An evaluation of major nutrients in dairy pasture in New Zealand and their effects on milk production and herd reproductive performance

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ABSTRACT

This thesis presents the results of seven experiments or trials between August 1990 and November 1994 designed to study the causes and effects of the variation in nutrient content within dairy pasture in New Zealand and their impact on dairy cow lactation and reproductive performance.

The work includes the results of two observational studies; a survey of seasonal variation in dairy pasture nutrients on four dairy farms; two controlled field trials of supplementation of pasture fed cows in seven commercial dairy herds (involving 1650 cows); an experiment recording changes in pasture nutrients with grazing, maturity and soil phosphate levels; and a replicated split plot trial measuring changes in pasture nutrients after nitrogen (N) application. Trials or experiments involved aspects of agronomy and pasture management, herd reproductive performance and dairy cow nutrition.

A common theme of the work was examination of factors affecting the high crude protein levels present in the diets of dairy cows consuming fresh ryegrass/white clover pasture, measurement of this and testing of some practices that may affect the productive penalties caused by these high protein levels.

Section 1 of the thesis deals with the initial observations (Chapter 1) and a survey of pasture nutrient changes through all seasons on four dairy farms (Chapter 2).

The first chapter describes the initial observational studies over two springs (1990 and 1991) in nine commercial dairy herds and additional survey information from 35 herds (1991). There was a strong negative relationship between urea levels in blood (or milk) and milk production in three separate datasets using principal component analysis (PCA). Milk urea levels related closely to pasture protein levels and especially protein/soluble carbohydrate ratios in pasture. Herd reproductive performance was also worse in the herds with higher urea levels. For example, the four herds observed in

1990 averaged 23.62, 24.09, 20.91 and 21.88% for pasture crude protein; 7.38, 8.20, 5.85 and 6.20 mmol/l for serum urea; and 0.74, 0.75, 0.94 and 0.91 kg milkfat/cow/day respectively over the 17 week period. "Empty" (non-pregnant) cow percentages for the herds were 10.6%, 4.2%, 1.8% and 3.1% respectively. Tentative conclusions were made on the basis of these findings relating especially to the potential negative effects of excess dietary crude protein in pasture on milk production and on herd reproductive performance. These conclusions were then explored in more depth and reported in subsequent chapters.

Seasonal changes in pasture nutrients on dairy farms were measured by analysing pasture collected over two years from four dairy farms of varying soil type and climate (Chapter 2). Two of the farms were at Massey University and two in the Waikato district. All farms were of above average productivity for their district. Samples were collected every two weeks from each farm and represented pasture about to be consumed by cows on these farms. These were analysed for major nutrients or analytes (crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), soluble carbohydrates (SOLCHO), pectin, digestibility (DOMD), potassium, calcium, phosphorus, and magnesium) using near infra red spectrometry (NIRS). Highest pasture CP, DOMD, and SOLCHO levels were found in spring and autumn (ranging from 23.6-25.8%, 75.4-78.1% and 9-12% DM respectively) with lowest ADF, NDF and pectin levels (ranging from 27-28%, 36-38% and 1.8% respectively). The converse applied to the summer period with 20-22% CP, 70-71% DOMD, 8-10% SOLCHO, and 29-31% ADF, 42-45% NDF and 2-2.5% pectin. Calcium and magnesium levels were highest in summer (0.8% and 0.2% respectively compared to 0.65% and 0.19% respectively), and potassium higher in spring and winter (3.2%). The potential consequences for milk production from dairy cows calving seasonally are discussed, with particular reference to the imbalance in the rumen between rumen degraded protein and fermentable carbohydrates. Especially notable were the seasonal differences in protein levels and the changes in the type of carbohydrate available in late spring/summer. Soluble carbohydrate decreased, and fibre expressed as NDF and ADF increased in late spring and summer.

Section 2 of the thesis deals with supplementation trials on 6 commercial dairy herds (Chapter 3) and another supplementation trial on a 7th herd involving maize silage and concentrates (Chapter 4).

Controlled supplementation trials on six commercial dairy herds (total 1380 cows) were carried out in spring 1992 to examine the reproductive and productive effects of supplementing pasture-fed cows with carbohydrates (either soluble carbohydrate or starch). Herds were split into treated and control groups on each farm to remove individual farm factors from the experiment and relatively low levels of either molasses (3 herds, 700 mls molasses/cow/day) or concentrate (3 herds, 1.3 kg concentrate/cow/day) were fed for an extended period in spring (approximately 90 days, from 1 September to 25 November). Significant milk production and reproductive effects were measured when results were pooled for all herds. Immediate responses were approximately 0.5 litres of milk per kg of supplement on average, but the main milk production response was observed later in the experiment (October and November) and was higher in better fed herds and those in better body condition. No effect was found on non-return rate or submission rate, but empty cow rates at the end of the mating season in the supplemented group were half those of the control groups (2.7% vs 5%). These results may indicate considerable productive and reproductive advantage in supporting pasture fed cows through October/November with appropriate supplement when ryegrass is in the reproductive phase, and has reduced digestibility which is likely to limit intake of ME. Improving diet quality or ME concentration at this stage may help reduce the monthly decline from peak lactation which typically occurs at this time in most districts in New Zealand.

Chapter 4 describes a controlled supplementation trial which was carried out in spring 1993 on a 240 cow commercial dairy herd where the diet was formulated according to recommended nutrient levels for high production (NRC, 1989). The diet was improved in content of "bypass" protein, soluble carbohydrate, lipid and minerals. The base diet for control and treated groups was pasture and maize silage. Both control and treated herds were offered the same amount of metabolisable energy (ME) - ie. the diets were iso-energetic. Improved milk production (2 litres milk) and reproduction (2.7% empty vs 6%) occurred in response to the addition of the balancing concentrate in the treated

group. There was a large carryover effect when the concentrate feeding ceased and the sole diet was pasture. Pasture dry matter assessment indicated the supplemented cows continued to consume more dry matter than control cows. The immediate response to supplementation was 1.25 litres/kg DM of supplement, and with the carryover response added exceeded 2.5 litres/kg DM of supplement. The immediate response improved after supplementation had continued for 2-3 weeks. This trial did not show substitution for pasture, but the converse. Improving the balance of dietary nutrients in pasture did improve performance.

Section 3 of the thesis deals with aspects of grazing management, agronomy and the effects of application of nitrogen to pasture on the nutrients within pasture (Chapters 5 and 6).

Variation in pasture nutrients from week to week was evident in the seasonal study presented in Chapter 2. More information regarding changes in pasture nutrients after grazing and as pasture matures was sought because this was considered a likely source of variation in productivity. In Chapter 5 nutrient levels in pasture were determined after grazing or in pasture left ungrazed by sampling every five days during spring from five sites located on two dairy farms. Sites were either grazed as part of normal rotation (3 sites) on the farm or were caged (2 sites) to prevent grazing. Conclusions from this study were limited by a lack of replication, but nevertheless highlighted reduced CP with maturity, increased NDF with maturity and immediately after grazing, reduced SOLCHO just after grazing and reduced digestibility with the advancement of spring into October. Pectin and calcium levels increased as spring advanced. The results were consistent with literature on the subject.

The effects of the level of nitrogen fertiliser and the timing of application in spring on pasture nutrient composition were examined in the final experiment reported in Chapter 6. Nitrogen was identified from the literature as one of the main external influences likely to affect pasture protein levels. Nitrogen was applied as urea to small (2 m^2) plots at 0, 20, 40 and 80 kg N/ha and at varying times (15 August, 31 August and 14 September) in late winter/early spring to dairy pasture at the Massey University Dairy Research Unit. The trial was a replicated split plot design with levels of N randomised

within starting dates. Significantly reduced ADF and NDF levels, reduced SOLCHO, reduced dry matter %, and increased CP levels occurred after N application. Higher N rates produced greater changes. Application of N earlier in winter resulted in greater effects on ADF (2% difference vs 6%), NDF (2% difference vs 6%) and CP (5% vs 7%) but lesser effects on SOLCHO (1.5% difference vs 0.5%) and these lasted longer in wintery conditions. Effects on SOLCHO were more marked later in the experiment. Brix values (a refractometer measurement of juice squeezed from the herbage sample) were also examined as part of this study to evaluate their usefulness as a rapid measure of SOLCHO concentration; results were inconclusive. The consequences of the effects of N on pasture for dairy cows are discussed and possible dietary or management improvements to minimise the consequences are suggested. The increased protein and reduced fermentable carbohydrate (reduced SOLCHO and reduced ADF or NDF) mean that poorer rumen fermentation could occur after N application, with lower amounts of bacterial tissue presented to the small intestine from ruminal fluid.

A final summarising chapter (Chapter 7) combines the conclusions from the various studies, indicates the need for further information and discusses how this might be obtained. Studies presented in this thesis have not conclusively shown that high CP in pasture has damaging effects on productivity, but have indicated strong associations and various factors influencing pasture CP and also other pasture nutrients.

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FOREWORD

This thesis began with on-farm observations made over a period of 16 years as a practising veterinarian on the frequency of dairy herd reproductive problems in seasonally calving dairy herds in the Waikato district. In adverse springs (very wet or overcast weather for prolonged periods in August/September/October) the incidence of anoestrus, poor conception and non-pregnant cows increased. Milk productivity was also correlated with herd reproductive performance, with better performance in high producing herds. Assessment of pasture suggested that poor performance did not necessarily relate to the quantity of dry matter available to the cows (as was often assumed), and the hypothesis formed was that changes in nutrients within pasture were at least in part responsible for differences in herd reproductive performance, and that these changes would reflect in selected blood parameters in cows within these herds.

The objectives of the studies reported in this thesis were a) to test these hypotheses in the context of commercial dairy herds, b) to evaluate the impact of alternative management practices on the nutrient balance of grazed pasture, and c) to assess the value of alternative supplementary feeding strategies in overcoming the limitations of grazed herbage as a source of nutrients for lactating dairy cows.

Studies began in 1990 when four herds were selected for their likely herd reproductive performance and herd milk production performance based on previous client records in the veterinary practice. These herds were monitored in detail for reproductive performance, milk production, changes in selected blood parameters, and the nutrients within the pasture consumed. Interactions between the measured data were then examined and interpreted. The observational study was repeated in 1991 in order to include dry matter intake and bulk vat milk urea measurements. The observational studies provided strong evidence of associations between weather conditions, pasture nutrients, blood parameters, herd reproductive performance and milk production.

The observational studies led to a survey establishing normal seasonal variation in pasture on dairy farms, controlled field trials with supplements designed to address

nutrient deficiencies identified in pasture, and studies designed to identify factors affecting nutrient levels in pasture like fertiliser application of phosphate and nitrogen, and also the effects of grazing and maturity on pasture nutrients.

The thesis consists of 3 sections. The first section includes the results of the initial observational studies and the survey of seasonal variation in pasture nutrients from four dairy farms. These serve as the basis from which the other work developed, although chronologically the survey of seasonal variation occurred after some of the other work was already complete. It was realised that this fundamental survey information (Chapter 2) was not available in the literature. The second section presents the results of controlled supplementation experiments in commercial dairy herds in spring where the pasture diet was supplemented with nutrients designed to correct imbalances in pasture identified in Section 1 when compared to recommendations for high producing cows. The third section presents the results of two experiments designed to clarify aspects of pasture management and fertiliser use likely to influence pasture nutrient status. In particular, the effect of grazing, pasture maturity, soil phosphate status, nitrogen (N) application to pasture and the timing of N application in the winter spring period were examined. A concluding chapter links the work in the 3 sections and suggests further studies to extend the results presented.

The subject matter of the thesis is varied, and therefore the normal thesis convention of an introductory literature review has not been followed. Instead, each chapter starts with an extended introduction in which the appropriate literature is cited. Chapter 7 then links the findings in the various chapters and makes conclusions. Physical assistance with the studies is acknowledged at the end of each chapter.

Commercial dairy farms were selected for most of the trial work in an attempt to keep the work relevant to practical circumstances encountered on farms. This made for difficulty in working with standard statistical design, but provided the opportunity to work with substantial numbers of cows (eg 1400 cows for the carbohydrate supplementation trial in Chapter 3) and a more powerful basis for ensuring effects on reproductive performance.

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SECTION 1

Consists of two chapters that develop the original hypotheses and provide information about seasonal variation in New Zealand dairy pastures

Chapter 1

Observational studies in commercial dairy herds linking pasture nutrients with milk production and herd reproductive performance

Pasture feeding as practised in the New Zealand seasonal dairy system is briefly described. Herd reproductive performance in this dairy system is summarised. An observational study of four dairy herds monitored over spring is described. Strong and consistent statistical correlations between pasture nutrients, milk production and milk (or blood) urea are established and associations with reduced herd reproductive performance were made.

Chapter 2

Seasonal variation in nutrient levels of NZ dairy pastures

Seasonal patterns in pasture nutrients are established on four dairy farms over 2 years, two at Massey University and two in the Waikato district. These are used to discuss nutrient inadequacies in pasture for lactating dairy cows.

CHAPTER 1

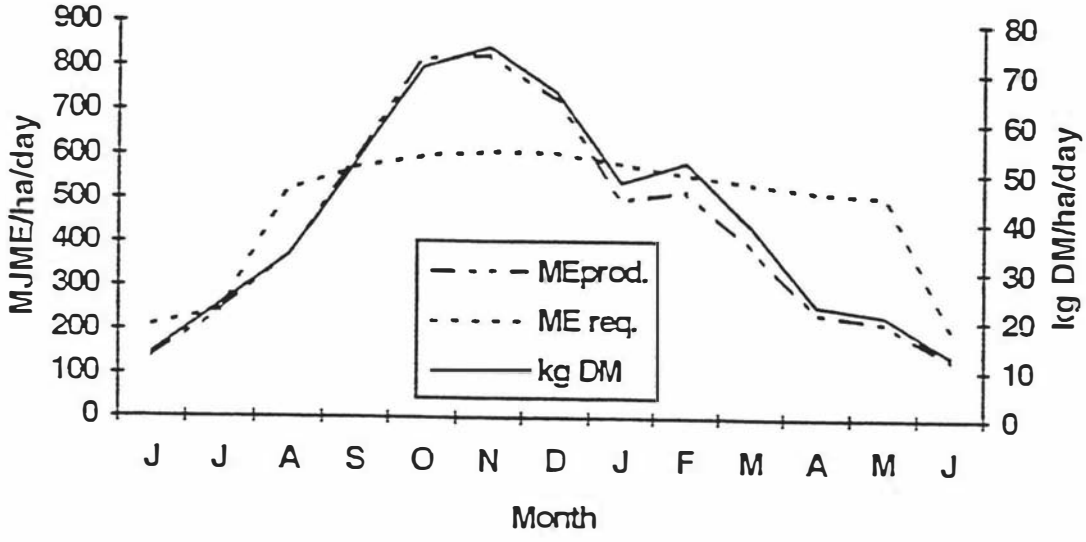
Observational studies in commercial dairy herds linking pasture nutrients with milk production and herd reproductive performance

