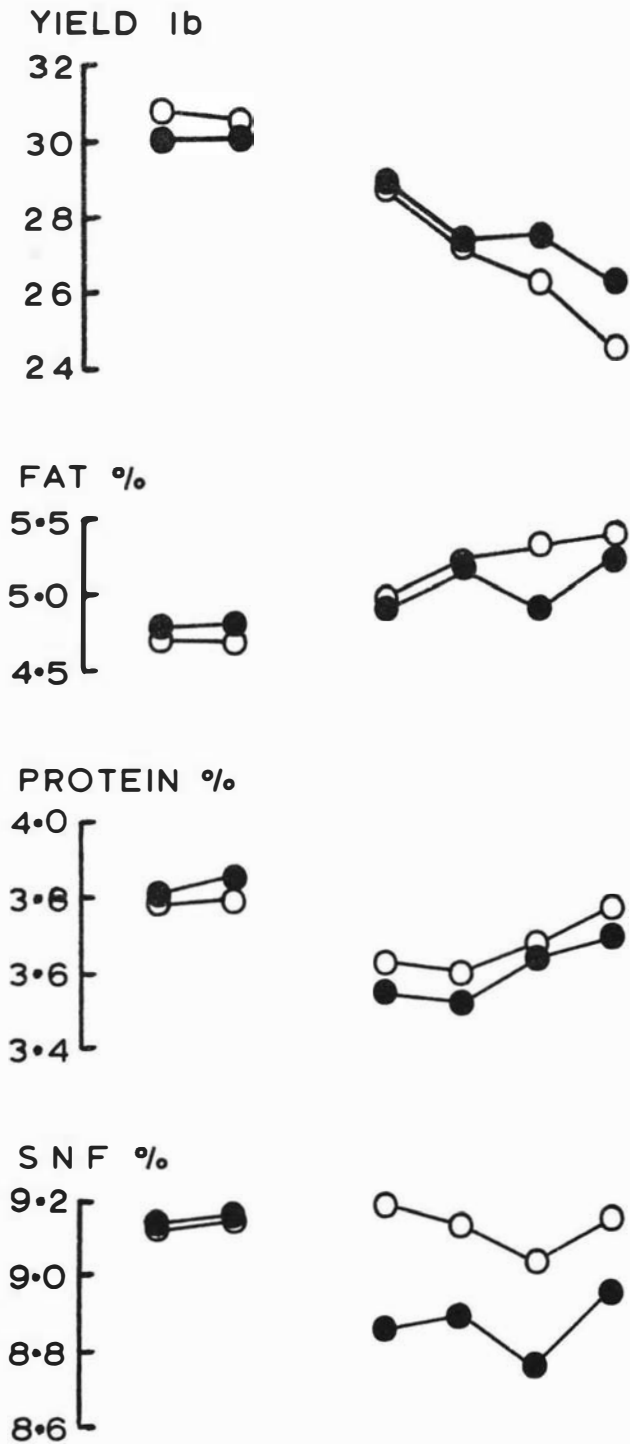


Fig.5.1 Average daily milk yields and milk compositions from two groups of twins grazing high (●) and low (○) nitrogen Ruanui ryegrass. (The first two samples, mixed pasture feeding).

TABLE 5.3 The yield and composition of milk from cows grazing Ruamui ryegrass pastures which had received two levels of nitrogenous fertilizer; adjusted mean values for experimental period (10/11 - 24/11/86).

	High N	Low N	S.E. of means	Signif. of diff.
Milk yield (lb./cow/day)	27.8	26.3	1.0	N.S.
Fat %	5.13	5.43	0.11	N.S.
Fat (lb./cow/day)	1.53	1.43	0.06	N.S.
Protein %	3.59	3.70	0.03	P < 0.01
Protein (lb./cow/day)	0.98	0.97	0.04	N.S.
Solids-not-fat %	8.87	9.13	0.05	P < 0.01
Solids-not-fat (lb./cow/day)	2.43	2.38	0.11	N.S.

TABLE 5.4 The yield and composition of milk from two cows given 100 g. KNO_3 daily (in two doses) via the rumen fistulae.

	Prelim. Period (Mixed pasture) 25/10 - 10/11	Exper. Period (Low N Ruamui rye) 10/11 - 24/11	100g. KNO_3 /day (Low N Ruamui rye) 24/11 - 30/11
Milk yield (lb./ cow/day)			
Cow 16	25.1	23.6	22.2
Cow 121	31.9	27.9	28.0
Fat %			
Cow 16	5.18	5.46	5.53
Cow 121	4.82	5.42	5.40
Protein %			
Cow 16	3.75	3.65	3.64
Cow 121	3.56	3.40	3.35

TABLE 5.5 Analyses on the rumen liquor from two pairs of twins grazing high and low nitrogen Ruwari ryegrass in Experiment 3.

Treatment	Cow	NH ₃ conc. mg. N/100 ml.	pH	Total V.F.A. mM/100 ml.	Molar proportions of V.F.A.		
					Acetic	Propionic	Butyric
High N (HN)	15	30.7	6.49	11.3	68.1	19.4	12.5
Low N (LN)	16	20.3	6.33	11.0	70.0	17.0	13.0
High N	122	31.2	6.44	11.9	69.0	18.4	12.6
Low N	121	19.8	6.34	11.3	70.9	16.8	12.3
Treatment differences signif. at							
10% level		-	-	-	HN < LN	-	-
5% level		-	-	-	-	LN < HN	-
1% level		HN > LN	HN > LN	-	-	-	-

the two pastures are given in Table 5.2. The high N pasture compared with the low N pasture was substantially higher in crude protein, and slightly higher in non-protein nitrogen, ether extract and ash contents. On the other hand the nitrogen-free extract, soluble sugar and crude fibre contents were slightly lower in the high N pastures.

5.32 Milk yield and composition.

The mean data for milk yield and composition are illustrated in Figure 5.1 and the adjusted data are given in Table 5.3. The milk yield produced by the cows grazing high N ryegrass was slightly higher than the yield produced by the low N group. The butterfat percentage in the milk produced by the high N group was slightly lower and the solids-not-fat and protein percentages significantly lower ($P < 0.01$) than the corresponding values for the milk from the cows grazing low N ryegrass. Fat, solids-not-fat and protein yields from the two groups did not differ significantly.

The yield and composition of the milk from the two fistulated cows was not affected by the administration of potassium nitrate (Table 5.4).

5.33 Rumen fermentation.

The rumen fermentation data are given in Table 5.5. The rumen fluid from the two cows grazing high N ryegrass had a higher pH and contained much higher concentrations of ammonia ($P < 0.01$) than the corresponding values obtained from the cows grazing low N ryegrass. There were only minor differences present between treatments for total volatile fatty acid concentrations and small, but significant, differences between the molar proportions of acetic and propionic acids in the rumen liquor. The cows grazing high N ryegrass had a lower proportion of acetic acid ($P < 0.10$) and a higher proportion of propionic acid ($P < 0.05$) in the rumen liquor, than those grazing low N ryegrass.

5.4 DISCUSSION

The two levels of nitrogen fertilizer produced pastures which differed in level of crude protein. Part of this difference was due to a higher content of non-protein nitrogen in the high N ryegrass. The high levels of nitrogenous fertilizers did not, on the other hand, lead to greatly reduced contents of soluble sugars and nitrogen-free extract. The differences between the chemical compositions of the high and low nitrogen ryegrasses were similar in direction (although smaller) to those between the short and long ryegrass used in Experiment 2A (see Table 4.2) and in both experiments the solids-not-fat content of the milk produced from the grass with the higher nitrogen content was reduced. Part of this difference in solids-not-fat percentage was accounted for by protein percentage but it was clear that the "lactose" component (S.N.F.% - protein %) also varied in the same direction as the protein percentage. Hence in all respects the results were also similar to those obtained in Experiment 1 (see Table 3.2) for the differences between Ruamui ryegrass and Paroa or Western Wolds ryegrasses.

A number of factors, some of which are associated with grass of high nitrogen content, may have caused the reduction in protein and solids-not-fat percentages, and these possibilities will be discussed in turn.

Plane of nutrition: In this experiment the intakes of the cows on the two treatments were probably similar as the milk yields from both groups of cows were similar. The concentrations of volatile fatty acids in the rumen liquor of the fistulated cows were also similar which supports the suggestion (but doesn't prove) that the intakes were similar. It is unlikely, therefore, that a reduced plane of nutrition was solely responsible for the changes in milk composition, but it is conceivable that a degree of under-feeding, as

occurred in both treatment groups in this experiment, is a necessary adjunct to the unknown process.

Harmful effects of non-protein nitrogen compounds: It is well known that non-protein nitrogen compounds, and in particular nitrate through the formation of nitrite, may lead to toxicity in animals (Garner, 1963). Because non-protein nitrogen levels in the pastures used in this experiment were relatively low (see Bryant and Ulyatt, 1965), and furthermore 100 g. of potassium nitrate produced no effect on milk yield or milk composition, it was considered unlikely that these compounds were involved directly in the present problem.

Alkaloid content: It is conceivable that the effect on milk composition may be brought about by an alteration in the rumen microbial population of the cows grazing high nitrogen pastures as it has been reported that plant alkaloids can destroy protozoa (Cunningham and Clare, 1943). However, as the alkaloid contents of the two grasses were not very different it would be surprising if this factor was of importance in this experiment, although clearly the possibility remains that the rumen population of micro-organisms was altered in some unknown way.

Volatile fatty acid proportions: The application of nitrogenous fertilizers in the spring has the effect of making the composition of the pasture more like that of autumn pastures, which are characteristically high in nitrogen and low in soluble carbohydrate (Waite, 1958; Thomson and Terry, 1965; Corbett, Langlands, McDonald and Pullar, 1966). Considerable attention has been paid to comparing the nutritive value of autumn and spring pastures and Corbett et al. (1966) demonstrated that autumn growth had the lower net energy

value and suggested that this appeared to be related to the relatively high ratio of acetic to propionic acids in the rumen liquor of the animals grazing this pasture. Bryant and Ulyatt (1965) also found higher ratios of acetic to propionic acids in the rumen liquor of sheep grazing high N pasture compared with low N pasture in the spring period. In the present experiment, however, presumably because the pastures did not differ by very much in soluble sugar levels, the proportions of individual volatile fatty acids in the rumen liquors from the fistulated cows were fairly similar. In actual fact the slightly lower ratio of acetic to propionic acid in the rumen liquor of the cows grazing high N ryegrass would be expected to give a higher, rather than lower, protein percentage in milk (see 1.56).

Utilization of grass protein: It is well established that dietary protein utilization and nitrogen retention are strongly influenced by metabolism in the rumen (McDonald, 1968). A high intake of soluble protein, like grass protein, results in high rumen ammonia concentrations and high urinary nitrogen losses. Chalmers and Marshall (1964) have demonstrated an inverse relationship between rumen ammonia levels and nitrogen retention, and furthermore, Head and Rock (1957) have shown that added starch is effective in controlling the urinary wastage of protein. Notwithstanding the high content of herbage nitrogen in the present experiment, the possibility exists that the low solids-not-fat percentages may have been due to a "physiological deficiency" of milk protein precursors, brought about by excessive catabolism of protein to form ammonia under conditions where insufficient fermentable carbohydrate is present as a source of energy.

The higher levels of ammonia in the rumen liquor of the fistulated cows grazing the high N ryegrass, in the present experiment, compared with the

values for their twin mates grazing low N ryegrass supports the above possibility, but the differences may have been merely due to the relative nitrogen contents of the two feeds. Because little direct evidence was obtained as to the possible importance of protein metabolism affecting milk protein percentage in this experiment, a number of further experiments were designed to examine this possibility.

In Experiment 4 a quantity of the high and low N ryegrasses not used by the cows in Experiment 3 was frozen and subsequently fed to two groups of three sheep in order to measure nitrogen balances.

The role and importance of the rumen metabolism of protein in respect to milk production was further studied in two ways. First, additional starch was given in order to balance the high nitrogen in the pastures (Experiments 5 and 6). Secondly, the effect of making additional protein available to the animal was studied (Experiments 8 and 9).

5.5 SUMMARY AND CONCLUSIONS

The effect on milk yield and composition of cows grazing Ruamui pastures which had received high and low levels of nitrogenous fertilizer was examined. The pastures which had received high, versus low, levels of nitrogen fertilizer contained higher levels of nitrogen and other extract and slightly lower contents of soluble sugars and nitrogen-free extract.

The cows grazing the high N pastures produced similar milk yields to those grazing the low N pastures but the milk contained lower percentages of solids-not-fat and protein.

These results confirmed the suggestion made following Experiments 1 and 2 that Ruamui ryegrass containing a high crude protein content (or some factor associated with this variable) can lead to low solids-not-fat contents in milk. While this change in composition was partly explained by a change in protein percentage, the "lactose content" (S.N.F. % - protein %) also varied in the same direction.

Additional non-protein nitrogen in the form of potassium nitrate (100 g./day) had no effect on milk yield or milk composition so that this characteristic of high N pastures was probably of little importance. The total alkaloid contents of the two pastures were also similar.

Differences in the molar proportions of individual volatile fatty acids in the rumen liquor from the cows on the two treatments did not account for the changes in milk composition obtained.

CHAPTER 6

EXPERIMENT 4 : NITROGEN BALANCE USING HIGH AND LOW NITROGEN
RUAMUI RYEGRASS FED TO SHEEP.

6.1 INTRODUCTION

In the previous chapter it was suggested that the cows grazing high N Ruamui ryegrass may have been unable to obtain a sufficient quantity of protein (or an essential amino acid) to maintain milk protein synthesis because of excessive catabolism of protein to ammonia in the rumen and subsequent loss of nitrogen in the urine. In order to examine this possibility further, sufficient of the high and low N pastures used in Experiment 3 were frozen so that nitrogen balances could be obtained using sheep. Nitrogen digestibilities and retentions from the two pastures were measured using two groups of three sheep fed indoors.

6.2 EXPERIMENTAL

6.21 Herbage

Sections of the high and low N pastures being grazed by the cows during Experiment 3 were cut with a mower on 20 November 1963. Sufficient green material (approximately 600 lb.) was obtained from each pasture to feed three sheep for three weeks. This was the maximum quantity of grass which could be stored. The grass was packed loosely into hessian bags and placed as quickly as possible on racks within a freezing chamber at -10°C . The grass was stored in the frozen state until 24 hours prior to feeding when it was transferred to a refrigerated room at 4°C .

6.22 Animals and design of experiment.

Six Romney wethers, aged about 10 months, were bought from the University Sheep Farm at the beginning of May 1967 and following treatment for internal parasites (Thiobendazole) they were put in metabolism crates and housed indoors. The metabolism crates, which were provided with a feed bin and water bucket, confined the movement of the sheep and enabled urine to be collected in a bucket beneath the sheep.

The experiment was divided into three periods:-

- (a) Training period 1/5/66 - 30/5 (mixed pasture feeding)
- (b) Preliminary period 30/5 - 10/6 (high and low N Ruamui ryegrass)
- (c) Experimental period 10/6 - 21/6 (Ditto)

(a) Training period: The object of the training period was to accustom the sheep to indoor feeding, the harnesses and the faecal collection bags, and also to establish voluntary intakes so that the sheep could be paired on this basis. The sheep were fed freshly cut mixed pasture to appetite twice daily at 9 a.m. and 5 p.m. and water was available ad libitum.

(b) Preliminary period: The sheep were "paired" on the basis of their voluntary intakes and the sheep within pairs were allocated at random to the two treatment groups. The three sheep within each group were then fed the high and low N Ruamui ryegrasses ad libitum during this period. The feeding times were as for the first period.

(c) Experimental period: During this period of eleven days faecal and urine output data were collected in addition to the intake data. Individual

animals were fed a quantity of the ryegrass equivalent to 90% of the daily voluntary intake established during the preliminary period. By feeding slightly below the ad libitum intakes, no feed refusals were obtained and hence "end point errors" would be expected to be minimised.

6.23 Sampling and analysis.

Herbage: Representative samples of feed (about 300 g.) were taken at each feeding time for the determination of dry matter intakes. The samples were dried at 100°C for 24 hours and the dried samples were bulked for chemical analysis. The bulked samples which had been stored in air tight plastic bags were subsequently ground (1mm. sieve) and analysed for nitrogen content (A.O.A.C., 1965).

Faecal samples: The faeces from individual sheep were removed twice daily and bulked to form two 4-day composite samples and one 3-day composite sample covering the 11 day experimental period. The faeces were frozen (-10°C) and stored in this form prior to the determination of dry matter outputs. The total faecal outputs of individual sheep for the composite periods were dried in an oven at 100°C for 48 hours and sub-samples ground prior to analysis for nitrogen content. A drying temperature of 100°C was chosen because Raymond and Harris (1964) concluded that 100 - 105°C was the optimum temperature to minimize losses of nitrogen with heating.

Urine samples: Urine was collected in H₂SO₄ (18 ml. 18N-H₂SO₄ / litre) in order to keep pH below 2 and prevent loss of nitrogen as ammonia (Martin, 1966). The urine from individual sheep was bulked as for the faeces and these samples were stored in a covered container at 4°C. At the end of each collection period

TABLE 6.1 Nitrogen balances and digestibilities for individual sheep fed high or low nitrogen Ruamui ryegrass.

Sheep	High N			Low N		
	1	2	3	1	2	3
Nitrogen intake (g./day)	22.69	17.91	16.22	13.27	9.50	11.42
Faecal N (g./day)	5.95	4.65	4.32	4.72	3.28	4.58
Urinary N (g./day)	15.18	12.32	11.30	7.30	5.37	5.89
Nitrogen balance (g./day)	+ 1.56	+ 0.94	+ 0.60	+ 1.25	+ 0.85	+ 0.95
Appar. N digestibility coef.(%)	73.8	74.0	73.4	64.4	65.5	59.9
Appar. D.M. digestibility coef.(%)	68.5	69.6	69.5	64.1	67.7	63.3
N retained /100g. N intake	6.88	5.24	3.70	9.42	8.95	8.32

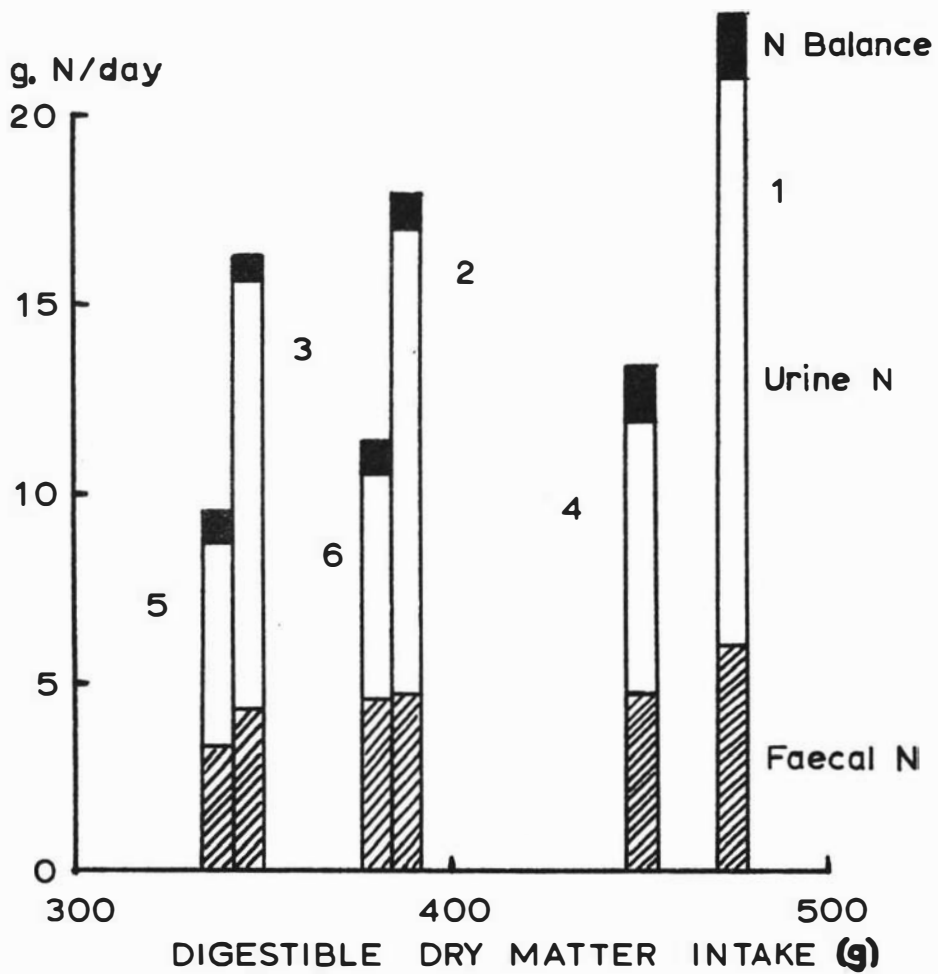


Fig.6.1 Fate of ingested nitrogen for groups of three sheep fed high (1, 2, 3) and low nitrogen (4, 5, 6) Ruanui ryegrass arranged according to their digestible dry matter intake.

the nitrogen contents of 5 ml. samples of urine were determined (in duplicate) using standard methods. Total nitrogen voided was then calculated from the total volume of urine collected.