1.1 INTRODUCTION

1.1.1 The dairy system practised in New Zealand

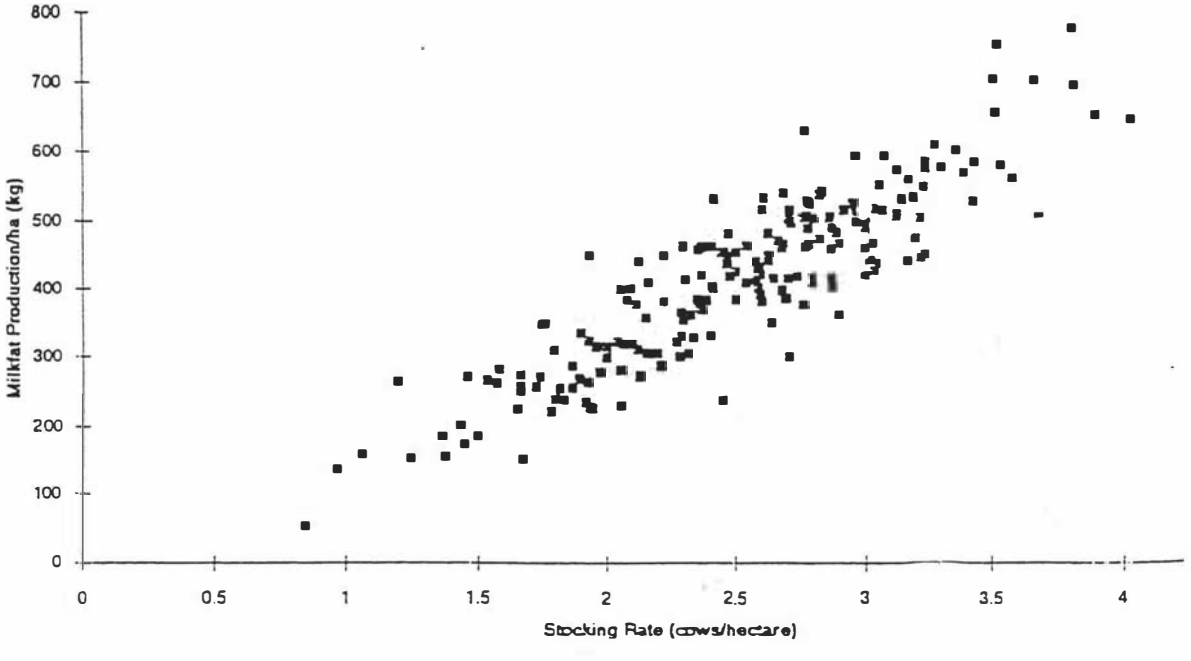
The dairy system practised on most seasonal calving dairy farms in New Zealand involves cows calving from late July to mid September in anticipation of increased spring growth rates of pasture that will match the cows' requirements for dry matter or metabolisable energy (ME) as closely as possible. Generally speaking cows only eat grazed pasture, but they may also be fed small quantities of stored pasture in the form of silage or hay for short periods. Pasture growth begins to accelerate in August and generally reaches maximum rates near the end of September or early October. All cows should have calved by this time to maximise efficiency of use of grown pasture by grazing. All cows are dried off in April/May to help conserve feed for dry cows in winter and save pasture for the following spring. Stocking rates are usually set to fully feed cows on pasture in spring and allow a small surplus to be conserved as silage or hay, which is then reintroduced to the cows at a later date when pasture growth is less than cow requirements (typically in a dry summer, late autumn or winter). Typical periods of feed surplus and deficit are presented in Figure 1.1.

Figure 1.1 **Average pasture dry matter and metabolisable energy production for No 2 dairy at DRC and the feed requirements for a herd stocked at 3.0 Friesian cows/ha and fully fed from peak to end of season** (peak 2.0 kg MS/cow/day and 7% monthly decline to a 305 day lactation; from Thomson and Holmes,1995)



The range of milkfat/ha and stocking rate for a group of farms in the 1991/1992 season are presented in Figure 1.2 (LIC Consulting Officer Survey data for 1991/1992). Generally, productivity per hectare lifts with stocking rate, and farm profitability often does also, although farm type (soils, terrain), cow quality and farmer management skills would also influence this relationship.

Figure 1.2 **Stocking rate versus milkfat production/ha** (Consulting Officer survey data from the 1991/92 season)



Various management techniques have been developed to conserve feed (as stored feed or stored in situ as ungrazed pasture) for periods of slow pasture growth, like alteration of rotation length, application of nitrogen in anticipation of higher requirement, and also use of cow's body reserves of condition (fat and protein) in periods of shortage. High utilisation by cows of pasture grown is an important requirement of profitable pasture farming, and generally this is most efficiently achieved by direct grazing of pasture. Ensiling or storage of surplus pasture by other means will result in increased losses of DM/ha, and excess pasture availability per cow will tend to increase losses from senescence and decay. As well as these losses, pasture digestibility may suffer when excess pasture is available and lead to lower intake and lower productivity. For these reasons high stocking rates should be maintained.

Based on experience as a dairy cattle veterinarian for 20 years, variation between herds in reproductive performance and milk production is considerable, with the same herds tending to repeat levels of reproductive performance every year at similar levels. However, in some years spring weather conditions can be overcast and wet for 1-2 months, with little sunlight until October. These conditions appear to be negatively associated with milk production, cow health and herd fertility even though the growth of pasture has appeared to be above average for this time of the year and dry matter allowances apparently sufficient to feed cows to appetite. It was hypothesised that these effects on reproduction and milk production were likely to be occurring indirectly through effects on the nutrients within pasture eaten. Direct effects of weather on animal performance seemed less likely.

The decision was made to investigate the potential links between pasture nutrient content, weather conditions, herd reproductive performance, milk production and cow blood parameters by observing these variables simultaneously in selected herds that were likely to have low productivity and poor herd reproductive performance or vice versa based on their history in previous seasons. This selection meant differences in variables were more likely to be observed, but extrapolation to other farms should be made more cautiously. There was limited literature linking these variables in local conditions and observation suggested there were likely to be interactions.

1.1.2 Nutritional quantity and quality in a pasture based dairy system

Dairy farmers in New Zealand have been taught to express dairy cow energy requirements and intakes in terms of dry matter (kg DM/day) and occasionally metabolisable energy (ME). Quality of pasture has been assessed mainly in terms of digestibility (in vitro). This simplification of dairy cow requirements may be adequate where low levels of per cow milk production are expected (the average litres/cow/lactation is 3360 litres in NZ (Holmes and Hughes,1993)), or where pasture and pasture derived supplements are the sole diet. The true requirements for dairy cows are more complex than this however and there are several known inadequacies or excesses in pasture that are likely to impact on production and reproduction (NRC, 1989; Muller, 1993; Ulyatt and Waghorn, 1993; Muller *et al.*, 1995; Moller *et al.*, 1996b; see Chapter 2 for fuller discussion).

Pasture is a variable food source, changing with seasons, growing conditions, maturity, species and management practices in the quality of nutrients available and also in amount available. The most notable excesses, imbalances or inadequacies that could restrict production or reproduction when compared with recommended diets would include:

- excess crude protein, especially the rumen degraded portion (RDP) (Danfaer *et al.*, 1980; Ulyatt and Waghorn, 1993).

- reduced levels of readily fermentable carbohydrates, like starch and soluble carbohydrates (Muller, 1993).

- sometimes low dry matter %, which may restrict intake (Verite and Journet, 1970; John and Ulyatt, 1987; Ulyatt and Waghorn, 1993).

- excessive fibre levels (as measured by acid detergent fibre (ADF) and neutral detergent fibre (NDF)) (Mertens, 1985) and reduced digestibility that will restrict dry matter intake (Hodgson, 1977; Wilson *et al.*, 1995).

- inadequate "effective" fibre, required to stimulate salivation and good rumen function (Muller et al, 1995; Mertens, 1996).

- a deficiency of some amino acids may limit high levels of performance (Muller, 1993).

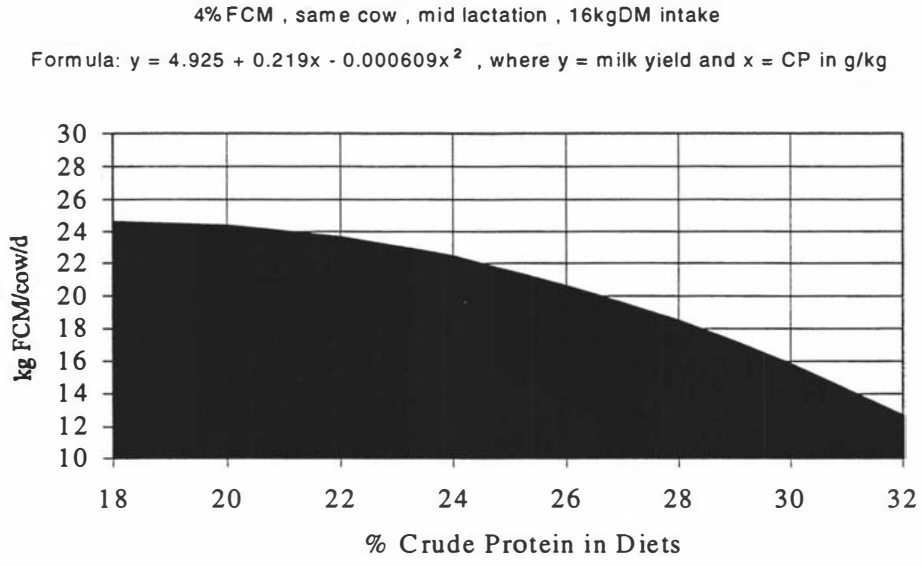
- macromineral deficiency of magnesium, sodium and less frequently calcium and phosphorus, but excess of potassium (Grace, 1983; NRC, 1989) and trace element deficiency of selenium, copper, and less frequently cobalt, iodine, and zinc (Grace, 1983).

Of all these imbalances the problem of excess crude protein in pasture diets appeared to be an important factor that was likely to restrict performance under New Zealand conditions. Milk production penalties have been predicted where dietary protein levels exceed 18% (NRC, 1989; Kelly *et al.*, 1993; Moore and Varga, 1996) and this penalty will escalate as protein levels in the diet approach 30% (see Figure 1.3; Danfaer *et al.*, 1980; Gordon, 1980). Pasture protein levels in New Zealand often exceed 25% in spring when most seasonal calving cows are nearing peak production (Moller,1996b). This factor may contribute to the relatively low peak seen in cows in New Zealand (Holmes and Hughes, 1993).

Allocation of lower amounts of dry matter intake than the cow's appetite would dictate are a feature of pasture-only dairy farming in New Zealand, because high stocking rates per hectare ensure a high utilisation of the total pasture grown (less senescence and decay, and in some circumstances more pasture grown and eaten with higher stocking rates; Fales *et al.*, 1995). Cows with the same genetic background are smaller than their cohorts grown in other dairy systems (Edwards and Parker, 1994). Maintenance of pasture quality (higher digestibility) is essential to maintain productivity in this system as this is a major determinant of intake of dry matter and metabolisable energy and hence production. Quality can be maintained mechanically (with topping or cutting and storing surpluses) or with less effort by high stocking rates. In order to apply sufficient stocking pressure to pasture to maintain quality a degree of underfeeding is usually required. In addition, growth rates of pasture do not perfectly match cow requirements

through the season (see Figure 1.1). With cows calving in July/August/September and drying off in April/May growth rates will not match requirements in May/June/July/August/part of September/January/February in most areas. Surpluses in October/November are stored or saved and fed later (see Figure 1.1), and other methods like nitrogen application, rotation length adjustment and purchased feed are employed to try to address the deficits. The farmer's individual skill in managing the above factors and others produces a range in productivity of approximately 300-700 kg milk fat/ha (500-1200 kg milksolids/ha), and a corresponding range in profitability per hectare (see Figure 1.2).

Figure 1.3 Graph based on data produced by Danfaer *et al.* (1980) predicting the degree of metabolisable energy loss caused by high protein diets



Footnote: The full milk production reduction predicted may not all occur as the cow may mobilise tissue to provide the metabolisable energy required. Other studies have also noted energy or milk production penalties from excess dietary protein (Blaxter, 1962; Gordon, 1980).

The following study was designed to identify differences between herds in pasture quality and blood parameters that may explain differences in herd milk production that did not necessarily relate to dry matter intake. Herds were selected that were expected to have differences in milk production per cow and per hectare.

1.1.3 Herd reproductive performance

Reproductive performance in herds can vary from year to year in the pasture based seasonal dairy system as practised in New Zealand, although the tendency is for herds to remain similar in ranking. Based on 20 years experience in dairy practice in the south Waikato district by this author and two colleagues, the range of performance is represented in Table 1.1 in terms of parameters commonly used by farmers and their advisors to measure herd reproductive efficiency.

Table 1.1 Range in herd reproductive performance experienced in the south west Waikato district:

Herd reproductive performance range	Average season	Poor season
Submission rate (21 days of AB)	60-95%	50-90%
Submission rate (28 days of AB)	75-100%	60-95%
60 day non return rate (NRR, or conception rate)	50-80%	45-75%
Cows "empty" at season end (not pregnant)	1-12%	3-15%

Footnote:

1. Submission rate is defined as % of cows submitted to mating from the start of the mating season within a defined period. eg. 28-day submission rate of 75% means 75% of cows to be mated were submitted for AB mating within 28 days of the start.
2. Non-return rate is defined as the % of cows apparently remaining in calf after a mating. This is usually for a specified period (eg. 45-day NRR, or 60-day NRR). This is not a true conception rate as some cows are actually not in calf even though they have not returned "in season".

In some springs the weather can be overcast and wet for long periods (1-2 months) in the Waikato district. This is often associated with poor reproductive performance and low milk production in many dairy herds, even though cows appear to be offered adequate or excess pasture for the production level they are maintaining. This phenomenon was also identified by McClure (1961). Based on client records in the author's veterinary practice, the comparative "cost" between a good reproductive performance and a poor reproductive performance in terms of cow wastage, reduced

milking days next season, expenditure to reduce anoestrus, induced calving in the following season are estimated at approximately $5,000 per 100 cows based on current milk prices and values for dairy cows. This calculation places no value on the inability to achieve genetic gain if high percentages of cows have to be culled because they are empty, which is another more long term disadvantage of poor herd reproductive performance. Any veterinary practitioner specialising in dairy cattle medicine in this district will service a range of clients with poor to good herd reproductive performance and there is a tendency for those with good performance to remain with good performance and vice versa every year.

What are the factors that ensure the good reproductive performance every year in some herds and not in others?

- Higher producing herds generally also have a better reproductive performance in New Zealand, although this is by no means absolute (Table 1.2, Table 1.6). This suggests that feeding levels play some part in the differences.

- Breed also plays some part - Jersey cows tend to have less anoestrus, but are more likely to have lower NRR (non return rate, "conception rate"), and Friesian cows are likely to have more anoestrus and higher NRR (Macmillan, 1985; McDougall, 1994).

- Individual herd management will also play some part, particularly in determining feed supply, the timing of feed supply and heat detection. In the author's opinion, heat detection and mating management have possibly been overestimated in importance in the past as major influences on herd reproductive performance, except in individual cases. Date of bull removal from the herd has some bearing on eventual empty cow rate because many cows will eventually conceive if they have regular cycles.

- Nutritional factors relating to total amount of dry matter supplied, the timing of supply of DM (McGowan, 1981), cow condition pre and post calving (McDougall, 1994), and the individual nutrients within the diet would seem to be the most

important factors. The nature of herd reproductive problems in other dairy systems with higher inputs tends to be different to that described above, with less problem with anoestrus and more problem with NRR . This may relate especially to higher productivity placing more stress on the reproductive system or difficulties in detecting heat in stalled animals (Haresign, 1980; Macmillan, 1985; Butler and Smith, 1989). A rising plane of nutrition during mating is also thought to improve conception.

As discussed above, there are multiple causative factors with herd reproductive problems, but current thinking in New Zealand is that anoestrus and conception rates are strongly influenced by feeding and cow condition before calving, at calving and in the month post-calving especially as identified by McGowan (1981) and McDougall (1994). Feeding levels and cow condition at mating appear to be of less influence than those around calving. That is not to say that management changes at mating time are ineffectual in controlling anoestrus or NRR, but that the main causes were likely to have occurred earlier. These comments apply to the dairy system practised in New Zealand for seasonal calving in spring.