6.3 RESULTS

The full results are given in Appendix G.1 and are summarized in Table G.1. The fate of ingested nitrogen for individual sheep is also illustrated in Figure G.1.

A greater proportion of the ingested nitrogen in the high N grass was apparently digested (73.7%, compared with 63.3% for the low N grass) but because a greater proportion of this nitrogen was excreted in the urine (92.9%, compared with 85.9% for the low N grass) the nitrogen retentions for the two types of grass were very similar. The nitrogen in the low N grass was clearly used more efficiently with an average of 8.9 g. of nitrogen being retained/100 g. of ingested N, compared with an average value of 5.3 g./100g. of ingested N for the high N grass.

6.4 DISCUSSION

Because the nitrogen retentions from the two types of ryegrass were similar, this experiment failed to support the hypothesis made in the previous chapter (3), and in the introduction to this experiment, that insufficient precursors for protein synthesis may be obtained from the high N pasture due to excessive catabolism of dietary protein.

The results did, however, show that the dietary protein of the high nitrogen pasture was used inefficiently and suggested that a higher proportion of carbohydrates in the ryegrass would lead to the fuller use of

the nitrogen present. This effect has in fact already been demonstrated by Head and Murdoch (1965) when steers were shown to retain more nitrogen with starch supplementation, in spite of a lowered crude protein digestibility. The similarity of the nitrogen retentions in the present experiment showed that the nitrogen content of both pastures was not the limiting factor to higher retentions, and the increased nitrogen retentions with increasing intake suggested that energy intake was the major limiting factor. The results are in agreement with those of Hogan and Weston (1967) who fed sheep high (13.8g.N/day) and low protein (5.5g.N/day) diets and found that the output of protein from the rumen (and hence available for absorption and utilization) was much the same in the two diets (8.8 and 8.1 g.N/day respectively).

6.5 SUMMARY AND CONCLUSIONS

The high and low nitrogen Ruamui pastures used in Experiment 3 were fed to two groups of three growing sheep in order to measure nitrogen balances and digestibilities.

Nitrogen retentions were similar from the two rations and increased with dry matter intake.

Although the nitrogen in the high nitrogen grass was used less efficiently than that in the low nitrogen grass, the experiment gave no direct evidence to support the hypothesis that the low protein percentage obtained in the milk, when cows grazed high nitrogen pastures, was due to a deficiency of nitrogen precursors for milk protein synthesis.

CHAPTER 7

EXPERIMENT 5 : EFFECT OF A STARCH SUPPLEMENT ON
MILK PRODUCTION IN THE AUTUMN.

7.1 INTRODUCTION

It was considered that a useful alternative approach to examining the possibility that the efficiency of utilization of the protein in high nitrogen ryegrass may affect milk protein percentage was to study the effect of supplementary carbohydrate on milk yield and composition. This was suggested by previous experiments in which grazing cows have been given supplementary concentrates (see 1.52). In addition readily available carbohydrate has been shown to lower rumen ammonia concentration (Head and Rook, 1957; Robertson and Hawke, 1964c) and that steers in the experiment of Head and Murdoch (1965) retained more nitrogen following starch supplementation.

The effect of a starch supplement (2 lb./day) on the yield and composition of milk produced by cows grazing high nitrogen Ruamui ryegrass was examined in this experiment. The experiment was carried out in the autumn as it is well established that autumn pastures are characteristically high in crude protein and low in soluble carbohydrates (Wilson and Dolby, 1966). It was considered that this type of pasture should provide conditions whereby the added starch was given the best opportunity of improving dietary protein utilization and possibly milk protein content.

Lactating cows with rumen fistulae were included in the experiment so that possible changes in the end-products of rumen fermentation, and in particular rumen ammonia concentration, could be measured and these correlated with any changes obtained in milk yield and composition.

Following the observation of "scouring" in the control cows, faecal dry matter determinations were made, and blood samples were obtained in order that magnesium concentrations could be determined as it was considered possible that mineral uptake may be affected by the changes in the physiological conditions in the gut.

7.2 EXPERIMENTAL METHODS

7.21 Animals and design of experiment.

Eight pairs of monozygous twins, including two pairs with rumen fistulae, which had calved in the spring were used in the experiments. Six pairs of twins were Jersey or Jersey cross animals and the remaining two pairs were Friesians. The preliminary period with all cows grazing mixed pastures commenced 28 March, 1967, and the Ruanui ryegrass feeding period of 14 days on 11 April. During this period one member of each twin set received 1 lb. of wheaten starch in water (800 ml.) at each milking.

7.22 Pastures.

Five paddocks of Ruanui ryegrass (see Table 2.1) which had been maintained with sulphate of ammonia were used in this experiment. Sulphate of ammonia was applied at the rate of 1 cwt. per acre about 3 weeks prior to the start of the experimental period. The cows were given sufficient pasture to ensure full feeding, a new break of grass being provided after each milking.

7.23 Sampling and analytical methods.

Herbage: Sampling and analytical methods were as described in 2.4 and 2.5.

Milk: Milk yields were measured at all milkings, and two-day composite samples were taken from individual cows twice each week and analysed for fat, solids-not-fat, and protein contents as described in 2.6.

Rumen Liquor: Samples of rumen liquor were taken from the two fistulated cows on each of the treatments on 19 and 20 April and treated as described in 2.8.

Faecal dry matter percentage: Grab samples of faeces were obtained from individual cows on one occasion during the second week of the experimental period. The samples were dried in the oven at 100°C for 48 hours in order to determine their dry matter content.

Plasma magnesium concentration: Jugular blood samples (10 ml.) were obtained from individual cows on one occasion at the end of the experimental period and plasma magnesium concentrations were determined by a technician from the Plant Chemistry Division of D.S.I.R. using atomic absorption spectrophotometry.

Statistical analyses: The milk yield and composition data, and the rumen liquor data, were analysed by analyses of variance (e.g. Appendix 3.2).

7.3 RESULTS

7.31 Pastures.

The Ruamui ryegrass pastures were 3 to 4 inches long at the time of grazing. The chemical composition of the pasture used, on a dry matter basis,

TABLE 7.1 Chemical analysis (on a dry matter basis) and dry matter content of Ruanui ryegrass used in Experiment 5.

	Mean	S.E. of means
No. of samples	5	
Cx protein (N x 6.25)	28.1	± 0.65
Cx fibre	20.8	± 0.70
Ether extract	5.9	± 0.28
Ash	10.1	± 0.30
Nitrogen-free extract	36.1	± 1.98
Total soluble sugars	6.9	± 0.43
Dry matter content	18.9	± 1.94

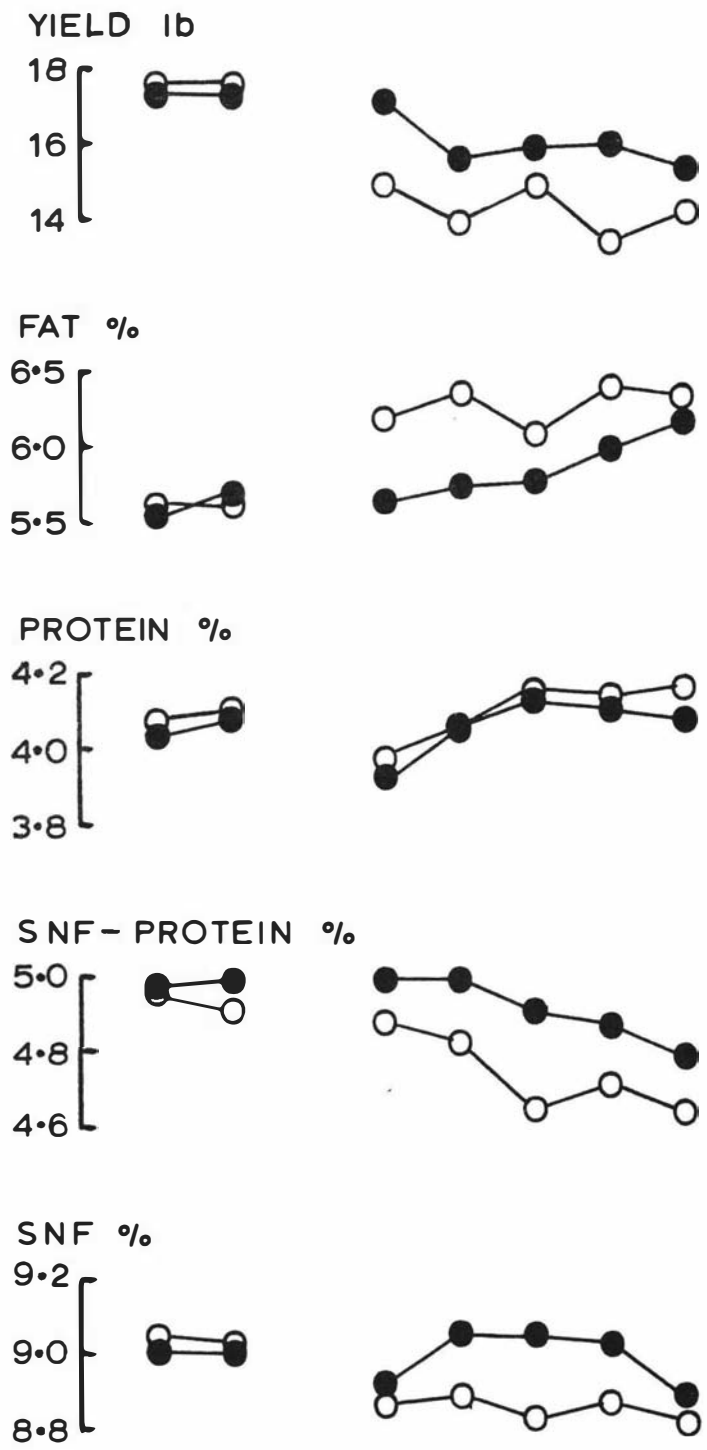


Fig.7.1 Average daily milk yields and milk compositions from groups of twins grazing autumn pastures supplemented with 2 lb. of starch/head/day. (Starch, ●; Control, ○). (The first two samples, mixed pasture feeding).

TABLE 7.2 The yield and composition of milk from cows given a starch supplement (3 lb./day) while grazing Ruani ryegrass during the autumn : adjusted mean values for experimental period.

	Control (C)	Starch (S)	S.E. of means	Significant differences P<0.05
Milk (lb./cow/day)	14.2	15.5	0.52	N.S.
Fat %	6.28	5.83	0.10	C>S
Fat (lb./cow/day)	0.86	0.87	0.02	N.S.
Protein %	4.04	4.07	0.05	N.S.
Protein (lb./cow/day)	0.60	0.61	0.02	N.S.
Solids-not-fat %	8.86	8.98	0.03	S>C
Solids-not-fat (lb./cow/day)	1.25	1.36	0.05	N.S.
S.N.F. % - protein % (lactose)	4.81	4.92	0.05	N.S.

TABLE 7.3 Differences between twin pair mates for milk protein and "calculated lactose"* percentages.
: Starch cow - Control cow.

Twin Cows	Protein %	Lactose %
15, 16	+ 0.17	-
43, 44	+ 0.11	+ 0.03
115, 116	+ 0.10	+ 0.11
111, 112	+ 0.02	+ 0.03
25, 26	0	+ 0.11
121, 122	- 0.02	- 0.04
81, 82	- 0.04	+ 0.01
131, 132	- 0.22	+ 0.18

* Lactose percentage = Solids-not-fat % -
protein %.

TABLE 7.4 Analyses on the rumen liquor of two pairs of twins with one member of each pair fed 1 lb. of starch.

Treatment	Cow	NH ₃ Conc. mg./100 ml.	pH	Total V.F.A. ml/100 ml.	Molar proportions of V.F.A.		
					Acetic	Propionic	Butyric
Starch	16	44.7	6.55	11.78	67.6	18.9	15.5
Control	15	46.8	6.53	11.56	69.8	15.2	15.0
Starch	122	60.3	6.21	14.21	68.5	18.2	15.3
Control	121	53.8	6.45	12.42	68.6	18.6	14.8
Treat. diffs. signif. at 5% level		-	-	-	-	-	-

is given in Table 7.1. The important features of the composition included the high content of crude protein (28.1%) and the low content of total soluble sugars (6.9%).

7.32 Milk yield and composition.

The original mean data for milk yield and composition are presented in Figure 7.1 and the mean values for treatment groups, after adjustment for preliminary differences, are given in Table 7.2.

Milk yield was higher for the group of cows which received 2 lb. of starch per day, but the difference was not significant. Fat percentage was depressed ($P < 0.05$) and solids-not-fat percentage raised ($P < 0.05$) by the starch supplement. The protein percentage in the milk was similar from the two groups and hence the calculated lactose percentage ($S.N.F.\% - \text{protein } \%$) was higher for the starch supplemented group. The differences between the values for individual pair mates in protein and lactose (calculated) percentages are given in Table 7.3. It is clear from this table that there was a considerable variation in the response in protein percentage to additional starch, and one pair of cows (131 and 132) in which the "control" cow had a much higher protein percentage had a considerable effect on the treatment means. This pair also differed most in lactose percentage (Starch > Control). The mean yields of all milk components were very similar for the two groups.

7.33 Characteristics of rumen liquor.

The mean pH, ammonia concentrations, volatile fatty acid concentrations and molar proportions of individual fatty acids in the samples of rumen liquor from the two cows on each of the treatments, are given in Table 7.4.

TABLE 7.5 The effect of a starch supplement given to cows, on the dry matter content of faecal samples, and on plasma magnesium concentrations (mg./100 ml.).

	Faecal D.M. %	Plasma Mg.
Control	8.9	1.61
Starch	12.8	1.82
S.E. of means	1.03	0.13
Difference signif. at 5% level	S > C	N.S.

The two cows which received the starch supplement had higher concentrations of volatile fatty acids in their rumen liquor than the control cows, but the difference was not significant. There were only minor differences in rumen liquor pH, and molar proportions of individual fatty acids, between twin pair mates. The concentration of ammonia in the rumen liquor of cow 122 which received starch, was considerably higher than that from 121 (Control) but the treatment effect was reversed for the other twin pair. In this case there was a significant ($P < 0.01$) difference between cows within treatment groups.

7.34 Faecal dry matter content and plasma magnesium concentration.

The mean dry matter contents of the faecal samples, and the plasma magnesium concentrations for the two groups of cows towards the end of the experimental period are given in Table 7.5. The dry matter percentage of the faeces from the cows receiving starch was higher ($P < 0.05$) than the corresponding value from the control group. Six of the eight cows which received starch had a higher plasma magnesium concentration than their twin pair mates in the control group, but the difference (0.21 mg./100 ml.) between the means was not statistically significant.

7.4 DISCUSSION

The mean soluble sugar content of the pastures was considerably lower than that in pastures used in previous experiments carried out during the spring periods but is comparable with previous data on autumn grown herbage (Wilson *et al.*, 1966). The supplementary starch probably increased the intake of readily available carbohydrate by at least 100%, and in doing

so made the autumn pasture ~~more~~ comparable in this regard with spring pasture. In spite of this relatively large increase in readily available carbohydrate there was no difference between the mean protein percentages of the milk from the two groups of cows. This "negative result" does not, however, disprove the hypothesis tested that differences in herbage protein utilization may effect milk protein, because there was a large variation in treatment effects between twin pairs.

Because intakes could not be measured it is not known whether the intake of grass was reduced (substitution effect), unaltered (additive effect), or even increased (interaction) by the addition of starch to the ration. It may be inferred indirectly, however, that the total energy intake of the cows receiving starch was increased above the control group, as milk yield was higher and fat percentage lower for this group of cows. The alternative possibility that the difference in milk fat percentage was due to a change in the ratio of acetic to propionic acids in the rumen liquor from the two groups, was also ruled out (Table 7.4).

The significant ($P < 0.05$) difference between the mean solids-not-fat percentages of the milk produced by the two groups of cows (Starch > Control) could not be accounted for by similar changes in protein percentage so that the lactose percentage in the milk probably differed. Lactose percentage is known to decline rapidly and protein percentage to increase towards the end of a lactation (Waite, White and Robertson, 1956), so that it may be suggested that drying off changes probably contributed to all the results and in particular accounted for the low lactose and high protein percentage of the milk produced by cow 131 (see Table 7.3). However, the possibility does remain that starch may ~~increase~~ lactose percentage during some periods

of the year. In this regard it should be remembered that lactose percentage, as well as protein percentage, was reduced by Ruamui ryegrass pastures in Experiments 1, 2 and 3.

The failure of the additional starch to induce a depression in rumen ammonia concentration was not consistent with many previous reports (Robertson and Hawke, 1964c; Christian and Williams, 1966). Because many other factors including intake of pasture and rate of passage may have altered (see also section 2.8) following supplementation with starch the discrepancy is probably of little consequence.

Observation of the cows suggested, and subsequent measurement of the dry matter content of faecal samples confirmed, that the added starch had a significant effect on the physical characteristics of the herbage residues as the fluid content of the faeces was lower from this group than the control group. Although there is some evidence to the contrary (Rook and Balch, 1959) this physiological difference suggested that mineral uptake from the food may have been altered. The plasma magnesium concentrations given in Table 7.4, and results from a subsequent experiment (Wilson, Reid, Molloy, Metson, and Butler, 1969), did in fact confirm that starch supplementation may have a beneficial effect in raising blood magnesium concentration. This finding may prove to be of considerable practical significance in the control of grass tetany in dairy cattle.