The literature suggests that conception rates may be depressed when excess urea is present in blood or milk (Jordan and Swanson, 1979; Ropstad and Refsdal, 1987; Ferguson *et al.*, 1988; Gustafsson, 1993), and problems are predicted when urea exceeds 7 mmol/l in blood (= 20 mg/dl blood urea nitrogen (BUN); Ferguson *et al.*, 1988). There is only limited evidence of a similar problem in New Zealand however, where urea levels can exceed 7 mmol/l in blood or milk during mating (Williamson and Fernandez-Baca, 1992; Moller, 1993). McClure (1961) noted a negative association between dairy cow fertility in the Waikato and total nitrogen content of the pasture, where pastures tended to be short, rapidly growing and ryegrass dominant. The problem lasted 1-2 months, and cows often did not gain weight. McClure (1968) has also linked lower blood glucose levels with lower conception rate.

1.2 EXPERIMENTAL METHOD

The following study was designed to identify differences in pasture nutrients and blood parameters between herds that were expected to have different herd reproductive performance based on previous herd history.

Observational studies were commenced in 1990 to monitor dairy herds through spring for milk production and reproduction with parallel measurement of blood and pasture nutrient data. Two herds were selected that generally had high submission rates to mating (low anoestrus levels) and low empty rates every year (Farms C and D), and two herds were selected that generally had low submission rates to mating (high anoestrus levels) and higher empty rates every year (Farms A and B). Calving start date was 7-10 days later in Farms C and D (1 August start vs 24 July start) and mating continued 40-50 days longer (see Table 1.2 for more detail on farms). Nutrient content of pastures was measured as crude protein (CP), acid detergent fibre (ADF), soluble carbohydrate (SOLCHO) and dry matter % (DM%). Analytical methods used were Kjeldahl N (TKN x 6.25) for protein, modified Van Soest method for ADF, and gas chromatography for individual sugars for soluble carbohydrate (see Appendix 1 for methods used). Drying methods were oven drying at 90°C for protein and fibre, and freeze drying for soluble carbohydrate. Soluble carbohydrate was included because it is one of the most labile components in pasture, and likely to be influenced by weather conditions which appear to have some effect on annual differences in production and reproduction. Pasture CP and ADF are more stable.

Blood chemistry analytes were included to provide extra information about metabolic changes within the cow and to assess the value of these analytes in monitoring nutritional differences in the herds studied. Albumin (Alb) was included as a measure of general nutritional state, condition and long term protein status (Kitchenham *et al.*, 1975; Manston *et al.*, 1975). Beta-hydroxybutyrate (BOH) and non-esterified fatty acids (NEFA) were included as measures of tissue (fat) mobilisation, urea as a measure of dietary protein excess and glucose as a measure of metabolic state (Baird, 1982; Whitaker *et al.*, 1983; Kolver and Macmillan, 1993). The herds were monitored weekly by blood testing 10 cows for parameters likely to be influenced by nutrition, using an

Hitachi 911 autoanalyser. Representative cows were selected (on the basis of age and previous production) and the same individuals were sampled each time. Pasture samples representing what cows ate on each farm were also collected weekly and analysed for crude protein, soluble carbohydrate, dry matter, and fibre [ADF]. Samples were collected from at least 8 points in a paddock about to be grazed, and were cut to "grazing height". Sampling continued for 17 weeks from 7 August - 20 November.

In light of the findings in 1990 five different herds were monitored in the spring of 1991 with measurements for milk urea, dry matter intake, condition score and milk production. Bulk vat milk urea was chosen in 1991 instead of blood urea because it was simpler to collect and close associations between the two have been found (Oltner and Sjaunja, 1982). Dry matter intake was estimated with the assistance of another dairy consultant in 1991 using a rising plate meter and the difference between herbage mass on grazed and ungrazed area measured. This was one important factor not measured in 1990. Reproductive performance was monitored in both seasons using the computer programme "Seasonal Dairy Herd Breeding Record Analysis" developed by J and S Grimmett, Matamata, New Zealand.

Further data were collected from 35 dairy herds in the district over 5 weeks in spring 1991 for daily per cow production of fat and protein, and also bulk herd milk urea twice weekly. This was designed to examine in more detail the relationship between these parameters highlighted by the 1990 study. In addition it provided information about the range of milk urea levels in dairy herds in spring.

Pasture samples were collected weekly in the morning between 8.00-9.30 am, and placed in a chillibin. Within 30 minutes of collection, a subsample was placed in a refrigerator for subsequent CP, DM and ADF analysis and another subsample was frozen for SOLCHO analysis.

Milk production was monitored using the daily dairy company returns divided by the number of cows milked on that day. Weather data (rainfall, sunlight hours and soil temperature at 10 cm depth) were obtained from the Ruakura weather station (less than 25 km from all farms) and applied to each farm. Different farms were visited daily

from Monday-Thursday each week, hence weather was slightly different on each farm in the previous 3 days, eg. Week 0 represents the sampling from 7 August-10 August on the four farms, Week 1 from 14 August-17 August, and so on. Dairy meal (concentrate) was fed in two herds briefly. Herd A fed 0.75 kg per cow per day during weeks 7 and 8, and Herd D fed 2 kg meal per cow per day in weeks 5, 6, and 7.

Statistical analysis was only possible to a limited degree due mainly to sample size (four herds in 1990 and five herds in 1991) and the lack of control of variables between these commercial dairy farms. The study was mainly observational and no attempt was made to standardise farm variables.

1.3 RESULTS AND DISCUSSION

Results and discussion have been combined to assist with presentation and interpretation.

1.3.1 Weather data for 1990

Weather data for 1990 are presented in Figures 1.4-1.6. Periods of higher rainfall (weeks 6, 12 and 16) coincide with increased ground temperature and reduced sunlight hours. Periods of lower rainfall (weeks 5, 7, 8, 11, 13, and 15) less consistently had increased sunlight hours and reduced soil temperature.

1.3.2 Herd details and results for 1990

Details on the four herds observed in 1990 are presented in Table 1.2. Stocking rate expressed as kg liveweight per hectare, was lowest for herd A at an estimated 1350 kg/ha and the highest was herd D at 1600 kg/ha. The breeding index (BI) measurement method was changed in 1990 and both values are presented. "Anoestrus" cows are defined as the number of cows examined at 3.5-4 weeks that had not been seen on heat. Blood parameters measured are presented in Figures 1.10-1.12 and mean values are presented in Table 1.2. Milk production per cow data are presented in Figures 1.7-1.9 and mean values in Table 1.2.

Herd reproductive performance was similar in each herd to that experienced in previous seasons, with good performances in herd C and D, and poor performances in herd A and B (see Table 1.2).

Figure 1.4 Mean daily rainfall (mm) for the 3 days previous to pasture sample collection on each farm through time (7 August-20 November).

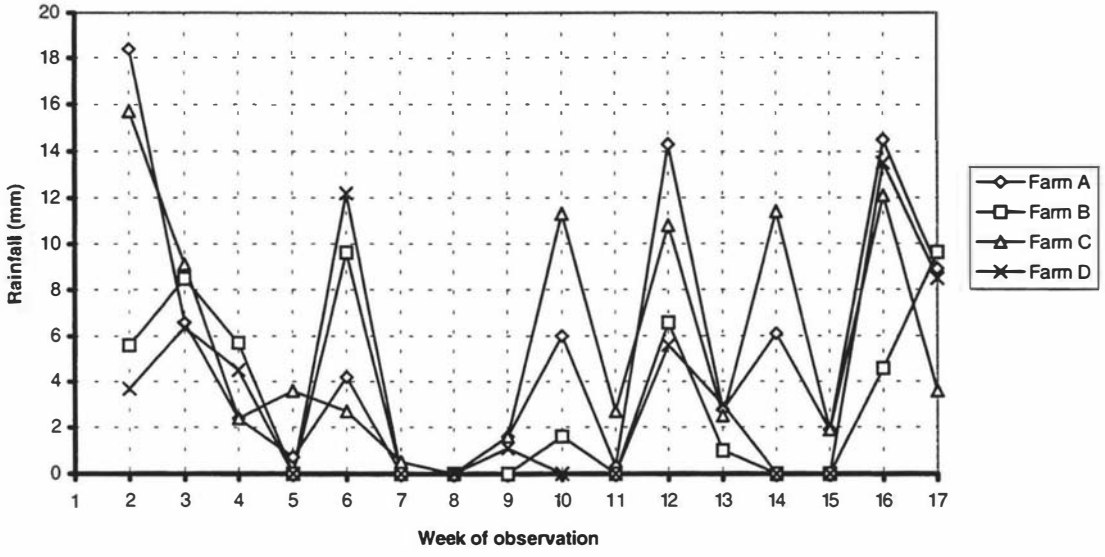


Figure 1.5 Mean ground temperature (10 cm) for the 3 days before pasture sample collection through time (7 August-20 November).

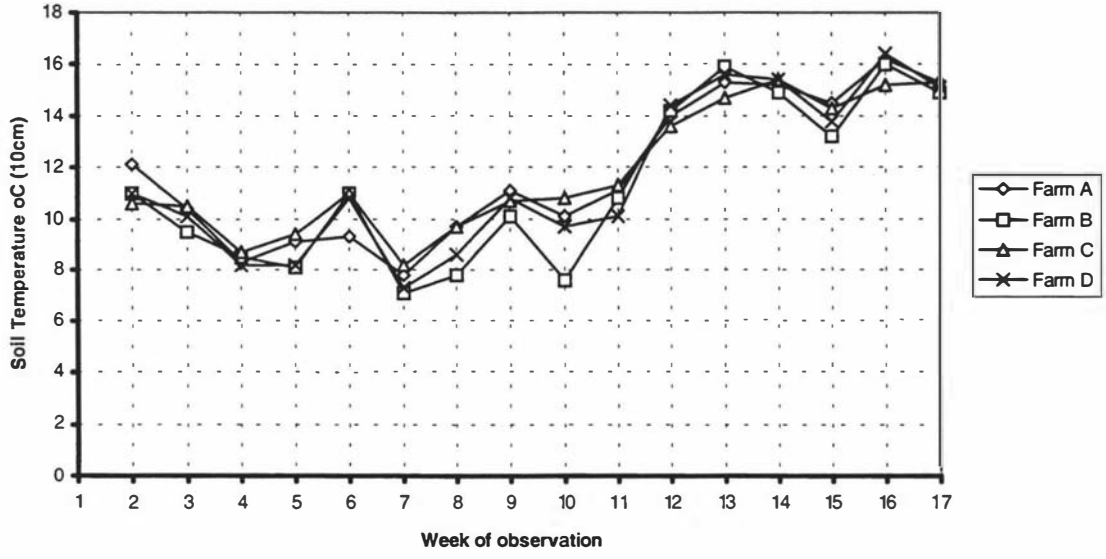


Figure 1.6 **Mean daily sunlight hours for the 3 days before pasture sample collection through time (7 August-20 November).**

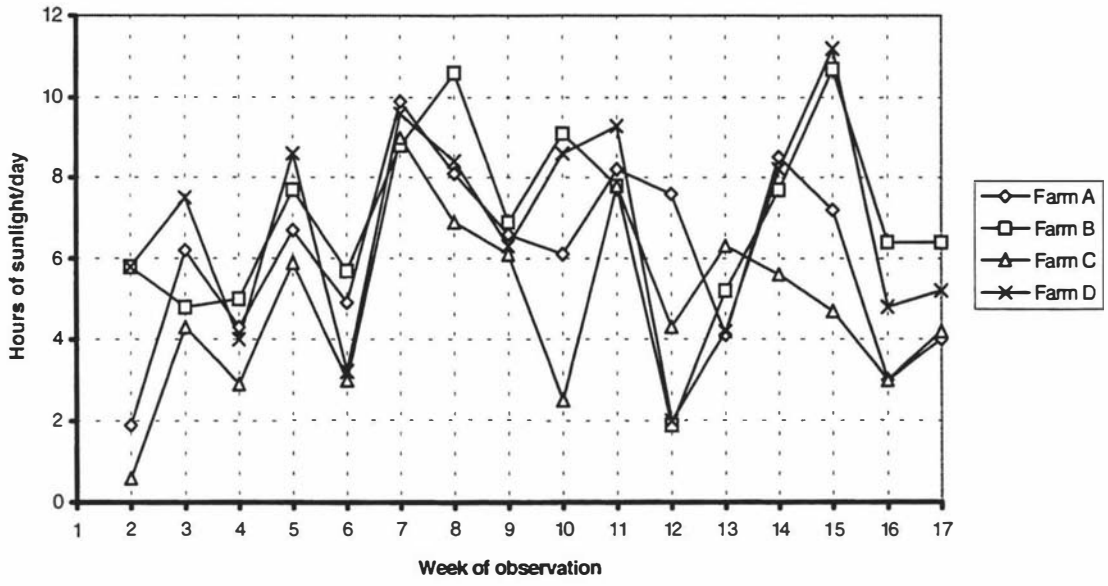


Figure 1.7 **Herd milkfat (kg) per hectare versus week of observation.**

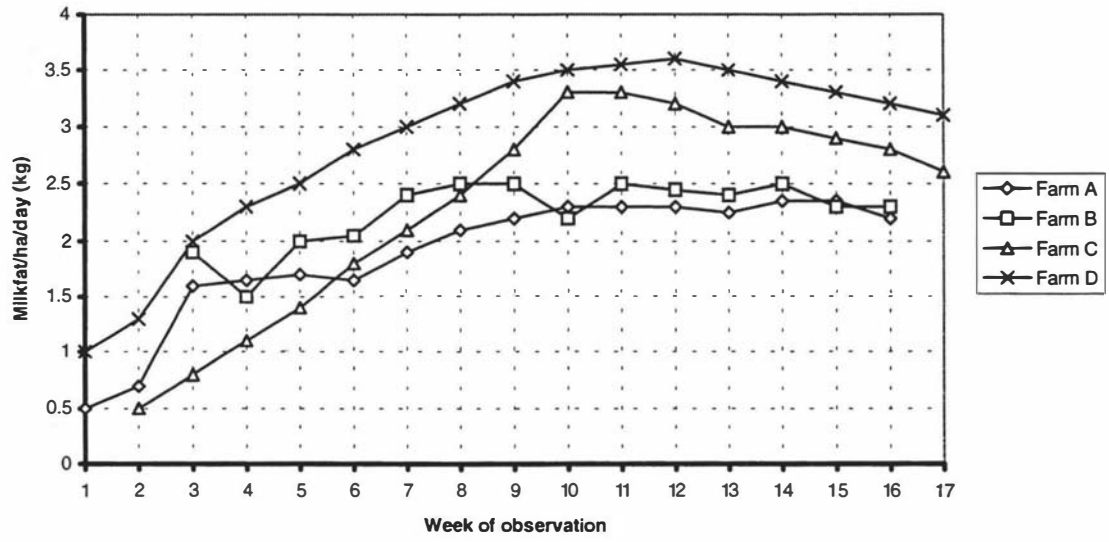


Table 1.2 Herd performance and descriptive data for the four farms in 1990.

Herd data	Farm A	Farm B	Farm C	Farm D
Herd Size	225	380	167	346
Breed	Friesian	Friesian	Friesian	Jersey
Stocking Rate (cows / ha)	3.0	3.3	3.4	3.75
kg Butter fat / ha 1989/1990	432	416	608	640
kg Butter fat / ha 1990/1991	432	443	560	700
Butterfat kg/cow/day (average)	0.74	0.75	0.94	0.91
Milk protein kg/cow/day (average)	0.60	0.58	0.74	0.68
Protein/Fat ratio in milk	0.81	0.77	0.78	0.75
Sampled cow BI - old units	129.9	103.2	128.5	128.1
Sampled cow BI - PBI	130.0	126.5	128.2	130.4
Condition score - start	4.75	4.75	4.75	4.75
-lowest	4.1	4.3	4.3	3.9
-end	4.5	4.8	4.7	4.5
Blood analyses				
Blood Urea (mmol/l)[1]	7.38	8.20	5.85	6.20
Blood Glucose (g/l)[2]	3.68	3.63	3.63	3.59
BOH (mmol/l)[3]	0.56	0.48	0.49	0.59
Albumin (g/l)[4]	32.6	32.0	32.0	34.2
NEFA (mEq/l)[5]	0.49	0.38	0.22	0.89
Pasture nutrients				
Pasture Dry Matter (%)[6]	16.15	14.47	15.50	15.59
Acid detergent fibre (%DM)[7]	24.97	23.71	24.96	24.72
Pasture SOLCHO (%DM)[8]	10.88	11.29	12.81	14.03
Pasture Nitrogen (%DM)[9]	3.78	3.86	3.35	3.50
Pasture Protein (6.25 x Nitrogen)	23.62	24.09	20.91	21.88
Pasture Protein: SOL CHO ratio	2.17	2.13	1.63	1.56
Reproductive performance				
Mating start date	15/10/90	13/10/90	23/10/90	21/10/90
Mating end date	14/1/91	15/1/91	26/2/91	8/3/91
Submission rate : 1st week of AB[10]	28% (30)	31% (30)	19% (30)	29% (30)
: 2nd week of AB	49% (60)	59% (60)	51% (60)	61% (60)
: 3rd week of AB	65% (90)	77% (90)	82% (90)	89% (90)
: 4th week of AB	72% (98)	80% (98)	94% (98)	95% (98)
Anoestrus cows (number seen)	63	56	8	5
% Herd "empty"[11]	10.6%	4.2%	1.8%	3.1%

[1-5] measured by an Hitachi 911 autoanalyser. [6-9] Measured as described in the text. Percentage units rather than g/kg were chosen for presentation of pasture nutrient data. [10] defined as the percentage of the herd submitted to artificial breeding (AB) in the specified time from the start of AB mating. [11] Diagnosed as not in calf at the end of the mating season.

Figure 1.8 Per cow milkfat (kg) per day versus week of observation.

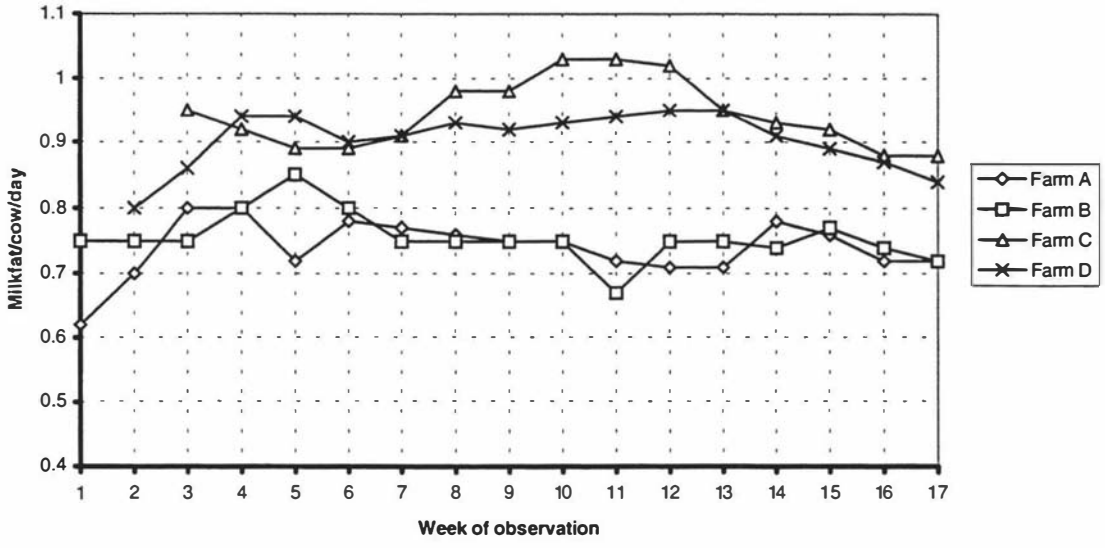
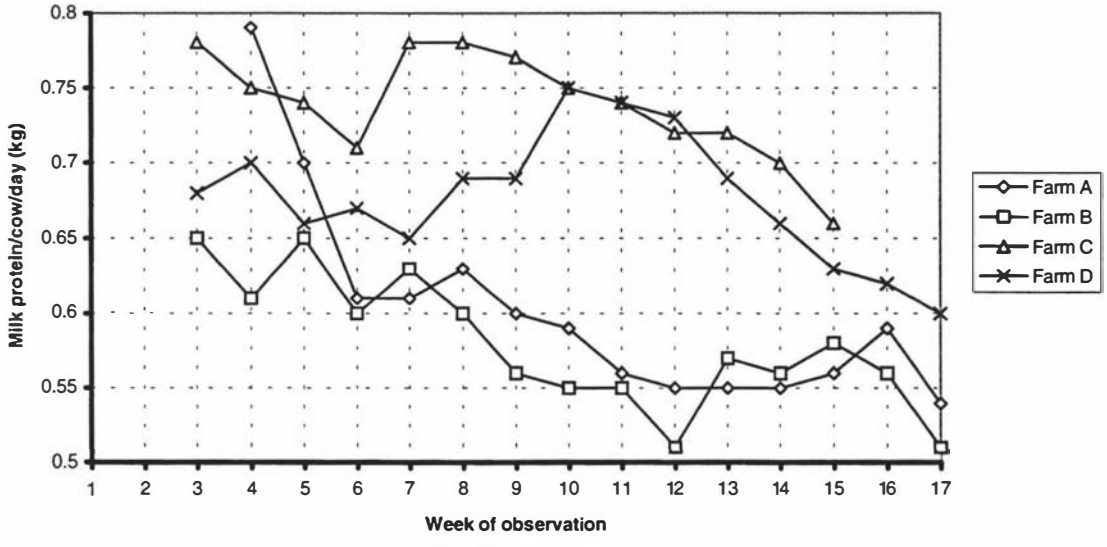


Figure 1.9 Per cow milk protein (kg) per day versus week of observation.



Dry matter intakes appeared suboptimal (this was assessed visually by difference between residual and grazed levels, but was not recorded in detail) in herds A and B. Intervals between grazings in these two herds reached 16-18 days in September/October, but remained at over 24 days in herds C and D. Visually herds C and D consumed longer pasture. In terms of pasture species, no obvious differences

between herds were noted, although clover content of pasture was generally considered to be low in that spring.

1.3.3 Milk production data for 1990

Changes in milk fat per hectare (Fig. 1.7) and fat and protein per cow per day over time are presented in Figs 1.8 and 1.9 (and means in Table 1.2). Milk production followed the previous year's pattern with herds C and D producing at higher levels than herds A and B both on a per hectare and per cow basis. Herd C had lower per hectare figures initially, possibly reflecting a slower calving rate, as daily per cow figures were the highest. Differences between herds became consistent from September (from weeks 6-10 approximately) onwards. No statistical analysis was used to support these observations.

1.3.4 Blood analyses for 1990 data

The results from the blood analyses are presented in Figures 1.10-1.14. Low points in Alb were present in weeks 1-2, 10 and 13 (Figure 1.10). Herd D had higher Alb throughout but still exhibited the same troughs as the other herds. This was the only Jersey herd. A low point in all herds (nadir) at week 10 (near 10 October) was followed by increases in all herds. Per hectare production and per cow fat production reached peak at week 10, when ground temperature began to elevate to a higher plane from a low point (Figure 1.5). The low point in Alb at week 12-13 did not appear to relate to cooler temperature but was associated with lower sunlight hours and increased rainfall in week 12 (Figures 1.6 and 1.7).

Mean serum urea concentrations are presented in Figure 1.11 with high points present around weeks 12-14 and low values near week 7 and 15-16. The high points were associated with a period of high sunlight hours and reduced ground temperature. All but herd D increased in urea levels from week 9 until week 12-14.

Again no statistical analysis was attempted to support the relationships with albumin and urea concentrations.

Figure 1.10 Mean serum albumin in each herd through time (7 August- 20 November).

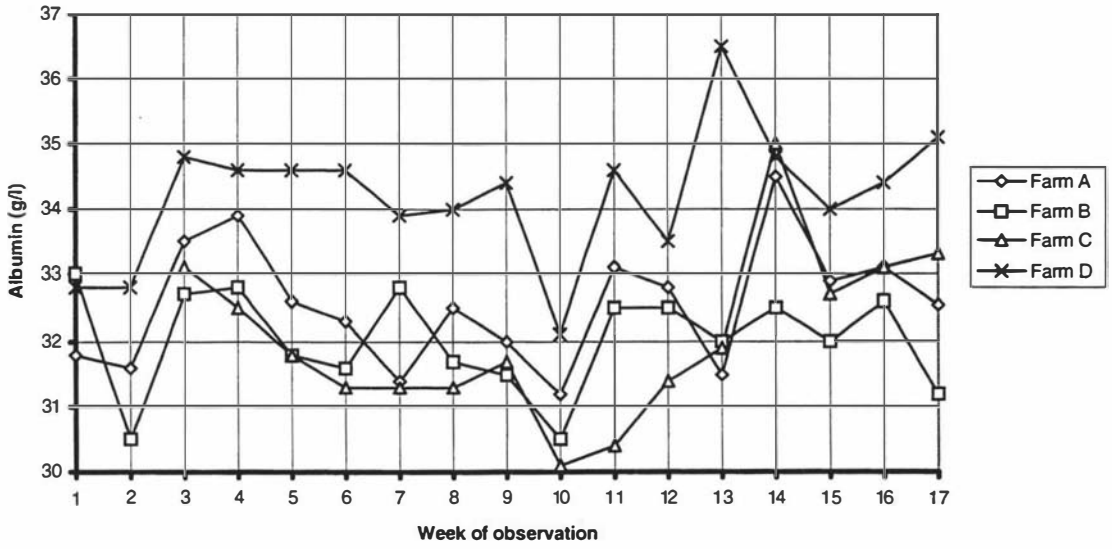
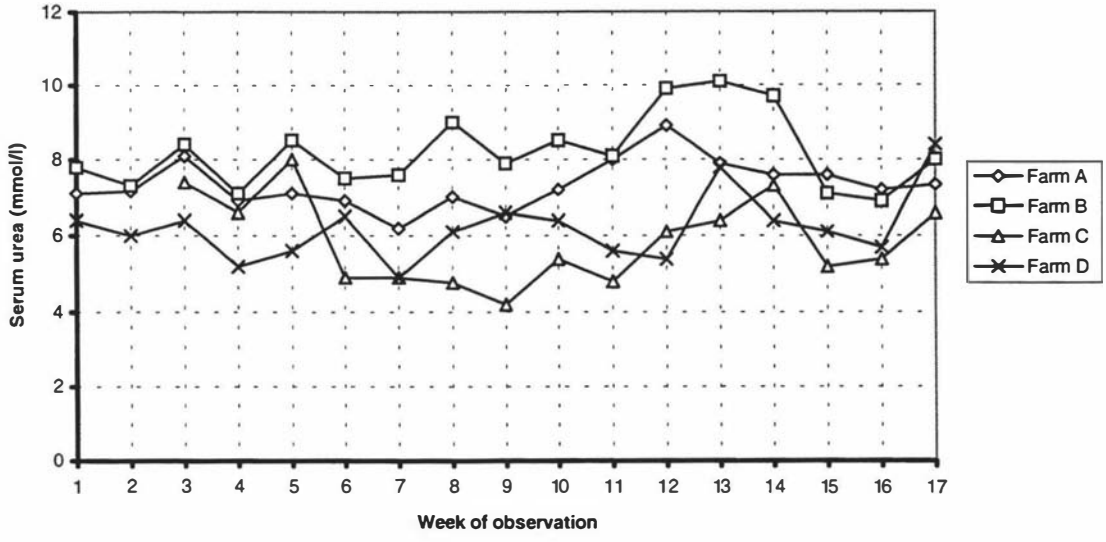


Figure 1.11 Mean serum urea in each herd through time (7 August-20 November).



Serum beta hydroxybutyrate (BOH) concentrations are presented in Figure 1.12 and NEFA (non-esterified fatty acids) in Figure 1.13. There were some high concentrations in both variables immediately post-calving and at weeks 13-14. These high points coincided with periods of lower sunlight and high rainfall.

Figure 1.12 Mean serum beta-hydroxybutyrate in each herd through time (7 August-20 November).

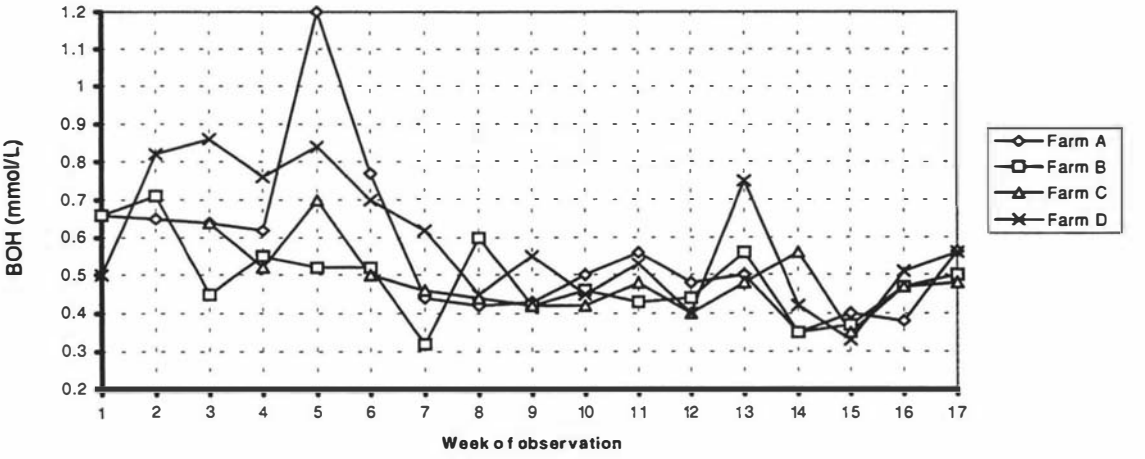
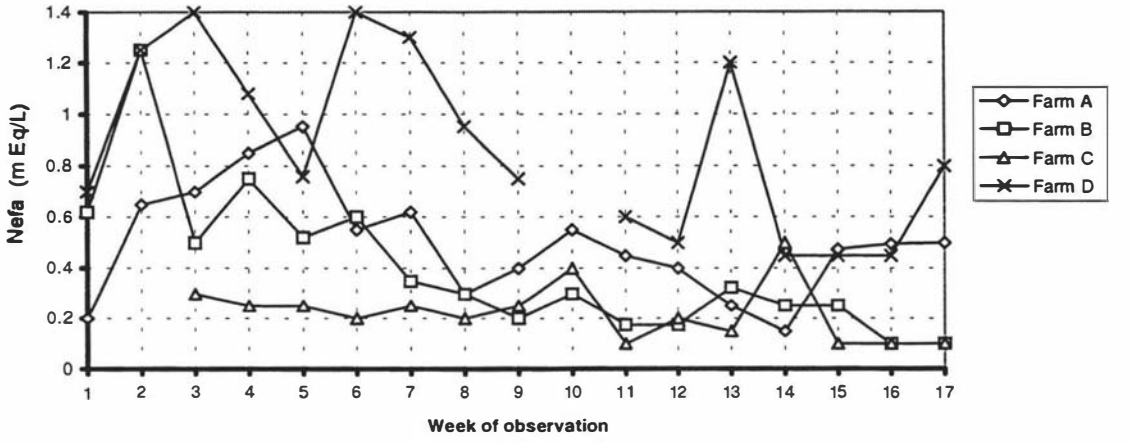
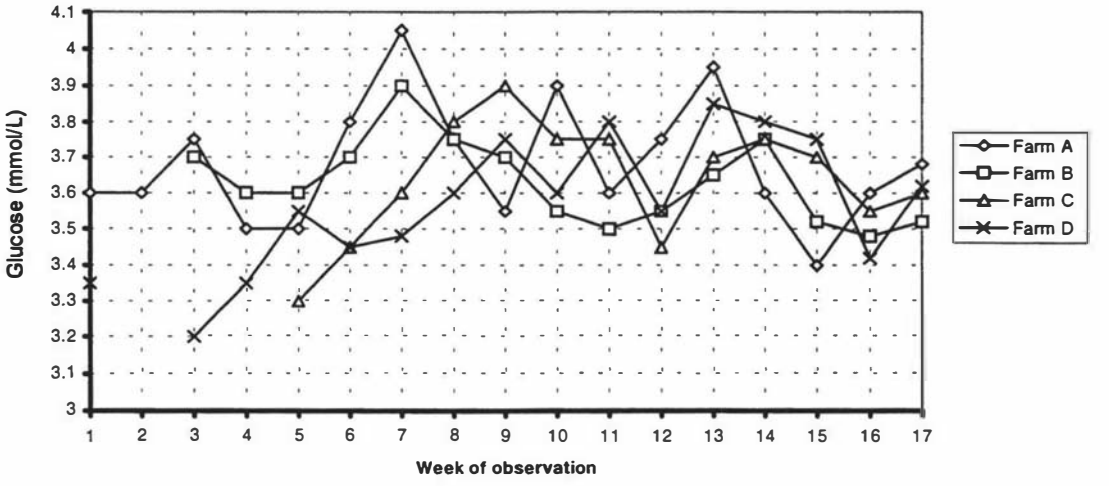


Figure 1.13 Mean serum non-esterified fatty acid from 10 cows in each herd through time (7 August-20 November).