7.5 SUMMARY AND CONCLUSIONS

The effect on milk yield and composition of a starch supplement (2 lb./day) given to cows grazing Ruamui ryegrass during the autumn period was investigated using eight pairs of identical twins.

The group fed starch produced a little more milk, with a lower fat percentage ($P < 0.05$) and a higher solids-not-fat percentage ($P < 0.05$) than that produced by the control group. Protein percentage, and the yields of fat, protein and solids-not-fat did not differ significantly between treatment groups. While the differences in milk composition within twin sets varied considerably between sets, the results were generally consistent with the starch having given an improvement in level of feeding which retarded the typical changes in milk composition that occur when cows are "dried off". This would explain the higher "lactose" (calculated) and solids-not-fat percentages in the milk from the group fed starch.

The experiment provided no evidence to support the hypothesis that starch improved the utilization of dietary protein and hence milk protein synthesis.

CHAPTER 8

EXPERIMENT 6 : EFFECT OF STARCH AND PEANUT OIL SUPPLEMENTS
ON MILK PRODUCTION.

8.1 INTRODUCTION

In view of the inconclusive nature of the results obtained in the previous experiment it was decided to investigate the effect of additional starch given to cows grazing high nitrogen Ruamui ryegrass during the spring. During this period the twin cows were at a more favourable stage of lactation than in Experiment 5 and it was during November that the high nitrogen Ruamui ryegrass pastures had previously resulted in low solids-not-fat contents in milk (chapters 3, 4 and 5).

Supplementary starch might be expected to increase the overall level of feeding of the cows relative to the non-supplemented control group. As total energy intake is known to be the most important factor which can influence the solids-not-fat content of milk (see 1.55) a third group of cows was given a non-carbohydrate supplement of similar calorific value to the added starch. Peanut oil was chosen in order that the experiment could also be used to investigate certain factors which may influence the occurrence of grass tetany in cattle. The results of starch and peanut oil supplements on magnesium, calcium, and phosphorus levels in the plasma of the cows used in this experiment, have recently been published (Wilson, Reid, Molloy, Henson and Butler, 1969).

The experiment was first carried out during early spring (September: Experiment A) when the results concerning plasma mineral levels were of

greatest importance (see Wilson et al., 1909), and then repeated in November (Experiment B). Six fistulated cows were also included in the November experiment so that the ammonia and volatile fatty acid concentrations in rumen liquor which resulted from the three rations could be measured.

8.2 EXPERIMENTAL METHODS

8.21 Animals and design of experiments.

Twelve pairs of monozygous twins which had calved in July or August were used in each experiment. During the preliminary periods of both experiments (28 Aug. - 4 Sept.; 6 Nov. - 14 Nov.) all cows grazed mixed pasture. The cows were then divided into three treatment groups (incomplete balanced block design) for the experimental periods (4 Sept. - 19 Sept.; 14 Nov. - 25 Nov.).

In the first experiment (A) five pairs of twins were two year olds, one pair was three years old and the remainder mature cows (5 years and older). For the second experiment (B) some of the cows used in the first had to be replaced. In this experiment three pairs of twins were two year olds and the remainder were mature cows. All cows used were Jerseys except for one pair of three year old Friesians in A and one pair of mature Friesians in B.

Treatments: One group of cows received no supplement (control group), a second group of eight cows peanut oil (205g./cow) following each milking, and the remaining cows wheaten starch (450g./cow suspended in water) following each milking. During both experimental periods the cows grazed "high nitrogen" Ruanaui ryegrass.

Pastures: The pasture used during the preliminary periods consisted of mixed ryegrass and white clover pastures with small quantities of cocksfoot and prairie grass. The five paddocks containing pure swards of Ruanui ryegrass (see Table 2.1) which were used during the experimental periods had been maintained with annual dressings of 3 cwt. superphosphate and 6-10 cwt. sulphate of ammonia per acre. On 20 July these pastures were topdressed with 2 cwt. muriate of potash and 3 cwt. of sulphate of ammonia per acre. In the period between the two experiments a total of 4 cwt. of sulphate of ammonia was applied.

The cows were given a fresh break of grass every day, the size of the break being adjusted to ensure that voluntary intake was not restricted. The Ruanui ryegrass pastures were grazed at a height of 6 - 8 inches.

8.22 Sampling and analytical methods.

Herbage: Representative pasture samples were taken from a number of paddocks of mixed pasture during the preliminary period of each experiment and similar samples obtained from each of the five Ruanui ryegrass pastures used. Freeze-dried samples were handled and analysed for crude protein, crude fibre, other extract and soluble sugar contents, as described in 2.4 and 2.5.

Milk: Milk yields were measured at all milkings and two day composite samples were taken from individual cows twice each week for milk analysis. Butterfat, total solids, and protein contents were determined as described in 2.6 and the lactose figures used were those obtained by use of I.R.M.A.

TABLE 8.1 Chemical composition of herbage dry matter during preliminary and experimental periods of Experiment 6.

	Experiment A (Sept.)			Experiment B (Nov.)		
	Mixed pasture	Ruamui ryegrass	S.E. of means	Mixed pasture	Ruamui ryegrass	S.E. of means
Crude protein %	28.5	24.8	0.81	22.6	25.4	0.75
Crude fibre %	16.6	16.2	0.97	19.4	19.7	0.60
Ether extract %	6.5	6.9	0.33	4.7	5.6	0.39
Ash %	8.5	8.8	0.53	8.1	9.6	0.45
Nitrogen-free extract %	39.1	43.3	2.30	45.2	39.7	2.38
Soluble sugar %	14.5	16.0	0.68	11.9	9.2	0.54
Number of samples	1	5		1	5	

Rumen Liquor: Samples of rumen liquor were taken from the six fistulated cows (15, 16, 121, 122, M45, M48), which had been randomly allocated to the three treatments, on two consecutive days during the second week of Experiment B. Samples were obtained and analysed as described in 2.8.

Statistical methods: These methods were as described for Experiment 1 (3.24) except that the relationship between volatile fatty acid and ammonia concentrations was examined for individual rumen liquor samples within treatment groups. The analysis used was the extension to the regression analysis as outlined by Snedecor (1955) in which the errors of estimate of the average within treatment group regression are divided into variation associated with the regression coefficients and to the deviations from individual treatment group regressions. In this way the test was made of the significance of the differences between the individual treatment group regression coefficients (Appendix 8.1).

8.3 RESULTS

8.31 Chemical composition of the pastures.

The results of the chemical analysis of the pastures are given in Table 8.1. In each of the two experiments the composition of the Ruamui ryegrass and the mixed pasture was similar in most respects, but the Ruamui ryegrass in Experiment B was higher in crude fibre and lower in ether extract, nitrogen-free extract, and soluble sugar contents than the corresponding values in Experiment A.

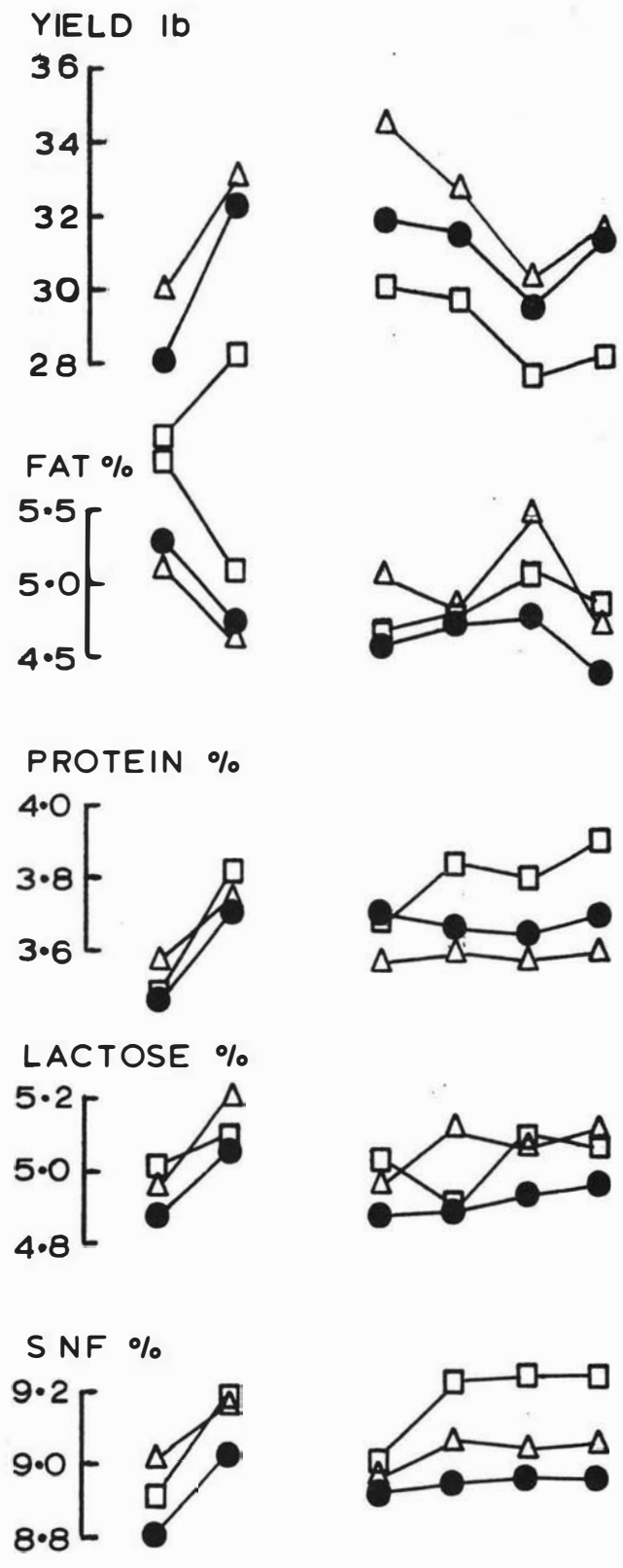


Fig.8.1 Average daily milk yields and milk compositions from groups of twins grazing Ruanui ryegrass in September, supplemented with starch (□) and peanut oil (△). (Control group, ●). The first two samples, mixed pasture feeding.

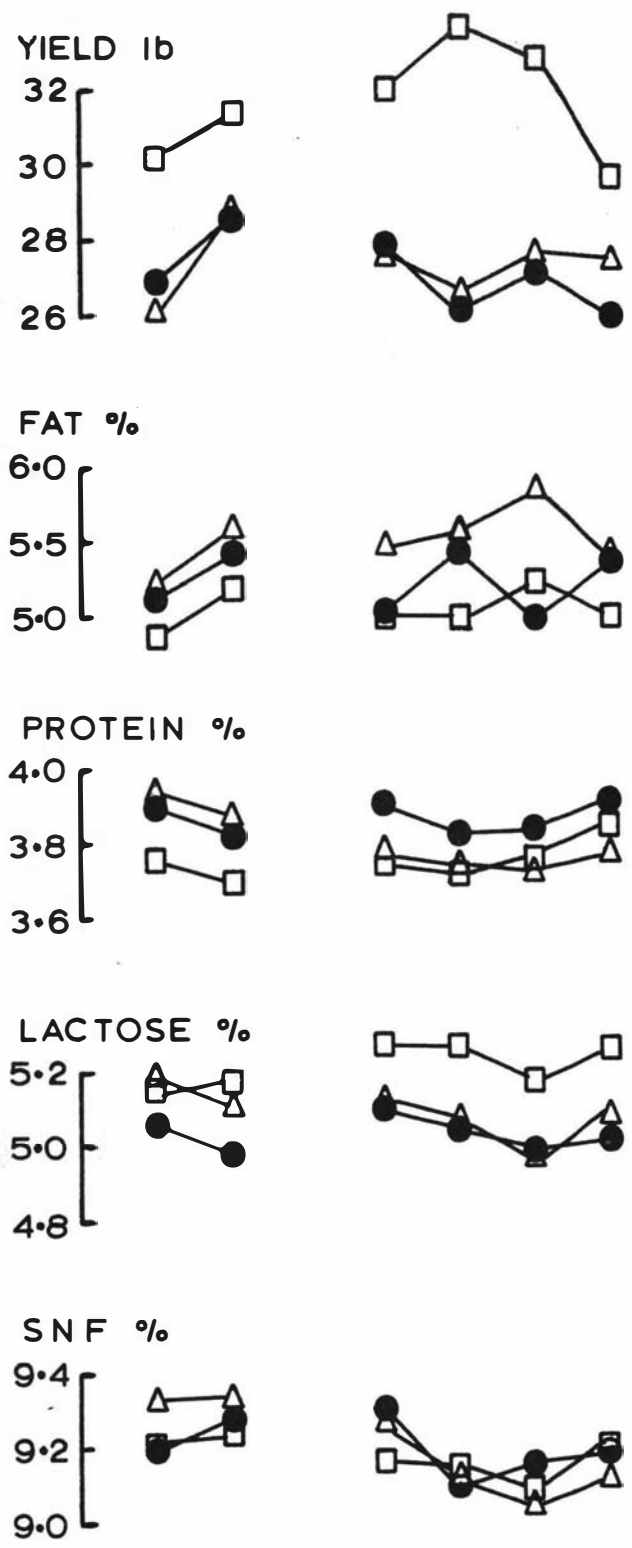


Fig.8.2 Average daily milk yields and milk compositions from groups of twins grazing Ruanui ryegrass in November, supplemented with starch (□) and peanut oil (△). (Control group, ●). The first two samples, mixed pasture feeding.

TABLE 8.2 The yield and composition of milk from cows given starch and oil supplements: adjusted treatment means.

	Starch (S)	Control (C)	Oil (O)	S.E. of means	Signif. of diffs.	
					P < 0.01	P < 0.05
<u>Experiment A.</u>						
Milk yield (lb./cow/day)	31.4	30.8	30.3	0.61	-	-
Fat %	4.65	4.72	5.17	0.12	O > C	O > S
Fat yield (lb./cow/day)	1.42	1.42	1.56	0.04	O > C	O > S
Solids-not-fat %	9.16	9.06	9.00	0.04	S > O	S > C
Protein %	3.81	3.73	3.57	0.03	S > O C > O	S > C
Protein yield (lb./cow/day)	1.19	1.14	1.08	0.03	S > O	S > C C > O
Lactose %	5.07	5.00	5.01	0.04	-	-
<u>Experiment B.</u>						
Milk yield (lb./cow/day)	29.1	27.7	28.5	0.70	-	-
Fat %	5.27	5.21	5.54	0.11	-	O > C
Fat yield (lb./cow/day)	1.53	1.41	1.55	0.06	O > C	S > C
Solids-not-fat %	9.20	9.22	9.10	0.06	C > O	S > O
Protein %	3.84	3.81	3.68	0.02	S > O	C > O
Protein yield (lb./cow/day)	1.11	1.05	1.03	0.03	-	-
Lactose %	5.16	5.13	5.07	0.03	S > O	C > O
Protein % (last 2 days)	3.92	3.85	3.68	0.03	S > O	C > O

8.32 Milk yield and composition.

The adjusted mean data for milk yield and composition are given in Table 8.2 and the original data are plotted in Figures 8.1 and 8.2 for the A and B experiments respectively.

In both experiments the mean milk yield and composition data for the control cows (eating Ruanui pastures) in the experimental period, were very similar to those produced during the preliminary period when the cows were grazing on mixed pasture (Figures 8.1 and 8.2).

In Experiment A there were no significant differences between treatments for milk yield, although the yield from the cows receiving starch was slightly higher than the yield from the other two groups. Peanut oil increased the butterfat percentage ($P < 0.01$) in the milk and also the butterfat yield relative to the other two groups. Starch increased the solids-not-fat percentage ($P < 0.05$) whereas peanut oil depressed it slightly. The protein yields and percentages in the milks were in the order Starch > Control > Peanut Oil, and all these differences were significant ($P < 0.05$), but lactose percentage was similar for all groups.

In Experiment B the milk yields were similar and peanut oil increased the yield and percentage of butterfat in the milk as in Experiment A. In this second experiment peanut oil clearly lowered solids-not-fat ($P < 0.01$), protein ($P < 0.01$) and lactose ($P < 0.05$) percentages whereas starch had no effect on the solids-not-fat percentage but raised the protein percentage of the milk, particularly towards the end of the experimental period ($P < 0.01$).

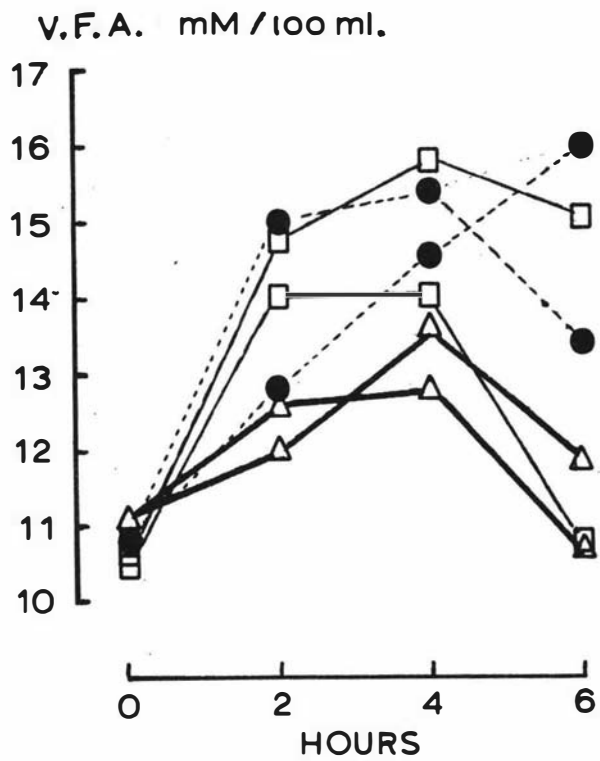


Fig.8.3 Diurnal variations in the volatile fatty acid concentration of rumen liquor from cows grazing Ruanui ryegrass supplemented with starch (□—□), and peanut oil (△—△). (Control cows, ●---●).

TABLE 8.3 Analyses on the rumen liquor from fistulated control cows, and starch and oil fed cows (Nov. expt.).

Treatment	Cow	NH ₃ conc. mg. N/100 ml.	pH	Total V.F.A. mM/100 ml.	Molar proportions (%) and concentrations () of V.F.A. (mM/100 ml.)		
					Acetic	Propionic	Butyric
Starch	15	32.0	6.13	13.0	68.3 (8.9)	18.1 (2.4)	13.6 (1.7)
Control	16	37.0	6.12	13.6	68.7 (9.4)	17.2 (2.4)	14.1 (1.9)
Peanut Oil	45	35.8	6.20	12.1	68.2 (8.3)	17.6 (2.1)	14.2 (1.7)
Control	48	34.9	6.10	13.5	69.1 (9.4)	17.2 (2.3)	13.7 (1.8)
Peanut Oil	121	36.4	6.24	11.8	69.3 (8.2)	17.4 (2.1)	13.3 (1.6)
Starch	122	35.8	6.24	13.5	67.7 (9.1)	17.5 (2.4)	14.8 (2.0)
Treatment diff. signif. at 5% level		-	-	S > O C > O	- (S > O)	- (C > O)	- (-)
1% level		-	-	-	- (C > O)	- (S > O)	- (-)

8.33 Rumen fermentation characteristics.

The mean pH, ammonia concentrations, volatile fatty acid concentrations, molar proportions and concentrations of individual fatty acids in the rumen liquors from the two cows on each of the experimental treatments are given in Table 8.3. The cows that were treated with oil had a lower concentration of total volatile fatty acids in their rumen liquor than either of the other pairs of cows ($P < 0.01$), the effect being present at all sampling times except prior to feeding (See Fig. 8.3). The molar proportions of the individual fatty acids were very similar for all cows, but because of the differences in total concentration, the concentration of acetic ($P < 0.01$), propionic ($P < 0.01$), and butyric acids (not significant) were lower for the cows receiving oil.