Blood glucose concentrations are presented in Figure 1.14. Herds C and D had low concentrations early in lactation. Low points also occurred in weeks 11-12 and 15-16 and high points near weeks 7-9 and weeks 13-14.

Figure 1.14 Mean blood glucose in cows from each herd through time (7 August-20 November).



1.3.5 Pasture nutrients (1990 data)

Pasture nutrient analyses through time are presented in Figures 1.15-1.18. Peaks in SOLCHO were present in weeks 7 and 16, with low levels in weeks 2 and 12-14. The low concentrations were only one third of the peak levels. Pasture DM% was at high levels in weeks 1-3, with a distinct low point at weeks 12-14. A gradual decline in SOLCHO and DM% occurred in weeks 7-14. Pasture CP showed high concentrations at weeks 12-14, and a low point at week 16. Pasture ADF showed a low point at week 7 (near 18 September), with a gradual increase from then on.

Week 7 had low rainfall, low soil temperature, and high sunlight hours (Figures 1.3-1.5), but conditions at week 16 were high rainfall, higher soil temperature and lower sunlight hours. The low DM% seen in weeks 12-14 (23 October-7 November) occurred when sunlight hours were low, soil temperature rising and rainfall was high. Reduced milk production, especially milk protein occurred at this time. Pasture ADF and CP were at a high point but SOLCHO at a low point at weeks 12-14.

Change in pasture composition in October/November would be expected in ryegrass/white clover swards because ryegrass is in the reproductive stage at this point

and ground temperature is rising. The increasing ADF concentrations and reducing CP levels in the second half of the observation period are probably indications of this. The high DM% early in the observation period is probably indicating more mature "winter saved" pasture.

Figure 1.15 Soluble carbohydrate % from pasture about to be grazed versus week of observation.

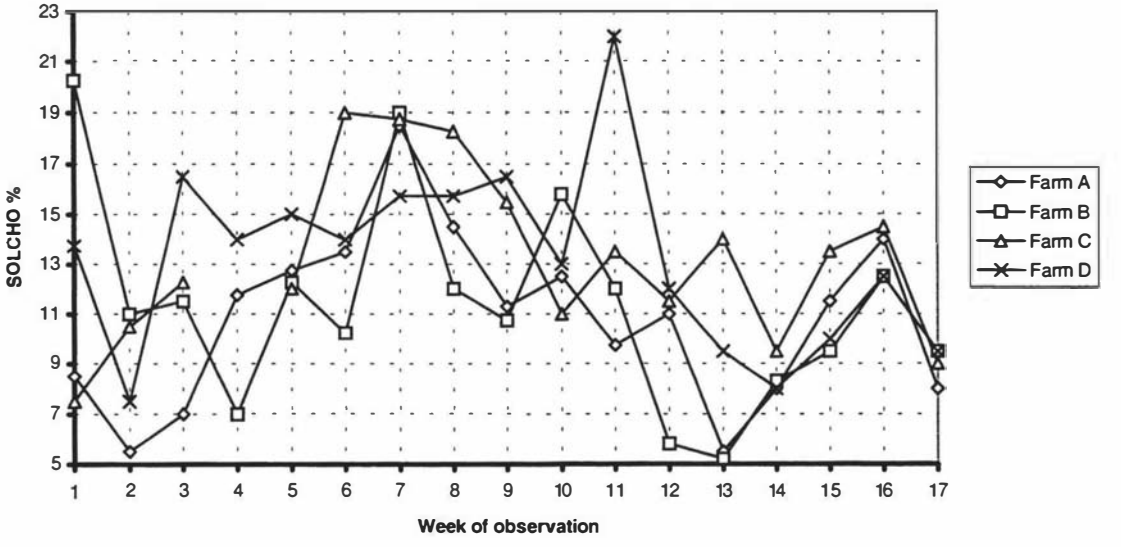


Figure 1.16 Dry matter % from pasture about to be grazed versus week of observation.

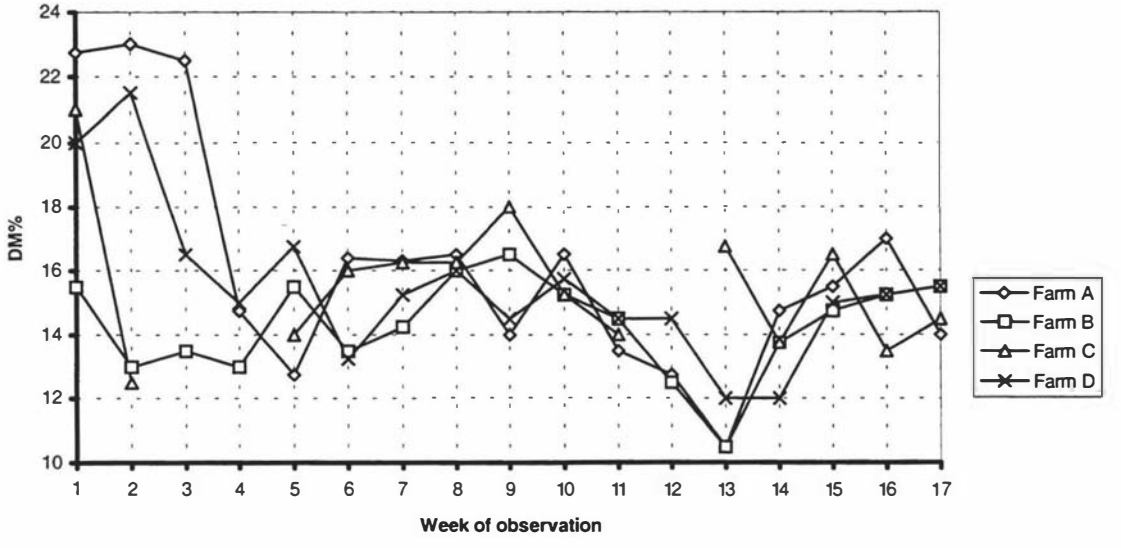


Figure 1.17 **Crude protein % in pasture about to be grazed versus week of observation.**

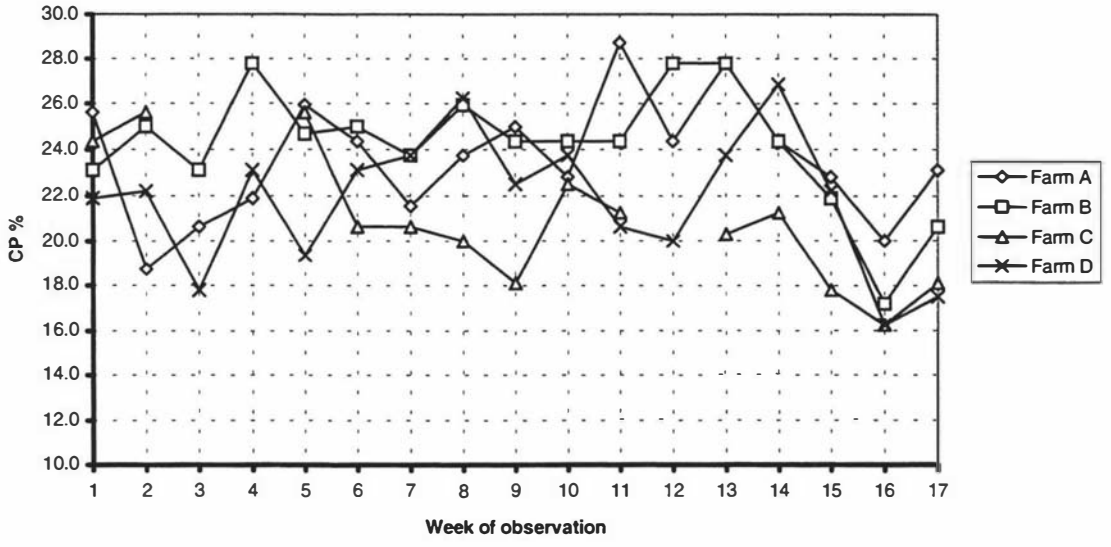
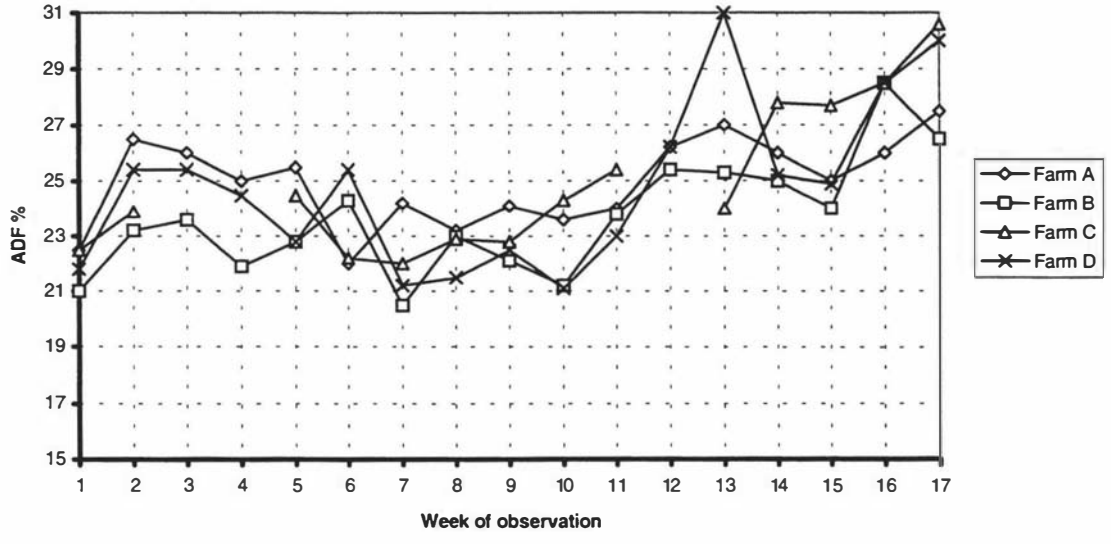


Figure 1.18 **Acid detergent fibre % in pasture about to be grazed versus week of observation.**



1.3.6 Combined interpretation of data from weather, production, blood parameters measured and pasture analysis - 1990 data

The apparent association of DM% and SOLCHO and the negative association with CP, especially in weeks 4-17 are of especial interest. The low point in pasture DM% near weeks 12-14 is of particular interest, and coincided with increased CP, reduced SOLCHO, increased blood glucose, increased BOH and NEFA, increased blood urea and a small decline in Alb. Milk production also showed signs of decline in this period. This occurred when high soil temperatures suggest high growth rates were occurring, and available dry matter for the cows was likely to be high. Changes in Alb, BOH, NEFA and production suggest inadequate ME intake and tissue mobilisation in response. This would suggest (but not prove) that the high CP, low SOLCHO, low DM % pasture present at this time was causing some problems for the cows.

Weather conditions appear to have played some part in producing this type of pasture with rising soil temperature and wet overcast weather (Figures 1.3-1.5). SOLCHO gradually declined from week 7-14, serum urea increased, milk protein per cow declined, sunlight hours declined, rainfall was frequent (especially from weeks 9-14) and ground temperature increased. These findings can be explained by weather conditions gradually reducing SOLCHO in pasture through lack of photosynthesis, thus affecting the dietary CP/SOLCHO ratio which in turn affects rumen fermentation and possibly bacterial growth. If fewer bacteria are digested as a protein source the consequence may be less milk protein is produced. High urea levels indicate this imbalance in the rumen, and also BOH and NEFA elevation, around week 12-14. If the higher urea levels indicated higher DM intakes after week 7-9 (as suggested by the generally rising Alb levels from then on) then milk production should have increased. Rises in Alb from week 10 may indicate weight gain from this time on (ie. partitioning away from production and into weight gain). It is unclear from the data available why this might occur but it coincides with increased pasture ADF, reduced pasture SOLCHO and DM %, and increased pasture CP plus increasing ground temperature.

Rises in blood urea occurred especially around weeks 12-14 and were associated with increased pasture CP, reduced pasture SOLCHO and DM %. The converse occurred in weeks 7-8. Elevated urea in blood or milk reflects dietary protein excess (Moore and Varga, 1996) and this seems the likely explanation here.

Lipid mobilisation appears to have occurred up till week 8-10 as evidenced by the raised BOH and NEFA values. This is a well established phenomenon where the cows propensity to milk exceeds the ability of the cow to consume sufficient dry matter for the first 8-10 weeks after calving (Muller, 1993). Albumin concentrations also declined in this period, especially in September, suggesting that some tissue protein was also being mobilised. The nadir in Alb concentration in week 10 in all herds suggests that some common factor (like weather conditions) to all farms instigated the change. At this point pasture growth probably accelerated in response to rising ground temperature and provided more dry matter for production and weight gain. Herd D was a Jersey herd and production levels were also high for this herd suggesting a breed difference, or better fed cows. No marked differences in condition score were detected between herds.

Blood parameters measured often changed on all four farms in the same week suggesting external influences like weather conditions played an important part in influencing the changes. Although DM intake was not measured in the 1990 data, it would seem unlikely that DM shortage would occur in the same week on all farms and improve at the same rate on all farms. Calving start date was similar (7-10 day difference in start date) on all farms, but this still does not really explain the unison in changes seen.

Pasture nutrient concentrations also changed in unison on all four farms, again suggesting external conditions were a major influence. Differences in cow productivity were associated with blood urea, CP and SOLCHO with higher milk production and herd reproductive performance occurring in herds with lower urea, lower pasture CP and higher pasture SOLCHO. Pasture CP/SOLCHO ratio was approximately 30% higher in herds A and B (Table 1.2).

1.3.7 Data from 1991- Results and Discussion

Data from 1991, from five farms sampled weekly, follow similar seasonal changes to 1990 (Figure 1.19), with the exception of the fluctuations in levels of SOLCHO and DM %. Milk urea is closely associated with blood urea (Oltner and Sjaunja, 1982). When pasture N/SOLCHO ratio versus time was superimposed over milk urea versus time there was a strong correlation ($r = 0.69$ for urea and N/SOLCHO ratio on 34 data points, relations significant at $P < 0.001$ when r exceeds 0.4), once again indicating that milk or blood urea levels reflect protein intake. Results of pasture analyses are presented as smoothed curves to make the interrelationships clearer (Flexi 2.0; Upsdell and Wheeler, 1992).