The average ammonia concentration in the rumen liquor of the cows receiving starch was slightly lower than the average for the other two treatments but the difference was not significant. There was a significant correlation (0.52, $P < 0.01$) between the volatile fatty acid and ammonia concentrations within individual rumen samples, and the within treatment group relationships between rumen liquor volatile fatty acid and ammonia concentrations did not differ significantly from the average within group regression (Appendix 8.1).

8.4 DISCUSSION

The effects of three separate factors on milk yield and composition warrant discussion: the influence of the change to Sunnui ryegrass feeding on the milk production of the control group of cows; the effect of supplementary starch; and the effect of peanut oil supplements on milk production.

Pasture effects: In contrast to previous experiments (1, 2 and 3) in which Ruamui ryegrass containing high levels of nitrogen caused low solids-not-fat contents in milk, this effect was not apparent in the milk of the control group of cows in either of the present experiments (Figs. 8.1 and 8.2). The reason for this difference was not apparent, but there were several major points of difference between the experiments. The mean milk yield of the cows used during the present experiment was some 10 per cent below the corresponding yields obtained in the previous experiments. Following the change to Ruamui ryegrass feeding, milk yields remained steady instead of declining as in the previous experiments. This may have been related to the fact that the crude fibre content of the Ruamui ryegrass was lower in the present experiment.

Supplementary starch: The supplementary starch increased the daily intakes of readily available carbohydrate by at least 50%. It was clear from the results that this supplement increased the yield of protein and the protein percentage in the milk (and S.N.F. percentage in Experiment A), but because the Ruamui pastures did not lower the protein and S.N.F. percentages in the milk from the non-supplemented control group the hypothesis regarding the cause of the low values associated with high nitrogen Ruamui ryegrass was not fully tested.

As in Experiment 7, the addition of starch to the ration did not result in as large a reduction in rumen ammonia concentrations as might have been expected from previous experiments (Head and Rook, 1957; Christian and Williams, 1966), so factors other than the intake of nitrogen and soluble carbohydrate must influence rumen ammonia levels. These may include the nature of the nitrogen present, the level of structural carbohydrates present,

and the level of supplementation relative to the carbohydrate and nitrogen contents of the herbage.

Rumen proportions and concentrations of individual fatty acids were not changed to any extent by the addition of starch (Table 8.3) so the change in protein percentage could not be explained on the basis of a change in the volatile fatty acids in the rumen liquor.

Although the differences in the milk yields between treatments were not statistically significant the starch fed cows produced most milk in both experiments. The relative importance of a small improvement in the supply of energy (relative to the control group) and the possible influence of an improved carbohydrate to protein ratio, could not be assessed with certainty from the results. However, the small changes in milk yield and composition obtained are in line with many previous experiments in which pasture fed animals have been supplemented with carbohydrate rich feedstuffs (e.g. Castle, Drysdale and Watson, 1960; Huber and Bowman, 1966; Corbett and Boyne, 1968) and indicate that the soluble carbohydrate content of grasses probably influences the protein content of the milk.

Supplementary peanut oil: The supplementary peanut oil increased the lipid intake of the cows by between 50 and 60%. This was equivalent to an ether extract in the ration of approximately 9 - 10%.

In the literature there are many conflicting reports of the effect of the amount and type of fat in the diet of cows on the yield and butterfat content of the milk. The feeding of highly unsaturated fish oils (e.g. cod liver oil) has generally reduced the fat content of the milk and sometimes also milk yield (McDowell, Reid and Patchell, 1967; Shaw and Enser, 1969). The reduction in milk fat may result indirectly through alteration in the proportions of volatile

fatty acids in the rumen, or from an effect via intermediary metabolism in the body or mammary gland (Sutton, 1965). The feeding of oils of plant origin has had variable effects on milk yield and fat content.

McDowell et al. (1957) obtained no effect of added peanut oil, linseed oil, soya bean oil (and tallow) on milk yield and composition, whereas in the present experiment milk yield was little affected, but butterfat yield and percentage were increased significantly in both experiments.

Butterfat production was clearly stimulated by the addition of oil. Because volatile fatty acid production appeared to be lower, rather than higher, it is likely that this stimulus resulted from an increased incorporation of long chain fatty acids into the milk fat (see 1.54).

In the two present experiments peanut oil given to cows caused a reduction in the protein and solids-not-fat percentages in the milk. A similar effect on solids-not-fat percentage was obtained with added tallow by Peters, Harris, Mulay and Pinkerton (1961), with Friesian but not Jersey cows, whereas other workers have found no changes in the S.N.F. percentage in milk (McDowell et al. 1957) when additional oils were fed.

The significant correlation (0.52) between the volatile fatty acid and ammonia concentrations within individual samples of rumen liquor, suggested that these variables were affected by a common factor such as feed intake. Furthermore, the relationship between the volatile fatty acid and ammonia concentrations did not differ for the rumen samples obtained from the cows within the different treatment groups. On the other hand, peanut oil was shown (Table 8.3) to have little effect on protein metabolism (ammonia concentration) but a significant effect on carbohydrate metabolism (lower concentration of volatile fatty acids). This observation, taken together with the fact that there was no evidence from

the milk yield data to indicate that the oil treatment led to a greatly reduced intake (as has occurred in some experiments; Robertson and Hawke, 1964a), suggested that the added oil may have altered the microbial population in the rumen. How a possible change in microorganisms might induce a change in milk protein synthesis was not clear, but the results from this experiment do indicate that the lipid content of some rations may be of more significance in influencing the protein content of milk than has previously been realised. In this regard it should be pointed out that the effects of short Ruamui ryegrass, and Ruamui ryegrass following heavy applications of nitrogenous fertilisers, on the protein content of milk (Chapters 4 and 5) may have been partly due to the higher levels of ether extract in these pastures compared with the control rations.

8.5 SUMMARY AND CONCLUSIONS

Starch (900g./day) and peanut oil supplements (410g./day) were given to groups of eight cows which were grazing Ruamui ryegrass during two experiments carried out during the spring period.

The cows receiving starch produced milk which contained higher yields and percentages of solids-not-fat and protein, indicating that the readily available carbohydrate fraction of pastures may be expected to have some influence on the protein content of milk. The additional starch did not influence the protein content by altering the molar proportions of individual volatile fatty acids present in the rumen liquor of these cows. Therefore, the process involved was probably a small improvement in the level of feeding or an improved utilization of dietary protein following the addition of starch. If level of feeding was the important factor of the two, it is clear that milk yield is not a sensitive indicator of the change.

The cows receiving peanut oil produced milk with a higher yield and percentage of butterfat, and lower contents of solids-not-fat and protein. The higher fat content of the milk was probably due to direct incorporation of dietary fat into milk fat. No adequate explanation could be made for the changes in the contents of solids-not-fat and protein so that further experiments were considered necessary, especially as the ether extract content of the high nitrogen ryegrass was higher than the content in low nitrogen grass (5.31).

The material presented in this chapter has been published (Wilson and Dolby, 1969) along with the plasma mineral data (Wilson et al., 1969) which does not form part of the present study. It is of interest, however, that the starch supplement reduced the extent of the fall in plasma magnesium concentration, whereas the peanut oil increased the reduction in plasma magnesium concentrations brought about by the high nitrogen Ruamui ryegrass. Hence the possibility exists that plasma magnesium status may affect milk composition, but at present there is no basis for suggesting that this was a casual relationship.

CHAPTER 9

EXPERIMENT 7 : UTILIZATION OF RUANDI RYEGRASS BY
SHEEP FED PEANUT OIL.

9.1 INTRODUCTION

In Experiment 6 (see 8.3) a peanut oil supplement was shown to stimulate the production of butterfat but depress milk protein and possibly lactose production. It was suggested that the effect on fat synthesis was due to an increased incorporation of long chain fatty acids in milk fat but no adequate explanation for the effect of oil on the protein content of the milk was made. Consequently a further experiment was conducted to provide more information concerning the effect of added oil on the digestion of ryegrass. In this experiment, sheep were fed indoors in order to obtain control over intake and so facilitate the measurement of the utilization of the protein, carbohydrate and fat components of the grass.

The effect of oil on the utilization of ration components has previously been studied extensively but results have been highly variable. Low levels of supplementation have usually led to the stimulation of carbohydrate digestion and given more efficient weight gains (Esplin, Hale, Hubbert and Taylor, 1963; Swift, Thacker, Black, Bratzler and James, 1947). High levels of fat, however, have generally resulted in a reduction in the digestibility of all feed components (Swift et al., 1947; Brooks, Garner, Gehrke and Muhrer, 1954; Brothour, Sirry and Tillman, 1958), and almost without exception the effect on carbohydrate digestion has been greater than that on protein digestion.

Apart from level of supplementation, the effect of oil varies with its composition (Brooks et al., 1954), method of administration (Pfander and Verma, 1957), quality of the basic ration (Esplin et al., 1963; Erwin, Dyer and Ensminger, 1958), and level of mineral ions present (Grainger, White, Baker and Stroud, 1957; Ward et al., 1957).

It has been suggested by several workers that the mechanism of action of oil is to coat the fibrous particles with a protective layer (Ward et al., 1957; Pfander and Verma, 1957) but White, Grainger, Baker and Stroud (1958) found that cellulose digestibility was not completely restored until 17 days after the removal of oil and interpreted this to mean that bacterial activity must be affected. This conclusion was supported by the results of Brooks et al. (1954) which included an increase in the proportion of cocci to bacilli in the rumen liquor following the feeding of oil.

There is less work reported on the effect of supplementary lipid on the concentration of fermentation end-products in the rumen. However, with few exceptions, acetic acid concentration has been reduced and propionic acid concentrations increased following addition of oils (Nottle and Rook, 1963; Shaw, Ensor, Telleches and Lee, 1960; Robertson and Hawke, 1964a). The reduced acetic acid concentration is consistent with a depression in the utilization of the fibrous fraction of the ration, but the increase in propionic acid concentration has not been adequately explained.

With the exception of the experiment of Robertson and Hawke (1964a), in which large reductions in intake complicated the results, none of the above mentioned experiments are absolutely applicable to the present situation as they were concerned with the effect of oil on the utilization of diets other than fresh pasture. This factor, together with the wide range of results obtained in previous work further emphasised the need for the present experiment.

TABLE 9.1 Experimental details for Experiment 7.

Week	1	2	3	4	5	6
Dates	19 Sept.-25	26 -2 Oct.	3-9	10-16	17-23	24-30
<u>Peanut oil supplement</u> (ml.)						
Sheep 1	-	-	-	23ml.	15ml.	23ml.
2	-	-	-	23ml.	23ml.	23ml.
3	-	23ml.	23ml.	-	-	-
4	-	23ml.	23ml.	-	-	-
	Preliminary period	First experimental period		Change- over period	Second experimental period	
<u>Period number for subsequent reference</u>	I	II		-	III	

9.2 EXPERIMENT METHODS

9.21 Animals and design of experiment.

Two mature Romney wethers (sheep 2 and 4) and two mature Romney X Cheviot ewes (sheep 1 and 3 with rumen fistulae) were used in the experiment. Following treatment for internal parasites (Thiabendazole) they were confined to metabolism crates and housed indoors. The sheep were fed throughout with freshly cut (once/day) Ruamui ryegrass pastures which had received 2 cwt. of ammonium sulphate two weeks prior to the commencement of the experiment. Following a training period of two weeks to accustom the sheep to indoor conditions and to establish voluntary intakes, the sheep were put onto the changeover experiment described in Table 9.1. During the six weeks of the experiment the four sheep were offered the same green weight of ryegrass each day. The quantity chosen was between 500 and 600 g. of dry matter per day as this was below the quantity consumed by the poorest eater during the training period. Approximately two fifths of daily ration was fed at 9 a.m., and the remainder at 4.30 p.m.

The quantity of peanut oil administered (a.m. 10 ml., p.m. 13 ml.) was calculated to give approximately the same ratio of oil to dry matter as was given to cows in the previous experiment (see 8.2).

The digestibilities of the dry matter, acid-detergent fibre, crude protein and ether extract, together with nitrogen balances for individual sheep, were obtained for each of the 6 weeks of the experiment. The extent of cellulose digestion was further examined using cotton thread inserted through the rumen fistulae of sheep 1 and 3 during each of the experimental periods and immediately following the end of the experiment. By covering half of the threads with peanut oil just prior to insertion into the rumen the importance of a protective

coating of oil in affecting cellulose digestion was assessed. As it was expected that digestibility could be altered by oil supplementation the rates of passage of food residues through the animals were measured. Part of the ration was stained with potassium permanganate solution on one occasion during each of the three experimental periods (see Table 9.1) in order to measure rates of passage.

A further measure of the influence of oil on the digestive processes was obtained by measuring volatile fatty acid and ammonia concentrations in the rumen liquor of the two fistulated sheep during each of the three experimental periods.

9.22 Sampling and analysis.

Herbage: Representative samples of feed (about 300 g.) were taken at each feeding time for the determination of dry matter intakes. The samples were dried at 100°C for 24 hours and the dried samples bulked for each week prior to chemical analysis. The bulked samples which had been stored in air tight containers were subsequently ground (1 mm. sieve) and analysed for nitrogen, ether extract (A.O.A.C. 1968) and acid-detergent fibre (A.O.A.C., 1963). Acid-detergent fibre, which is a measure of cellulose plus lignin, was measured in preference to crude fibre in this experiment as it is a more precise measure of the least digestible portion of the pasture plant (Van Soest, 1965).

Faecal samples: Faeces were collected using bags in the case of the wethers and from the ewes by deflecting the faeces away from the urine with a sloping gauze mesh mounted beneath the animals. The faeces from each sheep were bulked for each week (stored at -10°C), then dried in an oven at 100°C for 48 hours, and sub-samples ground prior to analysis for nitrogen, ether extract,

and acid-detergent fibre contents. From these data and the corresponding herbage data, digestibilities were calculated allowing a lag of one day between intake and faecal measurements in an attempt to account for the time taken for food to pass through the digestive tract.

Urine samples: Urine was collected in H_2SO_4 (18 ml. 18N- H_2SO_4 /litre) in order to prevent loss of nitrogen as ammonia. The urine from individual sheep was bulked for a period of a week and the bulk samples were stored in covered containers at 4°C . At the end of each week the nitrogen contents of 5 ml. sub-samples of urine were determined (in duplicate) using standard methods (A.O.A.C., 1935).

Rumen liquor: Samples of rumen liquor were taken from the two fistulated sheep on two consecutive days during weeks 1, 3 and 6. The first samples were taken just prior to the morning feeding and subsequently three further samples were obtained at two hourly intervals. Samples were stored and analysed for ammonia and volatile fatty acid concentrations and molar proportions of individual volatile fatty acids as described previously (2.8).

Digestion of cotton threads: Four "coils" of cotton thread (each weighing about 0.20 g.), two of which had been immersed in peanut oil, were weighed down to the bottom of the rumen of the two fistulated sheep on four separate days (9, 23, 30 and 31 October). The loss of dry matter which occurred during the course of 30 hours in the rumen was measured for individual coils of cotton. The last two occasions were included to see whether there was a carry-over effect of oil on cellulose digestibility.

Rate of passage of food residues: The rate of passage of dried ryegrass (stained with potassium permanganate) was followed on one occasion during each of the three experimental periods.

TABLE 9.2 Dry matter intakes (g./day) of individual sheep for each week of Experiment 7.

Week	1	2	3	4	5	6
Sheep 1	596	536	510	439	549	548
2	631	573	555	550	584	503
3	629	599	555	550	584	580
4	612	573	552	547	584	564

TABLE 9.3 Chemical composition of the Ruamui ryegrass used within each week of Experiment 7 (percentage of dry matter).

Week	1	2	3	4	5	6
Crude protein (Nx6.25)	32.8	28.4	27.4	26.5	25.3	22.9
Ether extract	6.5	6.9	6.8	6.5	6.1	5.5
Acid-detergent fibre	22.6	21.7	23.6	23.9	27.2	25.0
Ash	10.7	10.3	10.9	10.8	10.7	11.2

About 6 kg. of Ruanui ryegrass was dried (1 kg. D.M.) and then soaked in a potassium permanganate solution (100 g. in 5l. H₂O) for 30 minutes. The grass was then placed in a muslin bag and washed thoroughly for 30 minutes. At this stage very little colour could be leached off by immersing the stained grass in a bucket of water. The grass was then dried and 30g. (about 3% of dry matter intake) fed to each of the four sheep before the normal morning ration. Faecal samples were obtained 12, 24, 28, 32, 48, 60, 72 and 80 hours after feeding the stained grass. The individual samples were dried and 2 g. sub-samples ashed. The manganese was removed from the ash with 2N-HCl and the filtrate made up to a suitable volume with distilled water to allow the measurement of manganese concentration using an atomic absorption spectrometer. The concentration of manganese present in each of the samples was expressed relative to faecal organic matter.

9.3 RESULTS

9.31 Intakes of herbage.

The Ruanui ryegrass pastures were 6-8 inches long at the time of cutting. The dry matter intakes of individual sheep during each week of the experiment are given in Table 9.2. The intakes were fairly similar from week to week apart from during the preliminary period when they were higher. The oil treatment adversely affected the intake of sheep 1 during the last two days of week 4 and first 5 days of week 5. For this reason the level of oil supplementation for this sheep was reduced from 23 ml. to 15 ml. per day for the first 4 days of week 5.