1.3.8 Reproductive results and discussion (1990 and 1991 data)

The herd reproductive performances are presented in Table 1.2. Weekly NRR (60d) are superimposed on the urea levels for each herd sample mean in Figure 1.21. The results from Farm B are of special interest as problems with conception rate have been reported where blood urea exceeds 7 mmol/l (Ferguson *et al.*, 1988).

Figure 1.19 Milk urea and N/SOLCHO ratio versus time for 1991 data (10% confidence bands included).

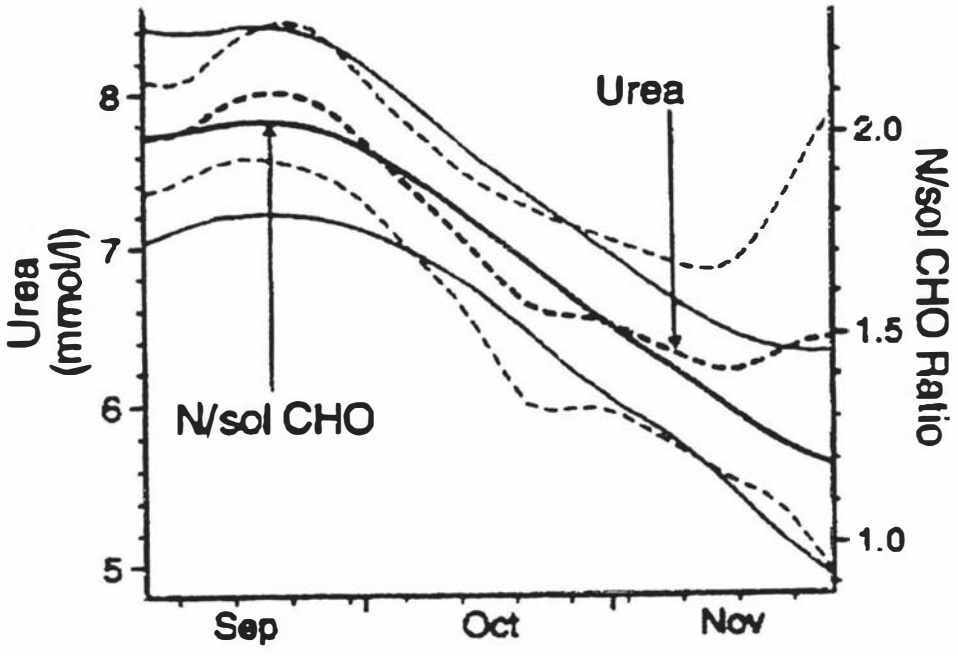


Figure 1.20 Chemical composition of pasture versus time for 1991 data.

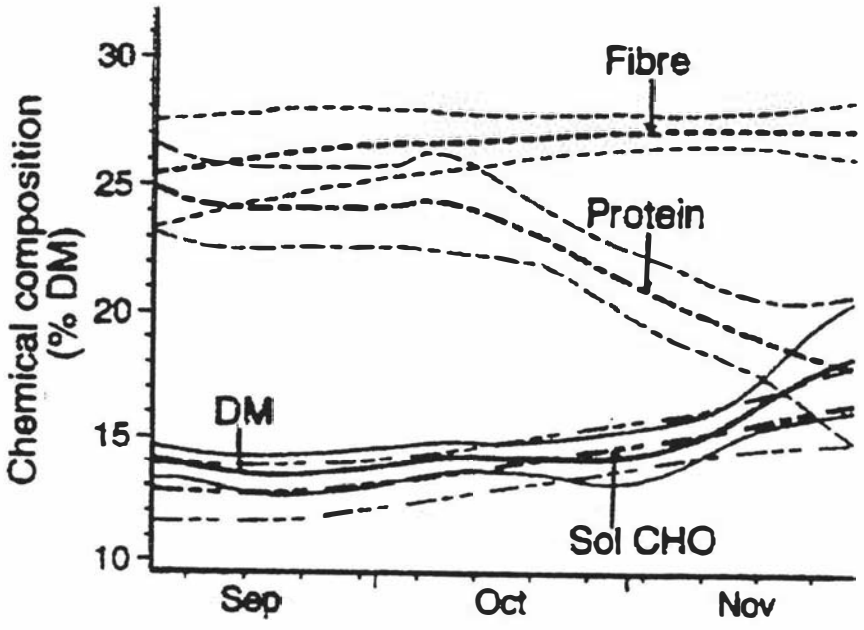
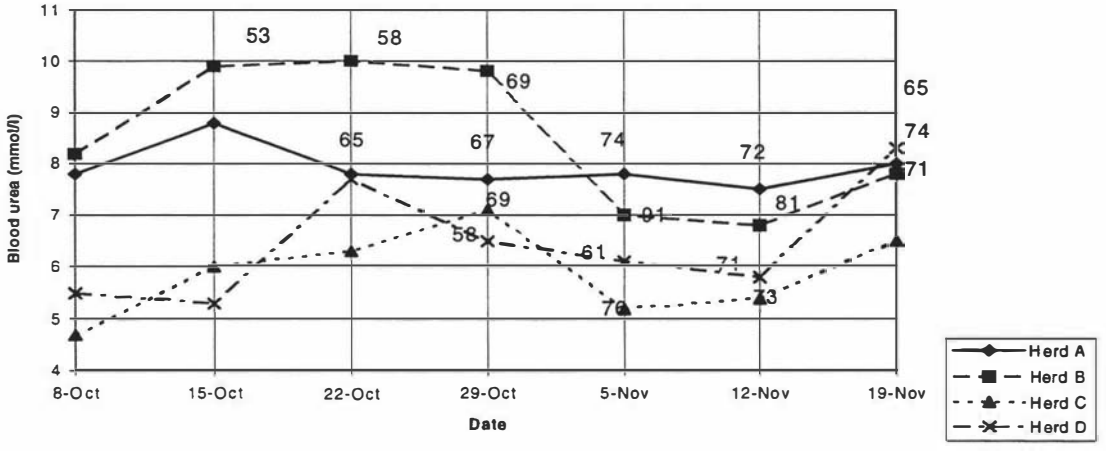


Figure 1.21 Weekly non-return rate (60 d) for the four herds superimposed on blood urea through time for the artificial breeding period (1990 data).



Footnote: AB mating started 20 October in herds C and D. Data not available for 4th week of mating in Herd D.

Conception rates (60d NRR) and anoestrus levels (submission rates) appear to be negatively related to urea levels in that higher urea levels occurred in herds with poorer reproductive performance (Table 1.2, Fig. 1.21). Anoestrus is not often referred to as a result of increased urea levels, but Moore and Varga (1996) state this as a result of prolonged negative energy balance caused by the high energy cost of conversion of ammonia to urea.

AB mating did not start till after 20 October in herds C and D. If the common observation that conception rates are higher when cows are on a rising plane is correct then this may have given advantage to herds C and D because Alb levels rose from this point on suggesting cows were gaining condition. The empty cow rates in herds C and D would be expected to be lower partly because the bull remained with the cows for longer.

1.3.9 Reproductive performance and milk production

The 35 herds surveyed in 1991 are grouped on the basis of milk production (Table 1.3). Note the association of higher milk productivity with higher herd reproductive performance. Empty cow % for lower producing herds in this data was unusually low in the writer's experience.

Table 1.3 Reproductive performance and milk production data from 35 herds in 1991.

	Highest producing herds (n=7)	Medium producing herds (n=21)	Lowest producing herds (n=7)
Submission rate (3 weeks)	93% s.d. 3.3	87% s.d. 9.3	81% s.d. 12.5
Kg milkfat/ha	622 s.d. 53	505 s.d. 26	416 s.d. 27
Empty cow %	2.5 s.d. 0.8	4.1 s.d. 1.9	4.3 s.d. 1.6

1.3.10 Relationship between pasture and animal variables

1.3.10.1 *Principal Component Analysis*

To examine the apparent relationship between urea levels, pasture protein and SOLCHO, and milk production principal component analysis (PCA) as described by Joliffe (1986) was applied to the three sets of data from 1990 and 1991. This identifies and describes independent patterns of association between the variables in the dataset

without any prior assumptions about what patterns exist or their cause. Analysis of variance of the PC scores was then used to determine the extent to which between-farm differences and seasonal change respectively contributed to the associations between the variables (PC variables) identified by PCA. Tables 1.4 and 1.5 summarise the results for 1990 (4 herds) and 1991 (5 herds) data.

Table 1.4 Principal Component Analysis on 1990 data.

Variable	Principal Component 1	Principal Component 2
Grass protein/SOLCHO ratio	- 0.38	0.85
Blood urea	- 0.53	0.13
Milk fat per cow per day	+0.53	0.39
Milk protein per cow per day	+0.54	0.34
Proportion of data explained	73%	19%
Farm effect (anova of PC scores)	P< 0.001	NS
Time effect (anova of PC scores)	NS	P< 0.01

Table 1.5 Principal Component Analysis on 1991data

Variable	Principal Component 1	Principal Component 2	Principal Component 3
Day of season	0.59	-0.07	0.16
Ratio of grass protein/SOLCHO	-0.54	-0.30	0.17
Milk urea	-0.44	-0.45	0.12
Milk fat per cow per day	-0.20	0.60	0.31
Milk protein per cow per day	-0.33	0.57	0.08
Cow DM intake	0.15	-0.12	0.91
Proportion explained by PC score	35%	33%	17%
Farm effect (anova of PC score)	NS	P< 0.001	-
Time effect (anova of PC score)	P< 0.001	NS	-

In Table 1.4, PC1 shows a negative association exists between blood urea and milk fat production or milk protein production. There is a positive association between pasture protein/SOLCHO ratio and urea level, and a negative association of pasture protein/SOLCHO ratio with milk fat and milk protein production per cow. PC1

explains 73% of the variation in the dataset. This PC1 was a between-farm effect and not a seasonal trend. PC2 describes a lesser seasonal effect (as shown by analysis of variance of PC scores) in 19% of the data.

The second dataset on five farms during spring 1991 showed similar relationships when subjected to PCA. Day of trial and estimated DM intake were also included in the 1991 dataset (Table 1.5).

The PC scores for the 1991 dataset are similar to those in the 1990 dataset except that the second PC contains the between farm effect and 33% of the variation in the data is explained by PC2. Dry matter intake does not feature strongly in the relationships in PC1 or PC2 but does feature in PC3 which explains 17% of the variation in the data (Table 1.5).

Additional data from twice weekly bulk vat milk urea and per cow milk fat and milk protein production were collected twice weekly for 5 weeks for 35 herds in spring 1991. These data were also subjected to PCA (Table 1.6), although farm and seasonal effects were detected in a different manner to the first two datasets. Data were averaged over the 10 dates to test for between-farm effects and were averaged over the 35 farms to test for seasonal effects, and two separate PCAs were generated. These results (Table 1.6) are very similar to those in the 1990 data (Table 1.4).

Table 1.6 Principal Component Analysis for 35 farm dataset in 1991.

Variable	Between farm effect	Seasonal effect	Seasonal effect
	PC1	PC1	PC2
Milk urea	+0.43	-0.56	0.65
Milkfat/cow/day	-0.64	-0.55	-0.31
Milk protein/cow/day	-0.64	0.61	-0.08
Proportion	75%	61%	22%

PC1 (seasonal effect) describes a milk protein per cow reduction as milk fat per cow rises to a peak in October. PC2 is related to a fall in milk production in late September.

1.3.10.2 *Correlation Matrix*

A correlation matrix of 59 complete data points from the 1990 data is presented in Table 1.7 which examines interrelationships between more data than the PCA analysis, including weather, milk production, pasture analyses and blood analytes. The week of observation variable displays the changes expected at the start of spring and early lactation. Alb strongly correlated with NEFA (r = 0.63). Blood urea strongly correlated with pasture protein (r = 0.54), but strongly negative with DM %, SOLCHO %, milkfat/cow/day and milkprotein/cow/day (r = -0.48, -0.60, -0.71, and -0.68 respectively). BOH correlated strongly with NEFA (r = 0.63). Dry matter % of pasture correlated strongly with SOLCHO (r = 0.60) and pasture N (r = -0.62). Pasture SOLCHO correlated negatively with blood urea, pasture N, pasture ADF, and ground temp (r = -0.6, -0.46, -0.49, -0.57). Pasture N correlated negatively with milk protein/cow/day (r = -0.34). The negative correlation of ground temperature with sunlight (r = -0.34) and positive with rainfall (r = 0.42) is also of interest. The correlation matrix detects the same relationships identified by PCA for urea and milk production.

1.4 INTEGRATING DISCUSSION

Three separate datasets over two seasons indicate a strong negative relationship between urea level and per cow production. Whilst this relationship is not necessarily causal, this seems a strong possibility.

Milk urea was closely related to protein level in pasture and even more strongly to pasture protein/soluble carbohydrate ratio (Figure 1.21). This fits well with published literature on the subject (Gustafsson, 1993; Moore and Varga, 1996; Fulkerson, 1996), although other factors also influence milk urea levels (Gustafsson, 1993), like lactation number, mastitis and calving date.

Table 1.7 Correlation matrix for complete data points (n = 59)[1]

	Week[2]	Alb	Urea	BOH	Glu	NEFA	DM%	S.CHO	N	ADF	Fat	Prot	Sun	Rain	Temp
Week	1.00														
Alb	0.09	1.00													
Urea	0.19	-0.05	1.00												
BOH	-0.47	0.29	-0.03	1.00											
Glu	0.16	-0.20	0.02	-0.35	1.00										
NEFA	-0.42	0.63	-0.16	0.63	-0.26	1.00									
DM%	-0.11	-0.17	-0.48	-0.14	-0.06	-0.08	1.00								
S.CHO	-0.42	-0.04	-0.60	0.07	0.01	0.17	0.60	1.00							
N	-0.22	-0.14	0.54	0.08	0.13	0.01	-0.62	-0.46	1.00						
ADF	0.62	0.39	0.18	0.09	-0.06	0.04	-0.32	-0.49	-0.29	1.00					
Fat	-0.15	0.22	-0.71	0.07	0.02	0.22	0.23	0.38	-0.40	-0.03	1.00				
Protein	-0.44	0.10	-0.68	0.24	0.00	0.25	0.24	0.54	-0.34	-0.17	0.80	1.00			
Sun	-0.03	-0.04	-0.01	-0.12	0.15	-0.09	0.21	0.28	0.07	-0.40	-0.10	-0.10	1.00		
Rain	0.23	0.13	0.11	0.03	-0.17	0.12	-0.10	-0.26	-0.24	0.49	-0.06	-0.16	-0.58	1.00	
Temp	0.86	0.23	0.24	-0.29	0.03	-0.29	-0.31	-0.57	-0.18	0.78	-0.10	-0.35	-0.34	0.42	1.00

[1]Week = week of observation, Alb = blood Alb, Urea = blood urea, BOH = betahydroxybutyrate, Glucose = blood glucose, NEFA = non-esterified fatty acids, DM% = pasture dry matter %, S.CHO = pasture soluble carbohydrate, N = pasture Kjeldahl N, ADF = pasture acid detergent fibre, Fat = milkfat/cow/day, Protein = protein/cow/day, Sun = sunlight hours/day for the 3 days before pasture sampling, Rain = rainfall/day for the 3 days before pasture sampling, Temp = soil temp at 10 cm depth.

[2]Relations significant at $P < 0.05$ when r exceeds 0.25, at $P < 0.01$ when r exceeds 0.3 and $P < 0.001$ when r exceeds 0.41

The associations between blood urea, pasture protein, pasture soluble carbohydrate and per cow milk production may relate to a difference in the nature of pasture fed to cows in herds that have less pasture available. That is, the reason for the differences could simply be that shorter pasture contains higher protein levels (Holmes, 1989), and that lower producing herds are offered less dry matter (DM) or metabolisable energy (ME). This explanation does not always agree with personal observation, where adequate dry matter seems to be available yet productivity may fall. The data from 1991 (not presented), where dry matter intakes were measured support the suggestion that DM intake is only part of the explanation for the associations found (Table 1.5).

The literature (Blaxter, 1962; Danfaer *et al.*, 1980; NRC, 1989; Moore and Varga, 1996) suggests that a milk production penalty will occur if dietary protein levels exceed 18% and that this penalty will escalate as protein level in the diet approaches 30% (Danfaer *et al.*, 1980; Gordon, 1980). With pasture protein levels in fresh pasture exceeding 25% frequently in spring when most of our seasonal calving cows are nearing peak production, this factor may contribute to the relatively low per cow peak production in New Zealand (Holmes and Hughes, 1993). The PCA analysis of data detected milk production drops when pasture protein is high and pasture SOLCHO is low.

There were apparent negative associations between herd reproductive performance (anoestrus, conception rate and empty rate) and urea levels also. Conception rate depression caused by excess urea or excess dietary protein has been noted by various authors (Jordan and Swanson, 1979; Ferguson *et al.*, 1988), but evidence in New Zealand is limited. Results presented here are not conclusive.

A relationship between anoestrus and urea levels has not been suggested previously in the literature, but anoestrus of the type seen in New Zealand is not common overseas either. This relationship could be indirect and not causal.

Seasonal trends in pasture nutrients probably relate to increasing pasture maturity as pasture growth rates exceed demand in October, as ryegrass reaches its reproductive stage and produces more stalk and less leaf, and as the pasture responds to increasing

temperature. Soluble carbohydrate (SOLCHO) content is a much more labile component of pasture, fluctuating from week to week (Figure 1.15). This appears to be associated with weather conditions, sunlight hours in particular (Figures 1.3-1.5). To some extent the increase in protein content of pasture when SOLCHO is low is a dilution effect, but this does not alter the fact that cows consuming this pasture will consume more protein and less SOLCHO. Pasture dry matter content appears closely associated with SOLCHO content (see Figure 1.21). This creates an additional problem for interpretation and presumably for the cow consuming this pasture. Cows need to consume 20-50% more wet matter of pasture to maintain the same intake of ME if presented with the low (10-12% vs 15%) dry matter pasture (Ulyatt and Waghorn, 1993). Pasture yields should be adjusted for the dry matter % change when estimating intakes, and this would not be the case with rising plate meter estimates of pasture present. SOLCHO is stored in the stem of ryegrass plants mainly as fructans (Chatterton *et al.*, 1989) and herds C and D (1990 data) both grazed more mature pasture than Herds A and B.

The consequences of reduced soluble carbohydrate and increased protein in the cow's diet will relate to rumen fermentation especially (Obara *et al.*, 1991). Rumen bacteria require readily available carbohydrate energy to metabolise protein into bacterial tissue. If lower than recommended levels of energy are present, less of the protein can be harvested by the bacteria. Non structural carbohydrate recommendations for optimal rumen function are over 30% of the diet (Obara *et al.*, 1991; Stokes *et al.*, 1991b). The excess pasture protein will be absorbed as ammonia, which is toxic to the cow, and converted to urea in the liver, which is then mainly excreted (Danfaer *et al.*, 1980; Visek, 1984; Moorby, 1995). The liver conversion process is relatively energy expensive, leaving less energy available for milk production (NRC, 1989). Reduced energy available for milk production will result in decreased milk production or increased weight loss in the cow as it attempts to adjust ME supply to requirements for production. Reduced bacterial supply to the small intestine could be another consequence of carbohydrate/protein imbalance in the rumen and this can lead to reduced milk protein production (Stokes *et al.*, 1991a). The ratio of CP/SOLCHO was 30% higher in herds A and B in the 1990 data. In absolute terms the cows in herds C and D would have consumed an extra 0.5 kg soluble carbohydrate daily.

Interpretation of blood parameters measured in the 1990 data is as follows: Higher urea levels in blood reflected the higher pasture protein and lower pasture SOLCHO. They could possibly have been elevated with weight loss or catabolism also, and weights were not recorded in any of the data. Albumin levels are a general reflection of cow condition. Wilson *et al.* (1985) noted reduced fertility with lowered albumin levels in New Zealand cows. There was no clear evidence of this in this study, although herd D had excellent fertility, and this herd had the highest Alb levels. The nadir in Alb in week 10 suggests condition gain from then on, but the reason for this is unknown. Whilst the blood parameters measured do provide some insight into what may have been occurring in these herds, they are not conclusive on their own. Further investigation of urea, albumin, glucose and BOH or NEFA appear to be warranted in relation to production and fertility but a much more controlled environment would be needed to allow interpretation. It is possible that the problems noted with excess pasture CP may relate to only part of the CP, ie. the non-protein nitrogen (NPN) component of the CP may be the most significant. The work by Danfaer *et al.* (1980) and Gordon (1980) involved elevation of dietary CP with non-protein nitrogen sources. Further work would be needed to clarify this.

Hyperglycaemia results from ammonia toxicity (Payne, 1977). Although ammonia was not measured, higher ammonia could occur with spillage when the liver is supplied with large amounts of ammonia. This may explain the increases in blood glucose around weeks 13-14.

A major problem with field data interpretation appears to be obtaining an accurate estimation of DM intake of individual cows, because low SOLCHO pasture, high protein pasture, low DM % pasture, elevated ketones and possibly lower DM intakes all tend to occur simultaneously.

1.5 CONCLUSIONS

Whilst the conclusions from this observational study must be tentative because no attempt was made to control differences between farms, the measurements and data

collected suggested that further investigation was warranted into protein and carbohydrate relationships with fertility and milk production and also the agronomic and pasture management factors influencing pasture protein and SOLCHO.

Strong associations were found (some of them in 3 datasets) between weather conditions, blood parameters, pasture nutrients, production and reproduction but no causation was proven. The most prominent associations were between blood urea (or milk urea), pasture protein (+ve) and pasture soluble carbohydrate (-ve), reduced milk production and possibly reduced conception rates. Periods where pasture SOLCHO and dry matter % reduced and pasture CP increased were associated with poorer milk production.

The hypothesis formed from this observational study is that high dietary protein or high protein/soluble carbohydrate ratio in pasture results in a considerable milk production penalty caused by the liver requiring extra metabolisable energy to convert excess ammonia absorbed from the rumen into urea so that it can be excreted. Ancillary hypotheses are that more mature pasture provides a better dietary balance of protein and soluble carbohydrate, and that supplementation of the pasture diet with sources of soluble carbohydrate should markedly improve milk production and dairy cow fertility. More knowledge of factors affecting soluble carbohydrate and protein levels in dairy pasture appears to be needed if steps are to be taken to minimise production loss from excess dietary protein.

The following chapters establish the seasonal patterns likely to be seen on dairy farms for the main pasture nutrients, test the effects on production and reproduction of supplementation of pasture with soluble carbohydrate or starch sources, test the effects of a "balanced diet" on production and reproduction, examine the effects of grazing and maturation on pasture nutrient analysis and finally test the effects of nitrogen application on pasture nutrient analysis.

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CHAPTER 2

Seasonal variation in nutrient levels of New Zealand dairy pastures

PREFACE

The previous chapter identified associations of high pasture crude protein in spring with reduced milk production and poor herd reproductive performance. More information was needed about the usual patterns of pasture nutrient content with season. Little information was available regarding nutrient change within dairy pasture over one whole season on dairy farms in New Zealand. Hutton (1961, 1962), Hutton and Jury (1964), and Hutton *et al.* (1967) reported proximate and mineral analyses for a large part of the dairy season from a Ruakura dairy farm, but this did not include measurements for soluble carbohydrates, acid detergent fibre (ADF), neutral detergent fibre (NDF), or pectin. This chapter describes a joint project between the author and Massey University staff (Moller *et al.*, 1996b) designed to more accurately define seasonal change in pasture nutrients and to study interactions between these nutrients. It was felt that this information was necessary to identify potential weaknesses in the pasture diet for dairy cows and assist research into enhancing dairy cow performance (production and herd reproductive performance) from pasture diets.

2.1 INTRODUCTION

Pasture nutrient composition and nutritive value are usually described in the following terms. Relationships to nutrient requirements for milk production are discussed for each nutrient.

2.1.1 Pasture digestibility

Pasture nutritive value has traditionally been assessed in terms of *in vitro* digestibility (OMD, DOMD), and this has been linked to dry matter intake (DMI) of pasture diets (Hodgson, 1977; Wilson *et al.*, 1995). Pasture digestibility is often converted to an ME value (metabolisable energy). The energy density (megajoules of metabolisable energy per kg of dry matter, or MJ of ME/kg DM) of the pasture diet will determine milk production and weight change if cows are fed to appetite. The importance of maintaining pasture digestibility in a pasture only dairy system should not be underestimated.

For example, a 450 kg Friesian cow with no weight gain or loss consuming 16 kg DM of 12 MJ of ME/kg DM pasture in mid October will consume 192 MJ of ME. Maintenance requirement for this cow is approximately 55 MJ of ME and there will be no requirement for weight gain or pregnancy (NRC, 1989). This leaves 137 MJ of ME available for milk production. One litre of 4% fat milk requires approximately 5 MJ of ME, so this cow should produce 27.4 litres of 4.0% fat milk or 1.09 kg milkfat. If, however, the cow consumes 16 kg DM of 11 MJ of ME/kg DM pasture the production will only be 24.2 litres of 4% fat milk or 0.97 kg fat. ie 3.2 litres milk difference simply because of a difference in ME value of the pasture consumed. This difference in ME consumed can also be diverted to other productive purposes like weight gain or less weight loss. In addition, intakes of 16 kg DM may be difficult to maintain in a 450 kg cow as this is near the cow's physiological limit. Lower digestibility pasture (like the 11 MJ pasture) could limit intake to below 16 kg DM and hence further reduce the likely milk production in the above example. Maintenance of pasture quality can have a large bearing on per cow productivity and hence profitability in a pasture only dairy system as practised in New Zealand.

The concentration of digestible organic matter in the dry matter of herbage (DOMD) is closely related to the concentration of metabolisable energy (NRC, 1989). Farmers would generally associate fresh green pasture with high DOMD, and though this is generally the case, it is not always so (Wilson *et al.*, 1995). While well managed dairy pasture does provide high quality feed, it does have a number of nutrient deficiencies

which are thought to limit milk production and herd fertility. These have been identified by various authors (NRC, 1989; Moller, 1991; Moller, 1993; Muller, 1993; Edwards and Parker, 1993; Ulyatt and Waghorn, 1993; Muller *et al.*, 1995; Wilson *et al.*, 1995; Moller, 1995).

2.1.2 Crude protein % (CP%)

Crude protein % often exceeds dietary nutrient requirements in pasture diets and the first chapter identified associations of high CP% with reduced milk production and poor herd reproductive performance. Metabolisable energy is required in the liver to metabolise excess ammonia absorbed from the rumen and gut. The extent of the energy requirement for metabolising excess protein is still not well defined, with predictions of 1.4 litres of 4% fat milk reduction when dietary protein changes from 19% to 23% by Danfaer *et al.* (1980) (see Fig. 1.3), and 7.2 kcal of ME/g of N (0.03 MJ of ME/g of N) in excess of requirements (Tyrell *et al.*, 1970). Recently Lobley *et al.* (1995) has found that in the formation of urea molecules, the liver requires one ammonia radical from an amino acid and one from absorbed ammonia. This may help to explain the apparent catabolic nature of high protein pasture diets (Muller, 1993). A large proportion of the protein in fresh pasture is rumen degradable (RDP) as opposed to rumen undegradable (RUDP, UDP, UIP, "bypass" protein) and is rapidly degraded in the rumen to ammonia, peptides and other forms of nitrogen (NRC, 1989; Muller, 1993; Muller *et al.*, 1995). For this to be a useful nutrient for the cow, rumen bacteria must assimilate it into bacterial tissue which then passes to the gut where it is digested and absorbed mainly as amino acids. Rumen bacteria require carbohydrate energy sources to assist with bacterial growth and multiplication and estimates for optimal fermentation are over 30% soluble carbohydrate or starch sources (Stokes *et al.*, 1991b). High yielding cows require more UDP because maximal bacterial protein production in the rumen is limited and cannot fulfil requirements over 20 litres milk (Orskov, 1981). Recommendations for high producing cows in the US are for 35% of protein to be UDP (NRC, 1989; Muller, 1993; Hutjens, 1995), and fresh pasture is probably nowhere near this (Muller, 1993). This would suggest that certain amino acids may be limiting in a pasture diet, but responses to methionine and lysine supplementation or fish meal supplementation

have not provided production responses to date in New Zealand (Penno and Carruthers, 1995; Salam *et al.*, 1996; Ulyatt, pers comm.). The non-protein nitrogen (NPN) component of pasture is of especial interest in that this will all be rumen degraded. In some circumstances this can be a high proportion of the CP% in pasture (Beever, 1993; Rulquin and Verite, 1993) and changes in NPN may play a large part in determining the protein energy penalty. The experiments of Danfaer *et al.* (1980) and Gordon (1980), where reduced production resulted from increased CP both involved addition of NPN to the diet to increase dietary CP. Both experiments used silage based diets, which may also have added to the non protein nitrogen concentrations in these diets.

2.1.3 Neutral detergent fibre (NDF) and acid detergent fibre (ADF)

NDF and ADF are both measures of the fibre or cell wall component of pasture. Neutral detergent fibre mainly represents the hemicellulose, cellulose and lignin fractions whereas ADF represents only cellulose and lignin. Hence the difference is an approximation of the hemicellulose component in pasture. Acid detergent fibre is less relevant as a representation of fibre in feeds but is often linked to the digestibility of feeds and many feed values are presented as ADF in standard texts. High performance diets in the US are formulated with NDF values in the 27-30% region and ADF near 20%. This is partly to allow a high energy density in the feed to maximise intake of ME. Higher NDF levels in feeds are known to limit DM intake (Mertens, 1985) with reductions in intake beginning near 35-40% NDF. This is a gut fill phenomenon, with more fibrous foods passing through the gut more slowly. "Effective" fibre is a more recent term describing the "scratch factor" function of stalky foods that promotes salivation and good rumen function (Muller *et al.*, 1995; Mertens, 1996). As yet, where pasture fits in the "effective" fibre range is unclear, although theoretical calculations using the Cornell net carbohydrate and protein model suggest fresh pasture diets may be low in this characteristic (Kolver, 1996).

2.1.4 Soluble carbohydrate and pectin

Soluble carbohydrate and pectin are two of the main readily fermentable carbohydrate components in pasture (Ulyatt and Waghorn, 1993), although other readily usable energy sources may also exist like organic acids. Starch is present in only low levels in ryegrass/clover pasture. Technically pectin is a fibre, but is readily fermentable. Recommendations for optimal rumen function for soluble carbohydrate and starch exceed 30% of the diet (Stokes *et al.*, 1991a). Pasture does not reach these levels (Ulyatt and Waghorn, 1993). Combined with the high CP%, the relatively low level of readily fermentable carbohydrate is a weakness of a pasture only diet as suggested in Chapter 1. The situation may exist where excess dietary protein in pasture could lead to insufficient amino acid supply to the small intestine for absorption and subsequently reduced milk protein production.

2.1.5 Dry matter %

Dry matter % is another characteristic of pasture likely to affect dry matter intake (DMI) with strong correlations between DMI and dry matter %, in the range from 12-25% dry matter as found by John and Ulyatt (1987). Verite & Journet (1970) found improved dry matter intake in milking cows when pasture DM% increased from 12 to 15% but little effect over 18%. Dry matter intake is progressively depressed by dietary DM% under 50% for silage and other fermented feeds, but this does not apply to pasture diets (NRC, 1989).