The crude protein, ether extract, acid-detergent fibre and ash contents of the ryegrass fed to the sheep during each week of the experiment are given

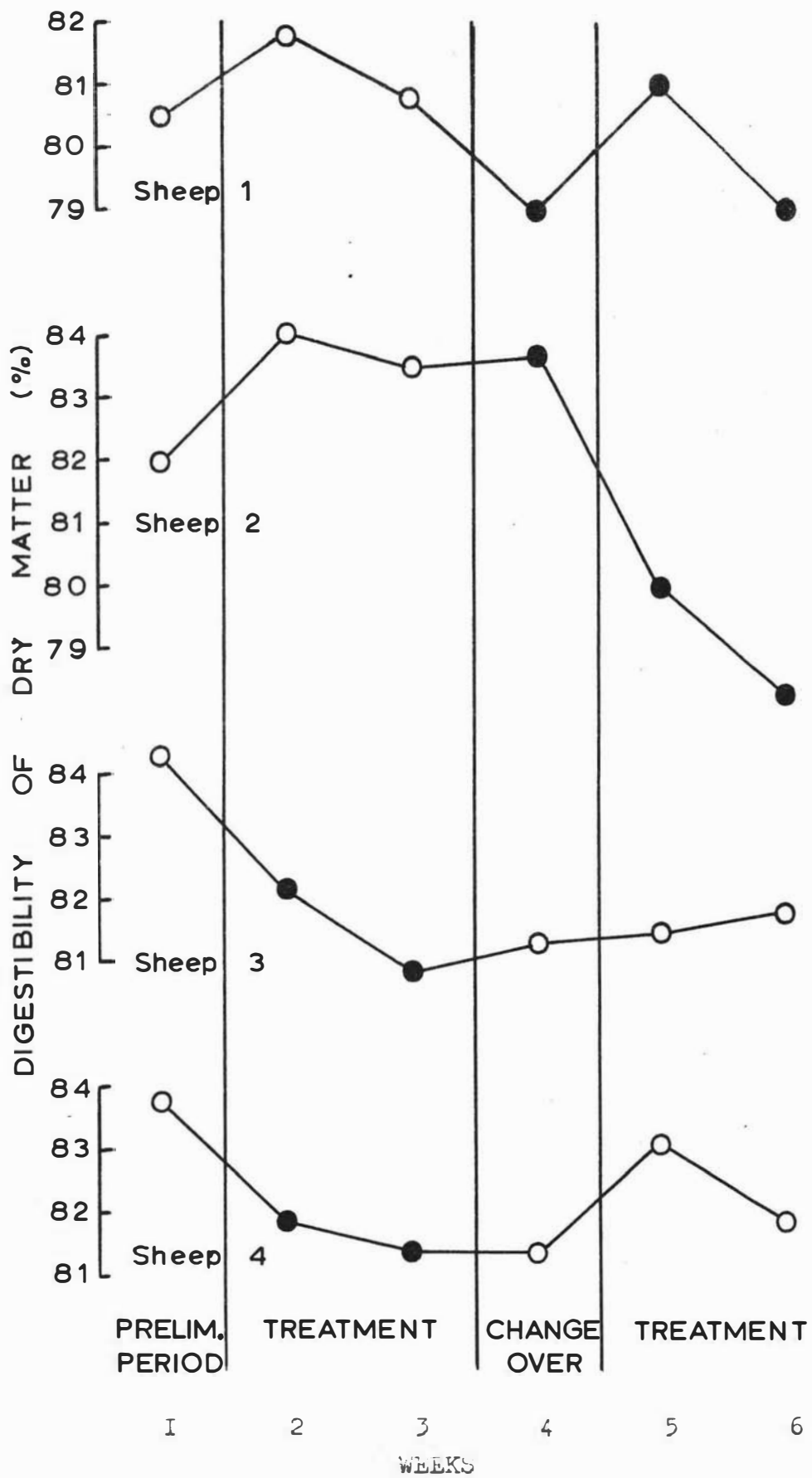


Fig. 9.1 The influence of a peanut oil supplement on the digestibility of the dry matter of Ruanui ryegrass for individual sheep over a six week period. (Oil, ●, Control, ○).

TABLE 9.4 Apparent digestibility (%) of ryegrass and ryegrass plus oil rations (dry matter basis) during the experimental periods (II and III).

	Dry matter		Crude protein		Acid-detergent fibre		Ether extract	
	Ryegrass	Rye + Oil	Ryegrass	Rye + Oil	Ryegrass	Rye + oil	Ryegrass	Rye + Oil
Sheep 1) Sheep 2) Oil given in Period III	81.27	79.00	84.25	82.65	76.02	74.00	62.0	76.0
	83.75	79.12	87.70	83.85	78.60	75.65	63.7	68.8
Sheep 3) Sheep 4) Oil given in Period II	81.37	81.50	84.60	85.05	76.00	74.65	63.5	76.0
	82.44	81.62	86.45	86.05	77.00	74.50	65.6	73.7
Overall means	82.21	80.31	85.75	84.55	76.91	74.70	64.44	73.63
Control mean - oil mean	-1.90		-1.20		-2.21		+0.19	
Significance of treatment differences.	N.S.		N.S.		P < 0.01		P < 0.05	

TABLE 9.5 Fate of dietary nitrogen for sheep fed ryegrass, or ryegrass and oil rations : mean values for 14 day periods.

Experimental Period	Ryegrass		Ryegrass + Oil	
	II	III	II	III
<u>Nitrogen intake (g./day)</u>				
Sheep 1 (Cheviot x Romney)	23.50	-	-	19.55
2 (Romney)	25.20	-	-	22.10
3 (Cheviot x Romney)	-	22.40	25.10	-
4 (Romney)	-	22.10	25.10	-
<u>Faecal output (g.N/day)</u>				
Sheep 1	3.70	-	-	3.40
2	3.10	-	-	3.55
3	-	3.45	3.60	-
4	-	3.00	3.50	-
<u>Urine output (g.N/day)</u>				
Sheep 1	17.15	-	-	14.80
2	20.80	-	-	18.70
3	-	16.05	16.50	-
4	-	18.75	19.50	-
<u>Retention (g.N/day)</u>				
Sheep 1	+2.65	-	-	+1.35
2	+1.30	-	-	-0.15
3	-	+2.90	+5.00	-
4	-	+0.35	+2.00	-
Mean	+1.80		+2.10	

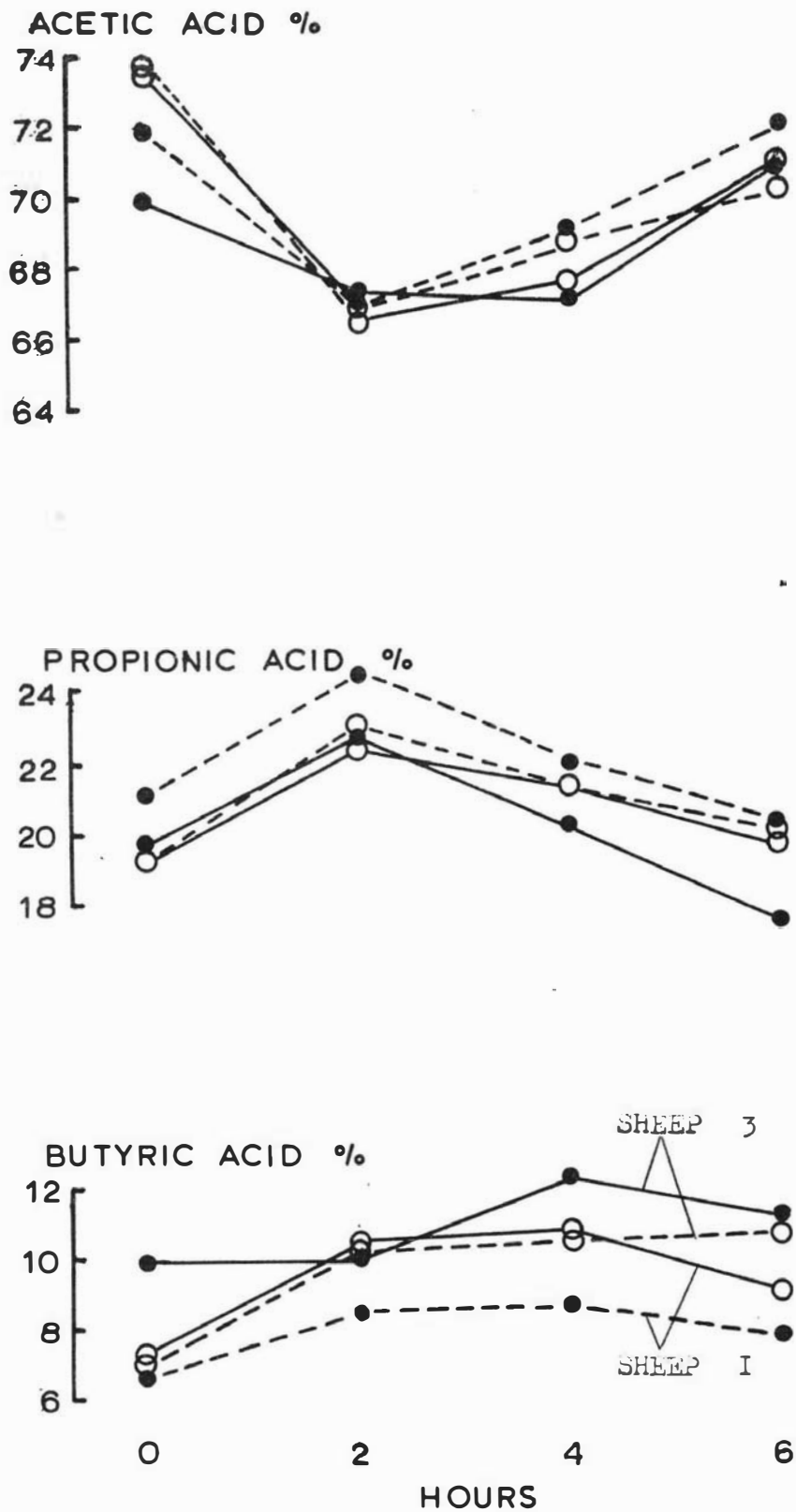


Fig.9.2 The effect of a peanut oil supplement (●) on the diurnal variations in molar proportions of V.F.A. of rumen liquor from two sheep (Control sheep, ○; Experiment 1,—; Expt 2,---).

TABLE 9.6 Analyses on the rumen liquor from the two fistulated sheep fed ryegrass, or ryegrass and oil : mean values of four samples on each of two days.

Experimental period	Sheep 1			Sheep 3		
	I	II	III	I	II	III
<u>Total V.F.A. (mM/100ml.)</u>						
Ryegrass	8.31	7.74	-	8.15	-	7.55
Rye + Oil	-	-	8.65	-	6.85	-
<u>NH₃ conc. (mg.N/100ml.)</u>						
Ryegrass	37.6	24.0	-	36.0	-	22.0
Rye + Oil	-	-	23.3	-	22.1	-
<u>Molar proportions of V.F.A.</u>						
Acetic acid) Ryegrass	67.3	69.9	-	68.8	-	69.8
) Rye + Oil	-	-	70.1	-	69.0	-
Propionic acid) Ryegrass	23.8	20.7	-	23.1	-	20.5
) Rye + Oil	-	-	22.0	-	20.1	-
Butyric acid) Ryegrass	8.9	9.4	-	8.1	-	9.7
) Rye + oil	-	-	7.9	-	10.9	-

TABLE 9.7 Digestion of cotton threads in sheep fed ryegrass, and ryegrass plus oil : percentage loss of dry matter over 30 hours.

	Sheep 1		Sheep 3	
	Oil covered cotton	Control cotton	Oil covered cotton	Control cotton
<u>First experimental period</u>				
Oil given to sheep 3 via mouth (9/10/68)	44.0	50.5	1.8	7.3
<u>Second experiment period</u>				
Oil given to sheep 1 via mouth (23/10/68)	1.3	6.7	40.9	42.6
(30/10/68)	7.1	8.5	45.1	50.4
<u>Post-experimental period</u>				
No oil via mouth (31/10/68)	18.7	14.6	31.1	43.9

TABLE 9.8 Length of time (hr.) for 25% and 50% of the stained hay to be eliminated from individual sheep (obtained from Figure 9.3).

Experimental period Treatment	I	II		III	
	Control	Control	Oil	Control	Oil
<u>25% of stained grass</u>					
Sheep 1	28	18	-	-	33
2	29	32	-	-	45
3	26	-	31	29	-
4	27	-	28	28	-
<u>50% of stained grass</u>					
Sheep 1	39	29	-	-	42
2	48	50	-	-	57
3	37	-	46	38	-
4	37	-	41	44	-

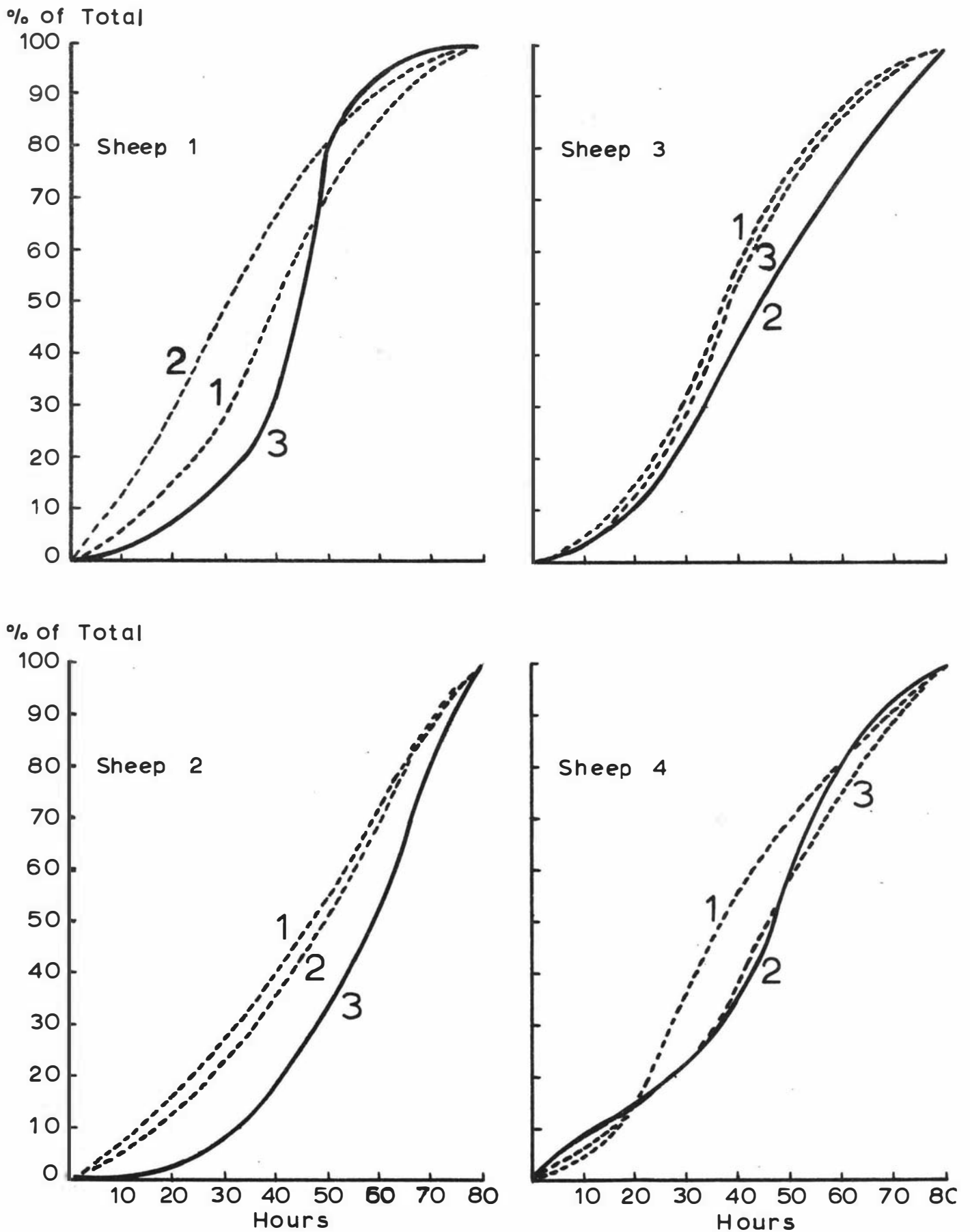


Fig.9.3 The rate of passage of Mn.-impregnated dried grass through four sheep eating Ruanui ryegrass alone (---), and supplemented with peanut oil (—), during each of the three experimental periods (1, 2, 3).

liquor samples taken from the two sheep during each of the experimental periods are given in Table 9.6. The diurnal changes in the molar proportions of volatile fatty acids for each of the sheep during the two experimental periods are illustrated in Figure 9.2. There were no consistent differences between any of the above mean values which could be attributed to the addition of oil. However, there were consistent differences between the two sheep irrespective of the presence or absence of additional oil. Volatile fatty acid concentration, ammonia concentration and the molar proportion of propionic acid in the rumen liquor were all consistently higher for sheep 1 than for sheep 3.

9.34 Digestion of cotton threads.

The extent to which ordinary cotton thread and cotton thread coated with peanut oil was digested when placed at the bottom of the rumen of the two sheep during the experiment is summarized in Table 9.7. Peanut oil given via the mouth depressed the digestibility of the cotton to less than a fifth of the level obtained in the non-supplemented sheep. Coating the cotton with oil also depressed the digestibility in all cases with the exception of sheep 1 on the day after administration of oil via the mouth had ceased (31/10/68).

9.35 Rate of passage of food residues.

The rates of passage of dried grass impregnated with potassium permanganate for individual sheep during the three experimental periods (I, II, III) are shown graphically in Figure 9.3. The curves show the hour by hour accumulated percentage of the total quantity of manganese eliminated in the faeces over an 80 hour period. The lengths of time required for the first 25 and 50% of the stained material (relative to total amount obtained

in 80 hours) to be voided were obtained by interpolation from the graphs and are presented in Table 9.8. These data in the graphs and in Table 9.7 indicate that the added oil depressed the rate of passage of food residues through the digestive tracts of the sheep.

9.4 DISCUSSION

The level of peanut oil administered was equivalent to raising the ether extract of the pasture from about 0% up to 10%. The ratio of oil to pasture dry matter intake was slightly greater than that reached during the previous experiment in which oil was given to lactating cattle. However, in other respects such as composition of the pasture, and method of administration of the oil, the present experiment replicated as far as possible the previous experiment.

The possibility that added peanut oil may reduce voluntary intake was indicated by the intake results from sheep 1 (Table 9.2). In this regard it was of interest that the effect on intake did not occur for several days after oil was first fed, and that after a period on a reduced level of supplementation a return to the full dose level did not then affect intake. This suggested some form of adaptation was necessary such as a slow change in the rumen micro-organisms present and is a possible explanation for the variable results obtained by Robertson and Hawke (1964a). By using short treatment periods (maximum of four consecutive days), their data were probably obtained during a transition period before the digestive system was accustomed to the very high levels of oil administered.

There were changes in the type of pasture used during the course of this present experiment and the higher contents of acid-detergent fibre and lower ether extracts during period III, compared with II, would be expected to be of

most significance. However, a definite treatment by period interaction was not apparent in the digestibility data (Table 9.4) possible owing to the small number of sheep used and the variations between the two sheep within treatment groups.

In accordance with previous experiments (see 9.1) the digestibility of acid-detergent fibre was decreased more than the crude protein fraction of the plant, which suggests that the oil had a rather selective effect on the least digestible portion of the ryegrass. These results were also in agreement with the finding that administering oil to cows (see 8.3) resulted in a lower volatile fatty acid concentration (derived mainly from carbohydrate), but caused no change in the ammonia concentration (derived from plant nitrogen) of the rumen liquor.

The relatively small differences between treatments in the digestibility of crude protein, the nitrogen retentions, and the ammonia concentrations of the rumen liquors from the sheep, confirm that nitrogen metabolism was little affected by peanut oil. It is reasonable to suggest, therefore, that the effect of oil in changing the protein content of the milk in the previous experiment was not brought about directly by a change in the utilization of dietary protein.

Of some general interest was the occurrence of clear differences between the sheep of the two breeds in the proportion of dietary nitrogen excreted in the urine (Table 9.5) and consequently differences in nitrogen retention. Further experimental confirmation of breed differences such as these could provide the means of developing new types of sheep capable of surviving on rations low in nitrogen.

In contrast to other components, the digestibility coefficient for ether extract was increased by the added oil. This may not necessarily mean, however, that the digestibility of the grass lipid was increased as it would be reasonable to expect that the added oil would be in a much more available form than the grass lipid. In fact the total output of ether extract in the faeces was increased by between 2 and 5g./day in individual weeks by the added oil.

The absence of an effect of oil on volatile fatty acid concentration in the rumen liquor is apparently in conflict with the changes obtained in digestibility, but the probable reason lies in the fact that with a highly digestible grass the greatest proportion of the volatile fatty acids would be derived from the readily available fraction of the dry matter.

While it is clear that the action of oil was mainly on the more fibrous fraction of the ryegrass residues the mechanism of action was not so apparent. The depression in the digestibility of the cotton thread after it was immersed in oil suggested that a protective layer of oil may be responsible, but because oil given via the mouth had a several times greater effect (Table 9.7), another mechanism was probably of more importance. This mechanism may have been an alteration in the type of micro-organisms present as was found by Dooks et al. (1954), or a specific detrimental effect of oil on the cellulolytic organisms. The partial recovery in the digestibility of the cotton thread for sheep 1, the day after the end of the experiment (oil not given orally), indicated that the oil had a carry-over effect which is also suggestive of a gradual change in the microbial population.

The results from the present experiment and those from the previous one (Expt. 6) differed in that the volatile fatty acid concentration in the rumen

liquor of the cows was lowered by oil whereas this did not occur in the case of the sheep. As the composition of the pasture was reasonably similar in the two experiments, and the level of supplementation was relatively greater in the case of the sheep, the most likely reason for the different response in volatile fatty acid concentration is that the voluntary intake of the cows was reduced. A lower digestibility and a slower rate of passage of food residues (Fig.9.3) might be expected to result in a lower intake under ad libitum feeding conditions (as for the cows) but these factors would be of no significance at the lower feeding levels used in the present experiment. A lower digestibility and/or a small reduction in intake could account for the lower protein content in the milk in the previous experiment.

9.5 SUMMARY AND CONCLUSIONS

A peanut oil supplement (23 ml./day) was given to four sheep eating Ruanui ryegrass in a changeover experiment in order to examine its effect on the utilization of dietary protein and carbohydrate.

The utilization of dietary protein as measured by digestibility, by ammonia concentration in rumen liquor and by nitrogen retention data, was not altered significantly by the addition of peanut oil.

The digestibility of the "fibrous" fraction of the ryegrass (acid-detergent fibre) was reduced by the peanut oil supplement ($P < 0.05$), and the oil also reduced the rate of passage of food residues through the animal.

In contrast to the previous experiment (6), the peanut oil supplement did not lower the concentration of volatile fatty acids present in the rumen liquor. It was considered that the volatile fatty acid concentration may have been lowered in the case of the cows by affecting intake, and that this did not occur

in the present experiment because the intakes of the sheep were held considerably beneath their maximum level. Such a change in intake, particularly if accompanied by a lower digestibility, would adequately explain the low protein content in the milk of the cows given peanut oil, provided that it is accepted (as was suggested in the case of starch supplements) that differences in milk yield were not a very sensitive indicator of changes in feeding level in Experiment 6.

CHAPTER 10.

EXPERIMENT 8 : EFFECTS OF PROTEIN SUPPLEMENTS ON MILK

PRODUCTION OF FRIESIAN COWS IN THE WINTER.

10.1 INTRODUCTION

In Experiment 6 (8.3) it was shown that cows given a starch supplement produced milk containing a higher protein percentage. The mechanism involved was not defined but it was clear that the change was not associated with an alteration in the molar proportions of volatile fatty acids present in the rumen liquor. A small improvement in level of feeding or an improved utilization of dietary protein in the rumen may have been involved. The effect of additional protein was examined in the present experiment to investigate further the latter possibility using a different experimental approach.

The main metabolic processes involving protein in the rumen have now been established (for recent reviews see Hungate, 1966; McDonald, 1968; Waldo, 1968). Proteins in the feed are rapidly broken down to amino acids, by proteolytic bacteria, and these are in turn quickly deaminated to form ammonia. Ammonia, which is absorbed through the rumen wall, is to a large extent a waste product and hence the animal depends for its protein supply on the digestion and absorption in the intestine of food protein which has escaped rumen digestion together with microbial protein. The factors which have been shown to influence the amount of dietary nitrogen that leaves the abomasum, relative to that lost as ammonia include: the level of nitrogen in the ration (see Experiment 4), the solubility and physical state of the dietary protein (McDonald, 1968), the amount of organic matter fermented in the rumen (Bogan and Weston, 1967) and the amount of available carbohydrate in the ration (see Experiment 6).

The importance to the animal of the amount of nitrogen leaving the abomasum has been emphasized by experiments such as those of Chalmers, Cuthbertson and Syngé (1954) and Little and Mitchell (1957) who obtained more efficient utilization of proteins and amino acids when they were administered to ruminants via the duodenum than via the rumen. With intact animals the basic problem is one of rendering the feed protein unavailable to rumen organisms without affecting the digestibility of the protein. Recently Ferguson, Hemsley and Reis (1967) have shown that treatment of casein with formaldehyde renders it resistant to microbial attack under alkaline or neutral conditions but that this process is reversed under acid conditions such as occur in the abomasum (pH 1 to 3).

In the present experiment supplements of formalin treated casein (treated casein) and ordinary casein (both in a concentrate mixture) were given to lactating Friesian cattle during the winter. Friesian cattle were used at this time of the year because they were expected to produce milk containing a low percentage of solids-not-fat and protein. Hence the hypothesis being tested was that milk with a low protein content may be produced when there is a shortage of protein reaching the abomasum. A positive result from the treated casein but not from the ordinary casein would support the hypothesis.

10.2 EXPERIMENTAL METHODS

10.21 Animals and design of experiment.

Twenty seven Friesian cows of mixed ages which had calved in April or early May were available for use in the experiment. Of these only seventeen regularly consumed the pelleted control ration (see below) when

3 lb. was offered to them at morning milkings over a five day period (1 to 5 June, 1968). These seventeen cows together with seven others chosen on the basis of a suitable calving date and a satisfactory milk yield were allocated at random to three treatment groups of eight cows with the restriction that only those that readily consumed concentrates were included in two of the three treatment groups. The experiment consisted of two feeding periods. During the preliminary period (9 - 21 June) all cows were fed mixed pasture together with a small ration of hay (approximately 6 lb./head/day) and silage (approximately 25 lb./head/day). During the experimental period (21 June - 5 July), feeding was similar to that during the preliminary period except that the cows in two of the three treatment groups received 2.5 lb. of pelleted concentrate per head at each milking.

10.22 Concentrate Mixtures.

The composition of the two concentrate rations were as follows:-

<u>Treated casein ration (TC)</u>		<u>Casein ration (C)</u>	
Formalin-treated casein	20 parts	Casein	20 parts
Barley meal	64 parts		64 parts
Bran	14 parts		14 parts
Molasses	2 parts		2 parts
	100		100

10.23 Preparation of the treated casein

The formalin-treated casein was prepared by the Dairy Research Institute with the co-operation of the Manawatu Co-operative Dairy Company Ltd.

A batch of dried unground lactic casein was divided into two parts, one of which was treated with formalin. Cold water (three times the weight of casein) was placed in a D - vat and the casein poured in. Formalin (2½% on

weight of water) was added and the whole mixed for 50 minutes. The slurry was then pumped via a screen to the casein press and thence fed to continuous vibrating screen driers. The material was then passed to a blending bin, mixed and bagged. The moisture content of the casein used was 12.2% and after formalin treatment was 14.3%.

10.24 In vitro test of the treated casein.

Although the nature of the complex which is formed in a specific case may not be known, the effectiveness of the formalin treatment in protecting the casein from proteolysis and denaturation in the rumen was gauged under in vitro conditions. Duplicate samples (0.5 g.) of treated casein, ordinary casein, and rennet casein (included for interest as this is known to be relatively insoluble at rumen pH of 6 - 7; Southward, 1968), were incubated with 50 ml. of strained rumen liquor in glass bottles (fitted with one-way lansen valves) held at 39°C in a water bath. The rumen liquor was obtained immediately before the in vitro fermentation from a rumen-fistulated cow grazing mixed pasture. Ammonia concentrations were determined (see 2.6) on sub-samples obtained from each of the bottles containing casein together with two blanks (rumen liquor only) after 3, 6, and 24 hours.

10.25 In vivo test of treated casein.

As a further check to the effectiveness of the formalin coating the treated and ordinary caseins (0.5 lb./day) were administered via the rumen fistulae of one pair of twins grazing mixed pasture at each morning milking over a six day period. Treatments were as follows:-

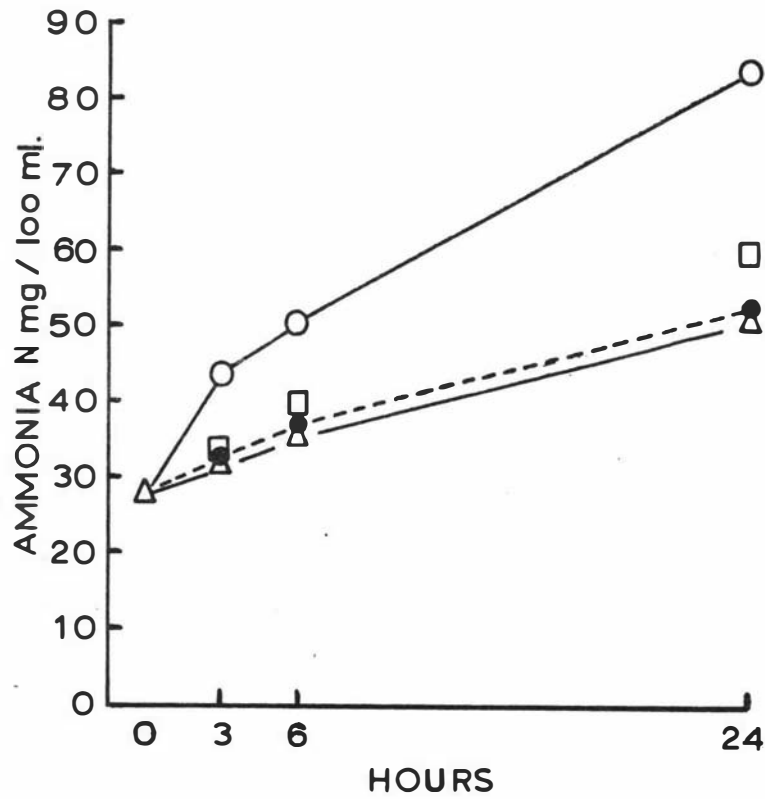


Fig.10.1 The production of ammonia in (50 ml.) samples of rumen liquor incubated with 0.5 g samples of casein (○), treated casein (●), and rennet casein (□), at 39°C for 24 hours. (Blanks with no casein, △).

<u>Day</u>	<u>Cow 121</u>	<u>Cow 122</u>
1	No supplement	No supplement
2	No supplement	No supplement
3	Treated casein	Casein
4	Treated casein	Casein
5	Casein	Treated casein
6	Casein	Treated casein

Samples of rumen liquor were obtained from the centre of the rumen from both cows immediately before administration of the casein and subsequently at 2, 4 and 6 hours, on each of the six days. Ammonia concentrations were determined on all samples of rumen liquor (see 2.8).

10.26 Milk sampling and analysis.

Milk yields were measured at individual milkings from the Sunday evening milking until the Friday morning milking during each of the four weeks of the experiment. Samples from individual cows at all milkings were analysed for fat, protein, and lactose percentages using I.R.M.A. These data were analysed by analyses of covariance as described previously (3.24).

10.3 RESULTS

10.31 In vitro test of treated casein.

The concentrations of ammonia produced in the in vitro incubations of the treated and ordinary caseins are presented in Figure 10.1. It was clear that after 3, 6, or 24 hours the ammonia produced from the treated casein was very similar to that from the blanks, indicating that the treatment was

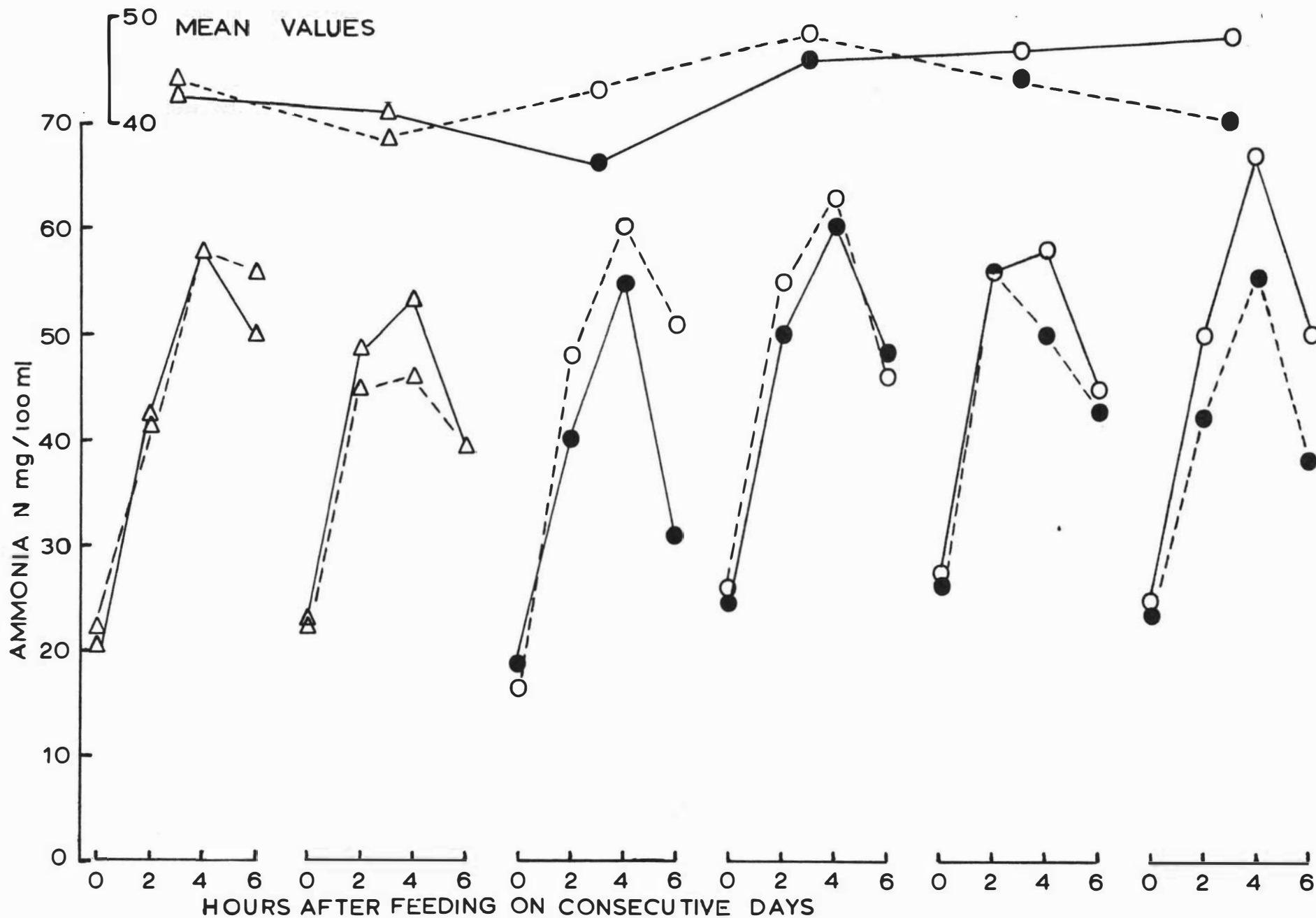


Fig.10.2 Diurnal variations in the ammonia concentration of rumen liquor, following administration of casein or "formalin treated" casein to a pair of rumen fistulated twin cows.

TABLE 10.1 The influence of 0.5 lb. of formalin-treated and ordinary casein on the mean ammonia concentration (mg.N/100 ml.) of the rumen liquor from two cows.

	No supplement	Treated Casein	Ordinary Casein
Cow 121	41.9	40.9	47.2
Cow 122	40.9	41.9	45.7
Overall mean (and standard error of daily means)	41.4 (1.6)	41.4 (3.7)	46.4 (1.2)

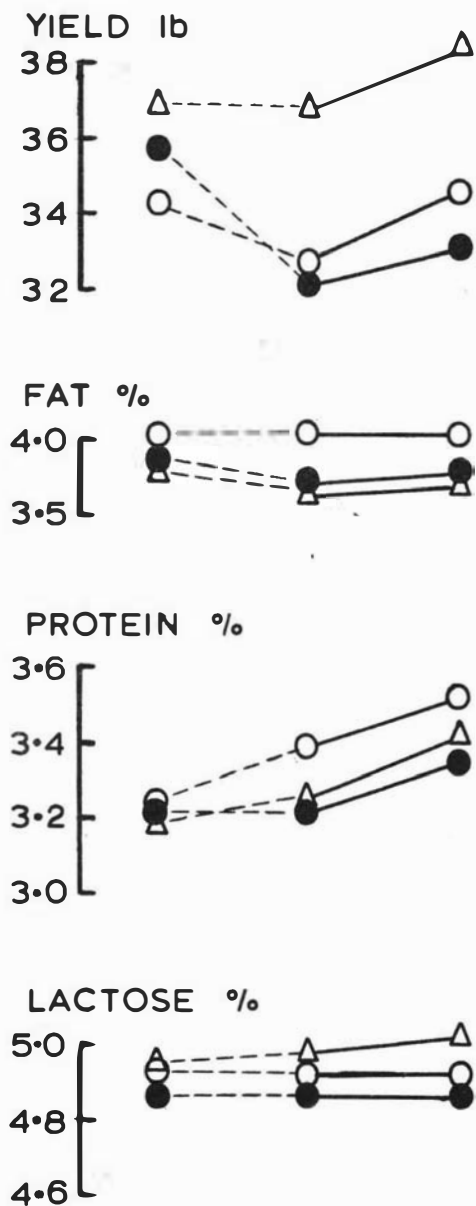


Fig.10.3 Average daily milk yields and milk compositions from groups of Friesian cattle given concentrate supplements containing casein (○), and formalin treated casein (△). (No supplement, ●). (First sample, mixed pasture feeding).

TABLE 10.2 The yield and composition of the milk from cows given two concentrate mixtures containing formalin-treated and ordinary casein : adjusted treatment means.

	Treated casein (TC)	Ordinary casein (C)	Non-supplemented (Ns)	Significant diffs.	
				P < 0.01	P < 0.05
Milk yield (lb./cow/day)	36.4	34.8	32.7	TC > Ns	C > Ns
Fat %	3.77	3.95	3.80	-	C > TC C > Ns
Fat yield (lb./cow/day)	1.36	1.34	1.26	-	TC > Ns
Protein %	3.33	3.44	3.25	C > Ns TC < C	TC > Ns
Protein yield (lb./cow/day)	1.22	1.19	1.06	TC > Ns C > Ns	-
Lactose %	4.97	4.91	4.91	-	-
Lactose yield (lb./cow/day)	1.80	1.70	1.30	TC > Ns	TC > C C > Ns

effective in preventing protein degradation in vitro. As a comparison, ordinary casein was shown to produce values substantially above the blank. Commercial rennet casein was apparently partially resistant to degradation as the increase in ammonia level was approximately one third of that obtained from the incubation of ordinary casein.

10.32 In vivo test of the treated casein.

The concentrations of ammonia in the samples of rumen liquor from the two cows, following supplementation with treated and ordinary casein are given in Figure 10.2. On the basis of the daily mean values for each of the cows (Table 10.1) the in vivo results confirmed that the formalin treatment was effective in reducing the formation of ammonia from the added casein.

10.33 Milk yield and composition.

By the end of the preliminary period it was clear that two cows were unsatisfactory experimental animals on account of mastitis infections, and therefore data were obtained from only seven cows per treatment group during the experimental period.

The mean data for milk yield and composition are presented in Figure 10.3, and the mean values for treatment groups, after adjustment by covariance for preliminary period differences, are given in Table 10.2.

The milk yield produced by the cows which received treated casein was higher than that from the cows which received the ration containing ordinary casein but the difference was not statistically significant ($P > 0.05$). Both the supplemented groups, however, produced significantly more milk than the non-supplemented group.

Butterfat and protein percentages were higher in the milk from the casein supplemented group than from either of the other two groups, but the protein percentage from the cows which received treated casein was also higher than that from the non-supplemented group ($P < 0.05$). Lactose percentages were similar for the milks from the three groups. The yields of all components were similar for the two supplemented groups and considerably higher than the corresponding values from the non-supplemented group of cows.

10.4 DISCUSSION

The in vitro and in vivo tests of the formalin-treated casein indicated that the treatment was effective in preventing the degradation of the casein by rumen organisms. There was not, on the other hand, any direct evidence from the results to confirm that the treated casein was made available for intestinal digestion and absorption. However, indirect evidence of two types suggest that this in fact occurred. The same treated casein was used in Experiment 9 and supplements given in water produced changes in milk composition which indicated that it was utilized. In addition another batch of treated casein, prepared by similar means as that used for this experiment, produced increases in plasma amino acid concentrations when fed to sheep by Carrico (1969). The concentrations of proline, citrulline, valine, leucine, and particularly an unknown amino acid increased considerably. The treated and the ordinary caseins used by Carrico were tested on his behalf in the in vitro experiment described (10.24) and the ammonia concentrations produced were almost identical to the corresponding values obtained from the material used in this experiment.

The principal comparison of interest was the yield and milk composition data from the treatment groups receiving the two types of protein supplement.

Because no "non-protein" concentrate ration was included as a fourth treatment group (due to insufficient cows being available) it was not possible to assess the effect of including the 20% casein in the ration independent from the effect of the "carrier" (barley meal and bran).

The composition of the milk from the two supplemented groups differed in protein ($P < 0.01$) and fat percentage and in both cases the supplement containing ordinary casein produced the higher value. However, the yields of these components were similar because of the higher milk yield from the treated casein group. The results for milk protein, therefore, did not support the hypothesis being examined, in that the treated casein did not result in a higher protein yield or content in the milk, relative to ordinary casein. A possible explanation for the higher protein percentage from the ordinary casein group may be that the carbohydrate fraction of the supplement provided a source of readily available carbohydrate which improved the utilization of the casein to form a larger microbial population which was subsequently available for digestion. Carrico (1969) found similar wool growth rates from treated and ordinary casein and also explained the unexpected result on the basis of the high amounts of readily available energy provided by the barley meal in the basic ration.

The low protein percentage in the milk from the non-supplemented group (3.25%) confirmed that the basic forage ration was capable of supporting only a relatively low milk protein content and was therefore deficient in respect to quality or quantity. Thus it was of some interest that both the supplements produced significant changes in milk yield and composition compared with the non-supplemented group. In fact the extent of the changes in milk yield and in protein percentage was generally greater than for several previous experiments carried out at Massey University (Taparia, 1966) in which supplements of 8.5 to

10 lb./day of a barley meal and bran mixture were fed to Friesian cattle, under similar circumstances to those used in the present experiment. The reduction in fat percentage (0.1 - 0.3%) obtained from feeding the carbohydrate supplements in the previous experiments did not occur in the current experiment. While it is tempting to suggest that the higher protein content of the concentrate mixture was responsible for the more satisfactory results obtained in this experiment, many other factors may have been involved. These include the fact that supplements were fed twice daily in the current experiment but only once in previous experiments, and the basic grass ration may have been quite different in the various experiments. A future experiment to compare concentrate supplements containing high and low levels of protein is indicated.

10.5 SUMMARY AND CONCLUSIONS

The effect on milk yield and composition of feeding Friesian cattle during the winter with two concentrate mixtures was examined. Five pounds per day of a concentrate mixture containing 20% commercial casein resulted in higher protein ($P < 0.01$) and fat ($P < 0.05$) percentages in milk than a similar mixture containing 20% formalin-treated casein. The yields of these components of milk, however, were similar for the two groups. Therefore, the results did not support the hypothesis that a low protein content in milk may be due to a deficiency in the amount of dietary protein that reaches the intestine.

It was suggested that the ordinary casein may have been efficiently utilized owing to the presence of large amounts of available carbohydrate in the remainder of the concentrate mixture.

CHAPTER 11

EXPERIMENT 9 : EFFECT OF PROTEIN SUPPLEMENTS ON
MILK PRODUCTION OF COWS GRAZING
HIGH NITROGEN RUANUI RYEGRASS.

11.1 INTRODUCTION

In view of the possibility that interactions between the carbohydrate and protein components of the concentrate supplements used in Experiment 8 (10.4) confounded the results it was considered necessary to examine further the effects of ordinary commercial casein and formalin-treated casein on milk production. In this experiment the two types of casein, suspended in water, were administered twice daily ($\frac{1}{2}$ lb./milking) to twin cows grazing Ruanui ryegrass containing a high content of nitrogen.

The experiment was carried out at the same time of the year and on the same pastures that in previous years induced low solids-not-fat and protein contents in milk. By giving the two types of casein the animals were provided with equal increments of energy but the site of digestion differed. The ordinary casein was subjected to metabolism in the rumen whereas the treated casein should only become available for digestion after reaching the abomasum (see also 10.1). If poor utilization of dietary protein in the rumen was a factor with pastures containing a high level of nitrogen, the protein yield or percentage of the milk would be expected to be increased by the treated, and not the ordinary casein.

11.2 EXPERIMENTAL METHODS

11.21 Animals and design of the experiment.

Twelve pairs of monozygous twins (two Jersey x Friesian pairs, and 10 pairs of Jerseys) which had calved in July or August were grazed together for a preliminary period on mixed pasture (9 Nov. - 22 Nov. 1968). Following this period the cows were divided into three treatment groups (incomplete balanced block design) for the experimental period (22 Nov. - 6 Dec.). During the experimental period the cows grazed Ruamui ryegrass pastures and one group of eight cows received no supplement (control group), a second group of eight 0.5 lb. of formalin-treated casein following each milking, and the remaining eight cows 0.5 lb. of ordinary casein following each milking. The casein was suspended in 800 ml. of water immediately prior to drenching.

Pastures: The pastures used during the preliminary period consisted of mixed ryegrass/white clover pastures with small quantities of cocksfoot and prairie grass. The five paddocks containing pure swards of Ruamui ryegrass (see Table 2.1) which were used during the experimental period had been top-dressed with 2 cwt. of sulphate of ammonia per acre on three occasions during the early spring period.

The cows were given a fresh break of grass every 12 hours, the size of the break being adjusted to ensure that the voluntary intakes of the cows were not restricted. The Ruamui ryegrass pastures were grazed at a height of 6 - 8 inches.

Casein supplements: The casein supplements used were from the same material that was described and used in the previous in vitro and in vivo experiments (see sections 10.3, 10.4, and 10.5).

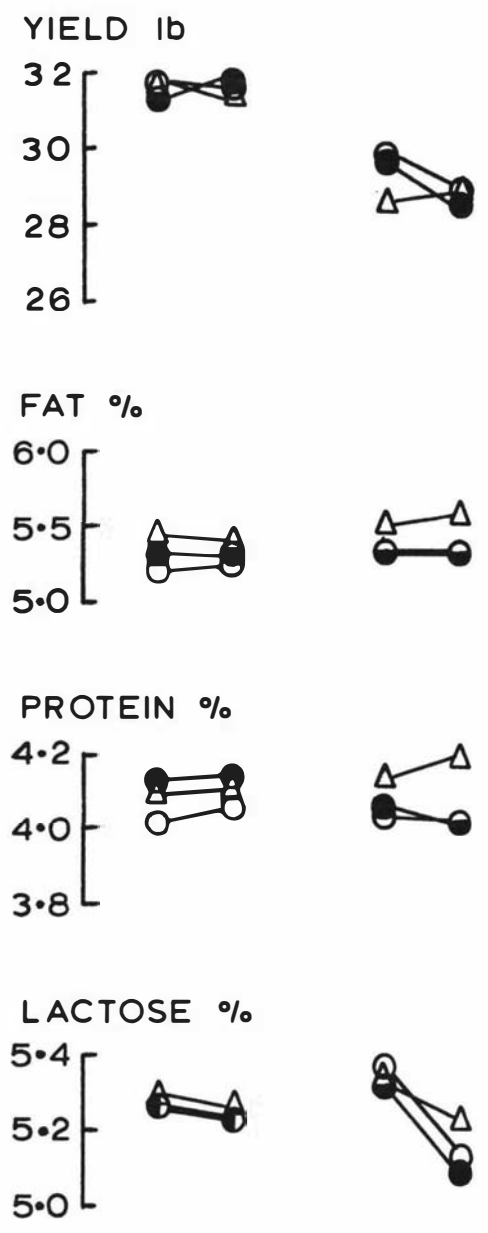


Fig.11.1 Average daily milk yields and milk compositions from groups of twins grazing Ruanui ryegrass pastures supplemented with casein (○), and "formalin treated" casein (▲). (Control,●). (First two samples, mixed pasture feeding).

TABLE 11.1 The yield and composition of the milk from cows given supplements (0.5 lb. twice daily) of formalin-treated casein and ordinary casein : adjusted treatment means.

	Treated casein (TC)	Casein (C)	Control (Ct)	Significant difference P < 0.05
Milk yield (lb./cow/day)	28.8	29.1	29.4	-
Fat %	5.45	5.45	5.35	-
Fat yield (lb./cow/day)	1.57	1.58	1.57	-
Protein %	4.15	4.08	4.00	Tc > Ct
Protein yield (lb./cow/day)	1.18	1.19	1.19	-
Lactose %	5.26	5.25	5.22	-
Lactose yield (lb./cow/day)	1.50	1.54	1.54	-

11.22 Sampling and analytical methods.

Herbage: Representative pasture samples were taken from the five Ruamui ryegrass pastures used in the experiment. Freeze-dried samples were analysed for crude protein, ether extract and soluble sugar contents, as described in 2.4 and 2.5.

Milk: Milk yields were measured at individual milkings from the Sunday evening milking until the Friday morning milking during each of the four weeks of the experiment. Composite daily samples of milk from each cow were analysed for fat, protein and lactose percentage, using I.R.M.A. These data were analysed in covariance analyses as described previously (3.24).

11.3 RESULTS

10.31 Chemical composition of the pastures.

The mean crude protein content of the Ruamui ryegrass pastures was 19.6 (\pm 1.3, S.E.), the ether extract content 4.8 (\pm 0.10), crude fibre content 20.6 (\pm 2.1), and soluble sugar content 12.9 (\pm 0.63).

10.32 Milk yield and composition.

The mean data for milk yield and composition are presented in Figure 11.1 and the mean values for treatment groups, after adjustment by covariance for preliminary period differences, are given in Table 11.1.

The yield of milk from the cows in the three groups was reduced following the change to pure ryegrass feeding and there were no differences between the mean values for the three treatment groups. Milk composition with the

exception of protein percentages was also similar for all treatments. The protein percentage of the control group was reduced by about 0.1% (see Figure 11.1) following the change to Ruamui ryegrass feeding, the protein percentage of the milk from the cows which received ordinary casein remained unaltered, and the value for the cows which received treated casein was slightly higher during the experimental period. The difference between the protein percentages for the treated casein and control groups was statistically significant ($P < 0.05$). The response to treated casein varied considerably between individual cows within the treatment group and ranged from a small negative effect (-0.09%) to a large positive effect (+ 0.27%).

11.4 DISCUSSION

Evidence for the effectiveness of the formalin treatment of the casein was presented and discussed in the previous chapter and it was concluded that the treated casein provided the animal with an additional supply of protein in comparison to the ordinary casein supplement.

Evidence was obtained in this experiment from the treated casein v. control comparison that the amount of protein reaching the abomasum and intestine may affect milk protein percentage. However, the effect was small and clear proof that high nitrogen pastures may lead to a low protein percentage in milk (because of inefficient use of dietary protein) was not obtained, as the protein percentage from the control group of cows was not greatly reduced following the change to Ruamui ryegrass feeding and the difference between the milk protein percentages from the treated casein and ordinary casein groups did not reach statistical significance. The minor nature of the change in protein percentage following administration of

treated casein is emphasized when it is considered that 450 g. of protein was given per day to cows yielding approximately 530 g. of protein in the milk. This comparison suggested that the total amount of protein absorbed, or the amount of a specific essential amino acid was not the major limiting factor to a higher milk protein production. On the other hand the variation in responses obtained from different cows indicated that differences may occur between cows in their ability to obtain sufficient protein (or a particular amino acid) to maximise milk protein synthesis. In this regard it is of interest to recall the differences in the proportion of dietary nitrogen lost via the urine by sheep of different breeds in Experiment 7 (9.3). Varying responses in milk composition (particularly protein content) between animals, to a given reduction in level of feeding, may possibly be accounted for on similar grounds.

The experiment of Martin and Blaxter (1960) indicated that protein administered via the duodenum provided about 40% more energy than when the same quantity was infused into the rumen. Hence in the present experiment the cows which received treated casein, were probably on a higher plane of nutrition than the cows in the other two groups. Although milk production was similar from all groups a higher energy intake may in itself be an alternative explanation for the change in the milk protein percentage of the treated casein group. If indeed, a change in the level of feeding was the cause of the change in protein percentage in the milk, it must follow that protein percentage is more sensitive to the change, than is milk yield. This possibility was also suggested to account for the positive effect of starch (8.5) and the negative effect of oil (9.5) on milk protein percentage. It is probably desirable, therefore, that further work on the effect of small changes in level of feeding on the yield and composition of milk from pastures be carried out.

11.5 SUMMARY AND CONCLUSIONS

The effect on the yield and composition of the milk of feeding cows grazing Ruani ryegrass with ordinary casein and formalin-treated casein (1 lb./cow/day) was examined.

The formalin-treated casein resulted in a slightly higher protein content in the milk than that from the ordinary casein and a significantly higher ($P < 0.05$) content than in the milk from the non-supplemented group. There were no other statistically significant differences between the yields or the compositions of the milk from the three groups of cows.

Clear proof that poor utilization of dietary protein was responsible for the low protein percentages in milk in the previous experiments (1, 2 and 3) was not obtained in view of the minor depression in protein percentage in the milk from the control cows following the change from mixed pasture to Ruani ryegrass feeding. Results did indicate, however, that the amount of protein absorbed by the animal may influence protein synthesis, at least to some extent or with some cows. The mechanism may involve an alteration in the availability of one or more amino acids or a change in the digestible energy intake of the animal. It was not possible to differentiate between these alternatives in this experiment, as the absorbed protein may be used for protein synthesis or deaminated and used as a source of energy. In this regard additional starch (as in Experiment C), while providing energy, would also be expected to spare protein and hence have the same effect as additional absorbed protein.

CHAPTER 12

GENERAL DISCUSSION

The individual experiments undertaken were in some respects independent and hence have been summarized and conclusions made at the end of each experiment. In this chapter the relevant results and conclusions will be integrated and discussed in respect to the original objectives outlined in the Introduction.

12.1 RYEGRASS VARIETIES AND MILK YIELD AND COMPOSITION.

The comparison between the milk yield and composition data obtained from the cows grazing two annual varieties of ryegrass and Ruamui ryegrass (Chapter 3) supported previous results (Wilson and McDowell, 1966) that differences between ryegrass varieties may contribute to variations in the yield and composition of the milk. The annual varieties supported a higher milk production than Ruamui ryegrass in both experiments, although the differences were not statistically significant. Two changes in milk composition associated with different varieties were of particular interest. The first, was the occurrence of a lower than normal fat content in milk from the cows grazing Western Waltha ryegrass compared with those grazing Paroa ryegrass during the winter, and the second, a lowered solids-not-fat percentage (protein and lactose) in the milk from the cows grazing Ruamui ryegrass compared with both annual varieties during the spring experiment. Both these changes in milk composition were independent of concurrent alterations in milk yield. The possible mechanisms which may be involved in bringing about these changes in the yield and composition of the milk are discussed in 12.3 and 12.4.

12.2 STAGE OF GROWTH AND MILK YIELD AND COMPOSITION.

In the two experiments in which the effect of stage of growth on milk production was examined, the short Ruamui pasture supported a higher milk yield than the longer and more mature pasture. The result was consistent with the well known reduction in the digestible energy value of pastures with increasing maturity (1.2). The intake of the short pasture would also be expected to be higher than that for the mature pasture if the results of Blexter, Wainman and Wilson (1961) apply to the feeding of fresh pastures. The changes in milk composition obtained, with one notable exception, were also consistent with the suggestion that the digestible energy intakes of the cows grazing the short pasture were higher than those grazing the more mature growth.

The exception mentioned above, was the significantly ($P < 0.01$) lower solids-not-fat percentage in the milk produced by the cows grazing the short Ruamui ryegrass in the first of the two experiments. This result was of particular interest and importance because high, rather than low, solids-not-fat percentages in milk are normally associated with high quality pastures. Several subsequent experiments (see 12.4) were designed specifically to explain this unexpected result.

12.3 PROCESSES CONCERNED IN THE PRODUCTION OF MILK CONTAINING A LOW FAT PERCENTAGE.

The lowered butterfat percentage in the milk from the cows grazing Western Woths ryegrass during the winter, was associated with a decreased proportion of acetic acid and an increased proportion of propionic acid in the samples of rumen liquor taken from the cows grazing this ryegrass. These changes in the molar proportions of volatile fatty acids, probably adequately explained the change in

fat percentage in view of the results from numerous experiments in which reductions in the fat content of milk have been obtained when cows were fed diets low in roughage and high in starchy concentrates. Such rations have been shown to result in low acetic acid and high propionic acid proportions in the rumen (e.g. Balch, Balch, Bartlett, Bartram, Johnson and Turner, 1955). The change in the ratio of the acetic to propionic acids in the current experiment was also adequately accounted for by the differences between the ryegrasses in carbohydrate composition. The ratio of readily available carbohydrates to the content of crude fibre was considerably higher for Western Wolths than for Paroa ryegrass (1.82 v. 1.52).

The possible biochemical pathways involved in the alteration in fat percentage following changes in the molar proportions of acetic and propionic acids were not investigated in this study, but they have been discussed by Van Soest in a recent review (Van Soest, 1963).

12.4 LOW SOLIDS-NOT-FAT PERCENTAGES ASSOCIATED WITH RUANUI RYEGRASS

The possible reason for the occurrence of low solids-not-fat percentages in milk when cows graze Ruanui ryegrass was investigated in a number of experiments. The separate measurement of the protein content, or protein and lactose contents in milk, rather than solids-not-fat content, indicated that a change in protein generally accounted for considerably more than half of the observed change in solids-not-fat content. For this reason the experiments undertaken were mainly orientated towards investigating changes in milk protein content.

The initial experiments (1 and 2), in which the changes in solids-not-fat percentages were demonstrated, provided some information on the chemical composition of the Ruanui ryegrass and also the characteristics of the cows at

the time. The pastures were high in crude protein and ether extract and low in nitrogen-free extract. The other consistent effect observed was that the reduction in solids-not-fat percentage was associated with, but not entirely due to, a reduction in plane of nutrition as indicated by a lowered milk yield (see 4.4).

Experiment 3 (Chapter 5), which involved the comparison of Ruamui ryegrass pastures which had received high and low levels of nitrogenous fertilizer, confirmed all of the above points and furthermore provided evidence which probably eliminated the possibility that the non-protein nitrogen content or the alkaloid content of the pastures was directly responsible for the observed effect. A major shift in the relative proportions of the individual volatile fatty acids in rumen liquor was also ruled out as a possible reason for the changes in solids-not-fat percentage in milk. Subsequent experiments were directed towards examining the possibility that the effect resulted from poor utilization of dietary protein in the rumen. When high and low nitrogen Ruamui ryegrasses were fed to young growing sheep (Chapter 6) the dietary nitrogen in the high nitrogen grass was used less efficiently than that in the low nitrogen pasture, but the absolute level of nitrogen retention was largely determined by dry matter intake and was not restricted by poor utilization of digestible protein. Because protein requirements for milk production are considerably larger than those for the synthesis of body protein it was considered that further experiments were warranted.

An attempt was then made to increase the utilization of dietary protein by adding additional starch (Chapters 7 and 8) and protein (Chapters 10 and 11). From the results in Experiment 6 (Chapter 8) it was clear that the added starch, which would be expected to increase the utilization of dietary protein in the

rumen (Head and Murdock, 1965) had a favourable effect on milk protein percentage. Direct evidence to suggest that the mechanism of action of starch was to improve the utilization of dietary protein was limited, however, to the finding of a low ammonia concentration in the rumen liquor obtained from one member of a fistulated pair of twins which received starch compared with its mate which received no supplement (Table 8.3).

The demonstration that supplementary protein, treated so that it was resistant to degradation in the rumen but still available for digestion in the abomasum and intestines, resulted in an increase in milk protein content (Table 11.1) supported the hypothesis that low protein percentages in milk may be caused, at least in part, by poor utilization of the digestible protein intake of cows grazing Ruanui ryegrass. Whether this effect (and the positive effect from starch) resulted from the amount of protein absorbed, or from the energy contained in the supplement, could not be determined in these experiments.

It may be concluded that no single factor was shown to be responsible for the low protein percentage in the milk produced from cows grazing Ruanui ryegrass. This may have been due to the fact that the change in solids-not-fat percentage could not be replicated in all experiments and, therefore, supplementary feeds were not given the best opportunity of reversing the change in milk composition. Alternatively, in view of the range of effects demonstrated in this study (positive effects on milk protein from starch and protein, and negative from oil) it is probably reasonable to postulate that the variable results obtained from the Ruanui ryegrass pastures were due to a number of factors associated with high nitrogen pastures which combined together to give large effects in some cases, and smaller effects in others. Interactions between the various components of the ryegrass (carbohydrate, fat and protein) which were not examined to any extent in this study may, therefore, be of considerable importance.

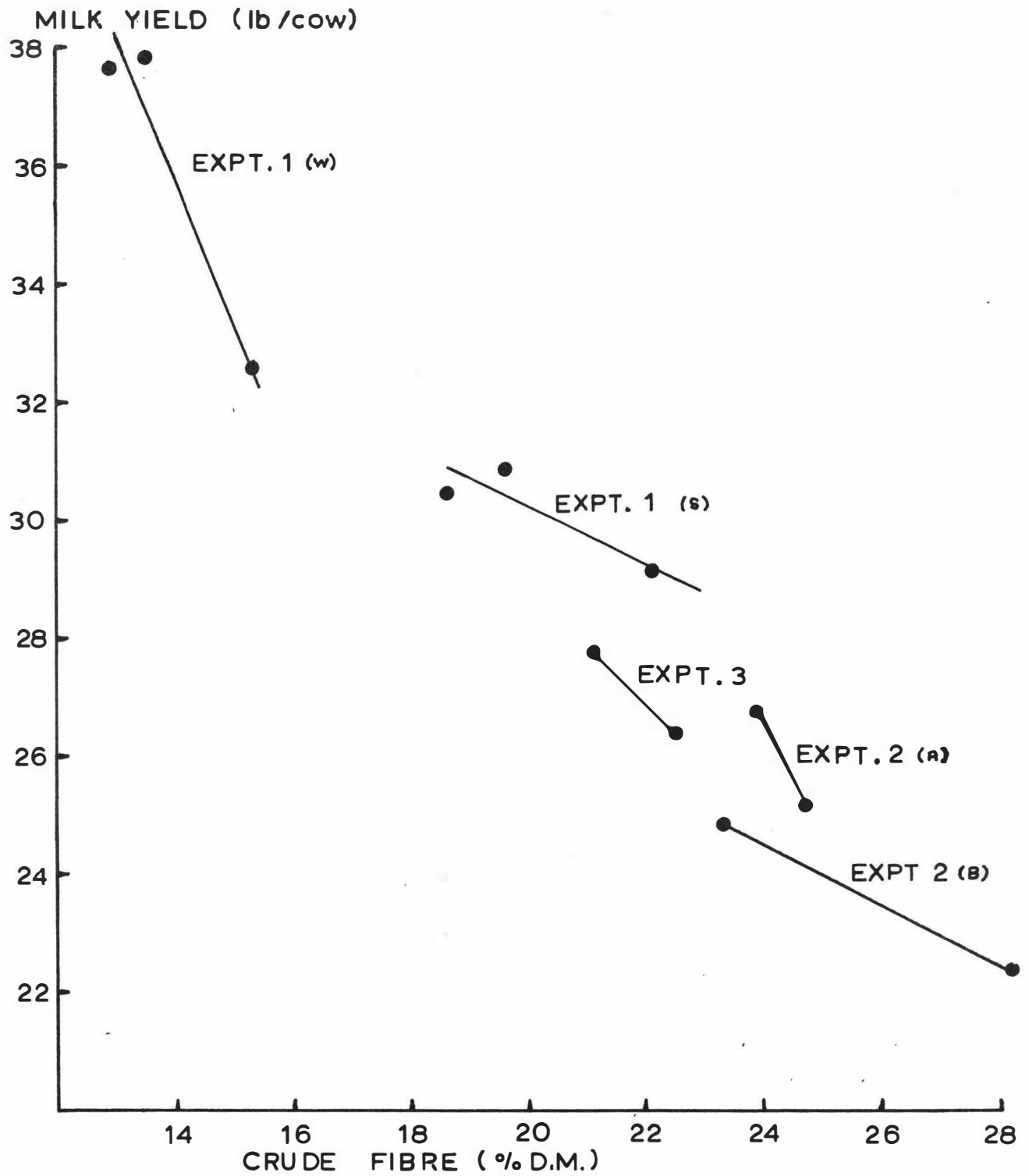


Fig.12.1 The relationship between the crude fibre content of ryegrass pastures and the mean daily milk yields from cows grazing the pastures, in a number of different experiments.

12.5 THE RELATIONSHIP BETWEEN THE CHEMICAL COMPOSITION OF PASTURE AND MILK PRODUCTION.

The data from the comparisons of three varieties of ryegrass, and Ruamui ryegrass at two stages of growth and two nitrogen levels, provided the opportunity to look for general relationships between plant composition and the yield and composition of milk which may be of value to plant breeders in deciding selection objectives when endeavouring to improve the nutritive value of pasture plants.

Because of the extreme difficulty involved in altering the concentration of a single specific component of ryegrass, experiments involving supplementary feeding (starch, protein and oil) were undertaken and these data also provide some information relating to optimum plant composition for milk production, provided that the supplements used are assumed to have similar effects to those which would be obtained if the supplement was an integral part of the plant material.

The relationship between plant composition and milk production will be examined separately for milk, fat, and protein production, as the optimum plant composition probably differs according to the type of production required.

Milk yield: The only component of pasture which was shown to be consistently related to milk yield was that of crude fibre. Within all the experiments undertaken milk yield was inversely related to the crude fibre content of the pasture grazed (see Figure 12.1). This relationship is probably adequately explained by the fact that, for feedstuffs of a similar type (e.g. ryegrasses), the organic matter digestibility is normally inversely related to the crude fibre content of the forage (Raymond et al., 1960). This was confirmed in this study in all experiments in which both variables were measured (Experiments 1 and 3). A low content of crude fibre in pasture is therefore required to provide a pasture

of high digestibility, which may also in turn lead to high intakes (Blaxter, Wainman and Wilson, 1961) and therefore high milk yields.

While it may be true that ryegrass pastures with low crude fibre contents are to be preferred for milk production, pastures of this type may be associated with health problems in dairy cattle as evidenced by the occurrence of grass tetany in Experiment 1. Two other problems associated with grass containing very low levels of structural carbohydrates may be of importance. Firstly, the dry matter content of the pasture, which is normally positively related to the crude fibre content, may be too low to enable the cow to satisfy its requirements for energy, within the limits of appetite. Secondly, rations with a low content of structural carbohydrates may lead to rumen dysfunction (Trenore and Merrill, 1967) and losses of production.

Fat yield: Fat production was closely related to milk production (see Experiments 1, 2 and 3) and therefore also inversely related to the crude fibre content of pastures. For fat production, however, the level of soluble sugars was also important as evidenced by the difference between Western Wolths and Paroa ryegrasses in Experiment 1, both of which had fairly similar contents of crude fibre. The ratio of soluble sugars to crude fibre in the case of Western Wolths was 1.82 compared with 1.52 for Paroa ryegrass. It might be expected that maximum fat yields will be obtained under conditions where the proportion of structural carbohydrates is as low as possible without affecting fat percentage by altering the proportions of individual volatile fatty acids produced. Fat percentage was reduced when the molar proportion of propionic acid reached 22.8% and this corresponded to a soluble sugar/crude fibre ratio of 1.82.

Milk fat production was increased by peanut oil supplements, the level given (440 ml.) being equivalent to changing the ether extract of the pasture from about 6% to between 9 and 10% in the two experiments. While these experiments with one level and type of oil do not provide conclusive proof of the importance of the lipid content of pastures, it should be noted that in several experiments (2A and 2B) the fat percentage of milk was correlated with level of ether extract in the pastures when it would have been more reasonable to expect fat percentage to be inversely related to milk yield. Further work to determine the optimum level of lipid in pastures seems warranted.

Protein yield: Protein yield was generally positively related to milk yield (Experiments 1 and 2B) but as discussed previously the protein percentage of the milk was not always positively related to milk yield, at least for Ruanui ryegrass. As high a ratio as possible of soluble carbohydrates to crude fibre in pasture would, on the evidence from this study, be expected to maximize milk yields and protein contents in milk. This conclusion was indicated from the variety comparisons (Experiment 1) and was endorsed by the experiments in which starch supplements were fed (Chapters 7 and 8). There is, however, probably a limit to the extent to which the crude fibre content of the ration could be reduced while still maintaining healthy animals (see previously).

With respect to the protein content of the pastures, it was clear that the levels were generally high, rather than too low, and that the real problem lies in how to improve the utilization of the protein present. Again a relatively high level of soluble carbohydrates in pasture should be beneficial (Chapter 8).

It seems reasonable to assume that there is an optimum lipid content for pastures to maximize milk protein synthesis since very low levels may result in poor utilization of dietary carbohydrate (see 9.1) but that high levels may reduce the digestibility of the carbohydrate and protein components of pasture or even reduce voluntary intakes (Chapter 9).

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APPENDICES

A copy of all the analyses carried out for this Thesis has been lodged with the Dairy Husbandry Department. An example of each type of analyses used is, however, included in this section.

LIST OF APPENDICES

- 2.1 Total soluble sugar content of two ryegrass swards sampled twice daily on three consecutive days.
- 3.1 Analysis of covariance and test of significance of adjusted treatment means. Milk yields adjusted for differences in milk yields during the preliminary period (winter experiment).
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- 3.3 Organic matter digestibilities (in vitro) of three ryegrasses.
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THE CONVENTIONAL ABBREVIATIONS USED IN THE TEXT

N.S.	Results not statistically significant at the 5% level.
$P < 0.05$	Results statistically significant at the 5% level.
$P < 0.01$	Results statistically significant at the 1% level.
F	The variance ratio for specified conditions.
S.E.	Standard error of the mean.
b	Regression coefficient.
df	Degrees of freedom.
SS	Sums of squares.
M.S.	Mean square.

APPENDIX 2.1

Total soluble sugar content of two ryegrass swards
sampled twice daily on three consecutive days.

Time of sampling	Fresh samples		Freeze-dried samples	
	9a.m.	3p.m.	9a.m.	3p.m.
<u>Long Ruanui ryegrass</u>				
26.9.65	16.5	18.4	16.6	17.8
	12.0	16.9	16.0	17.0
27.9.65	16.5	16.7	14.6	15.5
	13.0	15.9	14.8	16.5
28.9.65	14.9	18.0	15.5	17.6
	15.9	17.0	15.9	17.6
Mean	14.8	17.1	15.6	16.3
S.E.	1.90	0.91	0.76	0.88
<u>Short Ruanui ryegrass</u>				
26.9.65	15.0	16.0	14.1	15.8
	12.8	18.0	13.9	15.8
27.9.65	11.9	15.2	13.6	14.8
	14.1	15.2	13.2	15.8
28.9.65	14.2	14.2	14.9	16.2
	15.6	18.8	14.1	17.0
Mean	13.9	16.2	14.0	15.9
S.E.	1.37	1.79	0.57	0.71

APPENDIX 3.1

Analysis of covariance and test of significance of adjusted treatment means. Milk yields adjusted for differences in milk yields during the preliminary period (Winter experiment).

Source	df	Sx ²	Sxy	Sy ²	Errors of estimate			F Ratio and Signif.
					SS	df	MS	
Total	16	820.647	792.241	1133.943	369.128	15		
Treatments	2	39.356	16.316	85.628				
Error	14	781.291	775.925	1048.315	277.719	13	21.36	2.14
					91.400	2	45.70	N.S.

Average within treatment group regression coefficient = $\frac{775.9}{781.3} = 0.99$

Adjusted treatment group means

Western Wolthe	35.32	+	(2.37 x 0.99)	=	37.09
Paroa	39.01	-	(1.12 x 0.99)	=	37.89
Ruanui	33.80	-	(1.25 x 0.99)	=	32.55

Analysis of error variance (Regression of milk yield during experimental period on milk yield during preliminary period).

Source	df	SS	MS	F Ratio	Signif.
Total	14	1048.315			
Due to regression	1	770.596	770.596	35.9	P < 0.01
Error	13	277.719	21.36		

APPENDIX 3.2

Analysis of variance for molar proportions of propionic acid in rumen liquor of individual cows during the winter experiment.

Source	df	SS	MS	F	Signif.
Total	11	60.01			
Treatments	2	42.61	21.30	12.40	P < 0.05
Error	9	17.40			
Cows : Treatments	3	5.15	1.72	0.84	N.S.
Within cows	6	12.25	2.04		

Duncan's multiple range test to determine which means differ significantly.

S.E. = 0.29

For 5% level of significance the shortest significant range is :- 0.29×4.5 (From Duncan's tables) = 1.31

Ruanui ryegrass	Paroa ryegrass	Western Wolths ryegrass	
18.28	20.25	22.88	
<hr style="width: 50%; margin: 0 auto;"/>		<hr style="width: 50%; margin: 0 auto;"/>	
1.97		2.63	
<hr style="width: 100%;"/>			
4.60			

That is all differences between the means were significant (P < 0.05).

APPENDIX 3.3

Organic matter digestibilities (in vitro) of three ryegrasses.

A small additive correction was made to the in vitro digestibilities on the basis of the difference between the in vitro value on the standard grass and its known in vivo value (69.20).

Winter experiment

	<u>Standard grass</u>	<u>Western Wolths</u>	<u>Paroa</u>	<u>Ruamui</u>
	70.40	85.14	81.08	83.45
	71.91	84.90	81.79	83.33
	71.51	84.42	82.96	82.38
	71.80	84.42	83.08	81.78
Mean	71.40	84.72	82.38	82.74
Corrected mean	69.20	82.52	80.18	80.54

Spring experiment

	<u>Standard grass</u>	<u>Western Wolths</u>	<u>Paroa</u>	<u>Ruamui</u>
	70.01	73.13	70.32	70.13
	69.51	71.69	71.99	71.96
	68.10	73.45	69.64	70.01
	69.86	68.53	71.01	68.46
Mean	69.37	71.70	70.64	70.14
Corrected mean	69.20	71.53	70.47	68.97

APPENDIX 6.1

Original data for Experiment 4 (Chapter 6).

Treatment Sheep	High Nitrogen			Low Nitrogen		
	1	2	3	4	5	6
Feed						
Dry matter intake (g./day)	693.9	547.8	496.1	698.7	500.0	601.0
Nitrogen content (%)	3.27			1.90		
N intake/day (g.)	22.69	17.91	16.22	13.27	9.50	11.42
Faeces						
Dry matter output (g./day)	218.8	166.5	151.2	251.1	161.4	220.3
Nitrogen content (%)	2.72	2.79	2.86	1.88	2.03	2.03
N output/day (g.)	5.95	4.65	4.32	4.72	3.28	4.58
Urine						
N output/day (g.)	15.18	12.32	11.30	7.30	5.37	5.89
Nitrogen balance (g./day)	+1.56	+0.94	+0.60	+1.25	+0.85	+0.95
N digestibility coef.(%)	73.8	74.0	73.4	64.4	65.5	59.9
Treatment mean		73.7			63.3	
D.M. digestibility coef.(%)	68.5	69.6	69.5	64.1	67.7	63.3
Treatment mean		69.2			65.0	
N balance/N intake (%)	6.88	5.24	3.70	9.42	8.95	8.32
Urinary N/N intake (%)	66.9	68.8	69.7	55.0	56.5	51.6
Urinary N/Digestible N(%)	90.7	92.9	95.0	85.4	86.3	86.1
Treatment mean		92.9			85.9	
Body weight of animals (lb.)	65.5	61.5	55.0	72.0	60.0	59.0

APPENDIX 8.1

Analysis of regression of rumen liquor ammonia concentration on volatile fatty acid concentration and test of significance of differences between regressions within treatment groups.

SSx 212.59 SSy = 8548.01 SPxy 895.81
 b = 4.21 r = 0.52 (P < 0.01)
 \bar{y} = 35.3 \bar{x} = 12.9

Test of significance of b

Source	df	SS	MS	F	Signif.
Total	47	8548.01			
Lin.Reg.	1	3774.76	3774.76	36.4	P < 0.01
Error	46	4773.85	103.78		

Test of significance of differences between regressions within treatment groups.

Source	df	Sx ²	Sxy	Sy ²	Errors of Estimate			F Ratio and signif.
					SS	df	MS	
Starch	15	90.20	394.74	3060.11	1332.02	14		
Control	15	67.92	308.83	2784.65	1380.41	14		
Oil	15	30.42	206.00	2656.12	1274.03	14		
Deviations from average regression (S+C+O)	45	188.54	908.57	8500.88	4122.49	44		
Deviations from individual regressions					3987.66	42	94.94	1.41
Differences between individual treatment group regressions					134.83	2	67.41	N.S.