2.1.6 Minerals

Dietary **calcium** levels recommended in NRC (1989) are 0.43-0.66% of the diet depending on production level, whereas Grace (1983) states the requirements at 0.32% for cows producing 30 litre of milk. Dietary **phosphorus** levels recommended by NRC are 0.28-0.41% of the diet depending on production level and according to Grace are 0.3% for cows producing 30 litres milk. The recommended **potassium** levels are 0.9-

1% (NRC, 1989) and 0.2-0.6% (Grace, 1983). **Magnesium** recommendations are for 0.2-0.25% (NRC, 1989) and 0.16% (Grace, 1983). The discrepancies between the two sources perhaps relate to the difference between safe recommendations and calculated requirements, sometimes at lower levels of expected performance. Magnesium deficiency is a commonly recognised phenomenon on dairy farms in New Zealand, and most farmers take preventative steps, usually supplementing cows with magnesium oxide. In part magnesium deficiency results from interactions between high potassium levels and magnesium (Feyter *et al.*, 1986), making magnesium less available to the cow. Deficiencies in calcium and phosphorus are less commonly recognised in cows grazing pasture in New Zealand.

Deficiencies or excesses for other mineral nutrients are also recognised. Sodium and the trace elements selenium, cobalt and copper are often deficient for dairy cattle. Excesses of molybdenum and iron can interfere with copper uptake. Iodine and zinc deficiency may also occur (McNaught *et al.*, 1968; Smith and Cornforth, 1982; Grace, 1983; Manktelow, 1984; Feyter *et al.*, 1986; Betteridge, 1989; NRC, 1989; Ellison, 1991).

2.2 EXPERIMENTAL DESIGN

Four dairy farms were selected for the study. These could be described as "well managed" dairy farms of average to high productivity for their area with stocking rates similar to those in their district, with high soil fertility, different soil types, differing climate and differing sward types (see Table 2.1).

Two farms were located in the Manawatu; Massey University's No. 1 and No. 4 farms and two properties were located in Waikato (near Te Awamutu). Sampling occurred over a two year period on three of the farms and for 12 months on the fourth farm.

Table 2.1 Farm details

	Farm 1 (Massey)	Farm 2 (Waikato)	Farm 3 (Waikato)	Farm 4 (Massey)
Soil P(1993)[1]	26	34	39	27
Soil K[2]	0.47(exch K)	13	1.06(exch K)	0.6(exch K)
Soil S [2] (Sulphate S)	12	17	-	15
Soil pH[4]	5.9	6.0	6.2	5.8
Fertiliser applications (kg/ha) 1993/94	Aug-Sept 50N Nov 50P, 0K, 5S, 40N	Aug-Oct 38P, 66K, 87S, 34N Mar-Apr 40P, 35K, 4S, 36N	Jul-Aug 36P Mar 36P	Aug-Sept 33N Apr 17P, 43K, 23S
Soil types	Manawatu silt loam and Rangitikei fine sandy loam	Mairoa ash and Puniu silt loam	Mairoa ash	Tokomaru silt loam
Farm productivity (kg mfat/ha/yr) 1993/94	406kg MF/ha	900kg MF/ha[5]	575kg MF/ha	440kg MF/ha
Main pasture species	Mixed ryegrass/white clover/prairie/ cocksfoot/fescue	Ryegrass/clover	Ryegrass/clover	Ryegrass/clover

[1]P by Olsen extraction, μg/ml - soil test done in 1993
[2]K by ammonium acetate extraction, MAF QT units
[3]S by potassium phosphate extraction, ppm
[4]pH by 1:2.1 V/V water slurry
[5]This farm purchased approximately 3000 kg DM/ha supplement

2.2.1 Pasture sampling procedure

Massey Farms - 1992/93:

During the 1992/93 season two representative paddocks at each of the No. 1 and No. 4 farms were sampled at approximately monthly intervals. Samples were not collected when paddocks were closed for silage or within one week after grazing. These samples were thus collected irrespective of growth stage apart from the extremes mentioned above.

Massey farms - 1993/94:

Commencing in July 1993 a modified sampling procedure was adopted. A third paddock was sampled fortnightly on each farm which was from a paddock about to be

grazed by the cows on the next day. This extra sample allowed a more complete picture of what cows were actually eating on these farms.

Waikato farms - 1993/94:

Fortnightly sampling commenced on Farm 2 in January 1993 and continued till July 1994. Sampling on Farm 3 commenced in August 1993 and continued till July 1994. Samples were collected from one representative paddock on each farm. A wire cage was used to protect the site so that if cows had passed through the paddock in the previous 2 weeks as part of their normal rotational grazing it was still possible to collect a representative pasture sample similar to what the cows had just eaten. The cage was then moved after the sample was collected so that the next sample would represent typical pasture regrowth offered to cows at the next rotation.

2.2.2 Sampling method

Pasture was cut at levels so that samples were representative of what cows were eating. Where possible (when the caged site was not needed) this was collected from different parts of the paddock. The composite sample was immediately placed in a chillybin containing crushed ice and transported to a deep freeze where it was stored until required for drying and Near Infra Red Spectrophotometry (NIR) analysis.

2.2.3 Sample preparation and analysis methods

Samples were freeze dried, ground through a 1 mm sieve, then stored in airtight containers ready for NIR analysis.

Original samples used for calibration of the NIR were analysed using the following methods:

- Crude protein was calculated from the Kjeldahl N value x 6.25. Kjeldahl N was measured using a Kjeltec Auto system.

- Acid Detergent Fibre and Neutral Detergent Fibre were determined using a modified Van Soest method as described in James and Theander (1981).
- Soluble carbohydrate was analysed by the "Nelson's determination of reducing sugars" method.
- Pectin was analysed using the serial extraction of carbohydrates method for D-glucuronic acid.
- *In vitro* digestibility was measured by the procedure outlined by Roughan and Holland (1977). Metabolisable energy (ME) was calculated using the formula (DOMD x 0.16) (AFRC, 1993).
- Minerals (Potassium, Magnesium, Calcium and Phosphorus) were measured by atomic absorption (AAS).

More detail on the laboratory methods is included in Appendix 1. In all 193 samples with results from conventional chemical analyses were available for calibration of the NIR. Not all variables had values for 193 samples (see Table 2.2). These values were checked against the NIR predictions and a variance (R^2) value calculated, giving some idea of the likely accuracy of the NIR predicted value. The NIR analyser (based on calibrations derived from the 193 samples) was then used to analyse all the samples collected from the four farms over the 2 year period.

Table 2.2. Variance values for the NIR prediction compared to the 'wet' chemistry result[1].

Variable	N	Mean	SE	r^2
ADF (g/kg)	52	269.2	13.1	0.48
NDF (g/kg)	132	406.4	12.9	0.95
Cr.Protein (g/kg)	176	198.8	7.2	0.97
SOLCHO (g/kg)	159	115.2	14.3	0.79
Pectin (g/kg)	132	20.9	2.9	0.84
Phosphorus (g/kg)	49	3.5	0.2	0.75
Potassium (g/kg)	46	27.1	1.1	0.88
Calcium (g/kg)	47	5.1	0.4	0.93
Magnesium (g/kg)	48	1.6	0.1	0.76
Dig (DOMDg/kg)	108	718.4	12.9	0.84

[1]units for this table are in g/kg as supplied by Sydney University, Camden Laboratory.

Although the R^2 value for ADF was not high, the SE value indicates that the predictive value on NIR was still likely to be good. The R^2 values for the other nutrients measured were within acceptable limits. Mineral predictions are more difficult on NIR because NIR only recognises organic bonds and thus is measuring minerals indirectly from molecules associated with them (Shenk and Westerhaus, 1994). However, NIRS analysis is highly repeatable and not subject to "human error" from the multiple handling that occurs in traditional "wet chemistry".

A bayesian smoother (Flexi 2.4) was used to more clearly identify seasonal trends. The bayesian smoother was instructed to look for 365 day patterns (Upsdell, 1994). The bayesian smoother is a mixed model with the time effect being a random component with high correlations between neighbouring points. The covariance function was chosen to find seasonal patterns (Upsdell, 1996).

2.3 RESULTS AND DISCUSSION

Results and discussion are combined for easier interpretation of results.

2.3.1 Seasonal changes

Soil tests were in or near the optimum recommended range for P, K, S and pH on all four farms (see Table 2.1). Each nutrient is tabulated for each farm individually.

There were no visible differences on x-y scatterplots between Massey samples collected from paddocks sampled all over the farm just before grazing (92-93) and those collected regularly from fixed paddocks (91-92). These scatterplots have not been reproduced here.

There was considerable variation in nutrient analyses within seasons both within and between farms. The seasonal trends were very similar on all four farms measured.

Basic statistics for the data are represented in the following tables split into farms and seasons respectively. SEM is expressed as a simple estimate of overall variation.

Table 2.3. Means and Standard Errors for all variables by farm and over all 4 farms.

	Farm 1		Farm 2		Farm 3		Farm 4		All 4 Farms	
n	76		54		23		84		237	
Variable	Mean	(SEM)	Mean	(SEM)	Mean	(SEM)	Mean	(SEM)	Mean	(SEM)
ADF (%)	27.7	(0.2)	28.8	(0.3)	29.0	(0.6)	28.1	(0.2)	28.2	(0.14)
NDF (%)	36.4	(0.7)	38.6	(0.8)	38.5	(1.3)	38.3	(0.7)	37.8	(0.41)
CP (%)	24.6	(0.4)	23.2	(0.5)	23.8	(1.0)	23.4	(0.5)	23.8	(0.28)
SOLCHO %)	10.9	(0.3)	10.4	(0.4)	11.1	(0.5)	10.6	(0.3)	10.7	(0.18)
Pectin (%)	2.05	(0.07)	2.04	(0.1)	1.81	(0.07)	1.93	(0.06)	2.0	(0.04)
P (%)	0.37	(0.004)	0.35	(0.005)	0.33	(0.01)	0.37	(0.004)	0.4	(0.002)
K (%)	2.65	(0.07)	2.85	(0.08)	2.72	(0.14)	2.79	(0.05)	2.8	(0.04)
Ca (%)	0.7	(0.01)	0.78	(0.02)	0.73	(0.03)	0.7	(0.01)	0.7	(0.01)
Mg (%)	0.2	(0.002)	0.20	(0.002)	0.20	(0.003)	0.2	(0.002)	0.2	(0.0009)
DOMD (%)	74.3	(0.4)	73.7	(0.5)	74.5	(0.8)	73.5	(0.4)	73.9	(0.3)

Mean nutrient levels found were very similar on all four farms. Pasture CP, ADF and NDF levels were slightly different on Farm 1 possibly indicating slightly better quality pasture.

Table 2.4. Seasonal changes in pasture nutrients.

	Jun-Aug1993		Sep-Nov1993		Dec93-Feb94		Mar-May1994		NRC[1]
n	29		52		50		49		recommendation
Variable	Mean	(SEM)	Mean	(SEM)	Mean	(SEM)	Mean	(SEM)	
ADF (%)	26.7	(0.35)	27.4	(0.25)	29.8	(0.3)	28.8	(0.27)	21%
NDF (%)	36.1	(0.86)	36.2	(0.73)	43.3	(0.69)	38.5	(0.90)	28%
CP (%)	22.9	(0.62)	23.6	(0.55)	21.4	(0.37)	25.8	(0.6)	16%
SOLCHO(%)	10.6	(0.24)	12.0	(0.32)	9.1	(0.25)	9.1	(0.38)	35% (NSC%)[2]
Pectin (%)	1.6	(0.04)	2.0	(0.07)	2.3	(0.08)	1.8	(0.08)	-
P (%)	0.4	(0.004)	0.3	(0.004)	0.4	(0.03)	0.4	(0.05)	0.33%
K (%)	3.2	(0.05)	3.2	(0.05)	2.3	(0.07)	2.4	(0.07)	0.9%
Ca (%)	0.6	(0.01)	0.6	(0.01)	0.8	(0.02)	0.8	(0.02)	0.58%
Mg (%)	0.2	(0.001)	0.2	(0.002)	0.2	(0.01)	0.2	(0.01)	0.2%
DOMD (%)	75.0	(0.51)	75.4	(0.39)	70.2	(0.4)	78.1	(0.55)	-

[1]NRC (1989) dietary recommendations for 450kg cow producing 25 litres of 4.5% fat milk;

[2]NSC = non structural carbohydrate by subtraction of NDF,CP,Fat and Ash.

The most marked seasonal changes occurred in late spring-summer, with ADF, NDF, pectin and calcium rising and protein, soluble carbohydrate, potassium and DOMD falling. Crude protein % and DOMD were highest in autumn, and pectin highest in summer.

Figures 2.1-2.10 represent data for the pasture nutrients measured. Flexi 2.0 has been used to present a smoothed curve. The shaded area represents the 95% confidence limit for the curve. The horizontal dotted line represents the recommended dietary requirement or limit for lactating cows producing near 25 litres of 4% fat milk (NRC, 1989; Muller, 1993). An upper limit where production or intake is likely to decline is inserted for NDF.

The variations through time for all variables were similar for all 4 farms in 1993-94. The variations were also present in the previous year (1992-93), although the number of data points in the previous season are limited. Considerable variation occurs with samples from the same farm but this does not mask a distinct seasonal pattern for the variables measured. These results suggest that similar results would be obtained from many other dairy farms in New Zealand.

2.3.2 Digestibility (Figure 2.1)

Digestibility (DOMD) decreased by 5 percentage units in the late spring-early summer period during the reproductive phase of ryegrass pasture. Digestibility has been linked to DM intakes on pasture (Hodgson, 1977; Wilson *et al.*, 1995). From a milk production point of view the timing of the reduced digestibility is detrimental for cows calving seasonally in spring that are reaching peak milk production in October and need to maintain high DM intakes to maintain production. Digestibility in ryegrass/white clover pasture is especially dependant on temperature and maturity of plants and decreases by 1% for every 1°C rise in temperature (Minson and Mcleod, 1970). Assuming that pasture management was of a high standard, then temperature influences are likely to be dominant in the variation seen in digestibility in this study. Digestibilities (*in vitro*) ranged from 65% to 80% with high values associated with high

protein, low ADF and NDF pasture, and low digestibility pastures with low protein, and higher ADF and NDF values.

Metabolisable energy values are presented in Figure 2.2.

2.3.3 Crude protein (Figure 2.3)

Very high CP levels were seen in spring and autumn especially. As discussed in the introduction these are likely to result in reduced milk production or weight loss. The high CP levels were especially marked in autumn on the two Massey herds. This phenomenon has been reported previously (Wilson, pers. comm.).

Crude protein levels in pasture generally seem sufficient to support milk production on the farms sampled, although suboptimal levels were present briefly in October/November on most of the farms and during February/March on the Massey farms. The adequacy of dietary CP may change dramatically if lower protein supplements are added to the diet in periods of shortage of dry matter such as summer. Addition of maize or maize silage to the diet can have a marked impact on dietary protein, as only 8% protein is present in maize silage (NRC, 1989). Addition of low protein (10-12%) grass silage would have a similar effect. Low CP levels in pasture in October/November could affect peak milk production as the highest dietary CP requirements are at peak lactation and should be near 17-18% (NRC, 1989). Supply of nitrogen (NAN) to the duodenum for absorption by the cow may be below optimum even with excess crude protein in the diet because pasture protein degraded in the rumen requires fermentable energy to assist with bacterial assimilation, and this was below optimum in this study. Grass silage protein tends to be even more rumen degraded and the fermentable energy lower because sugars have been converted to volatile fatty acids which are not usable by bacteria (Thomas, 1996).

Figure 2.1 **Seasonal trends for *in vitro* digestibility (DOMD) for the four farms.**

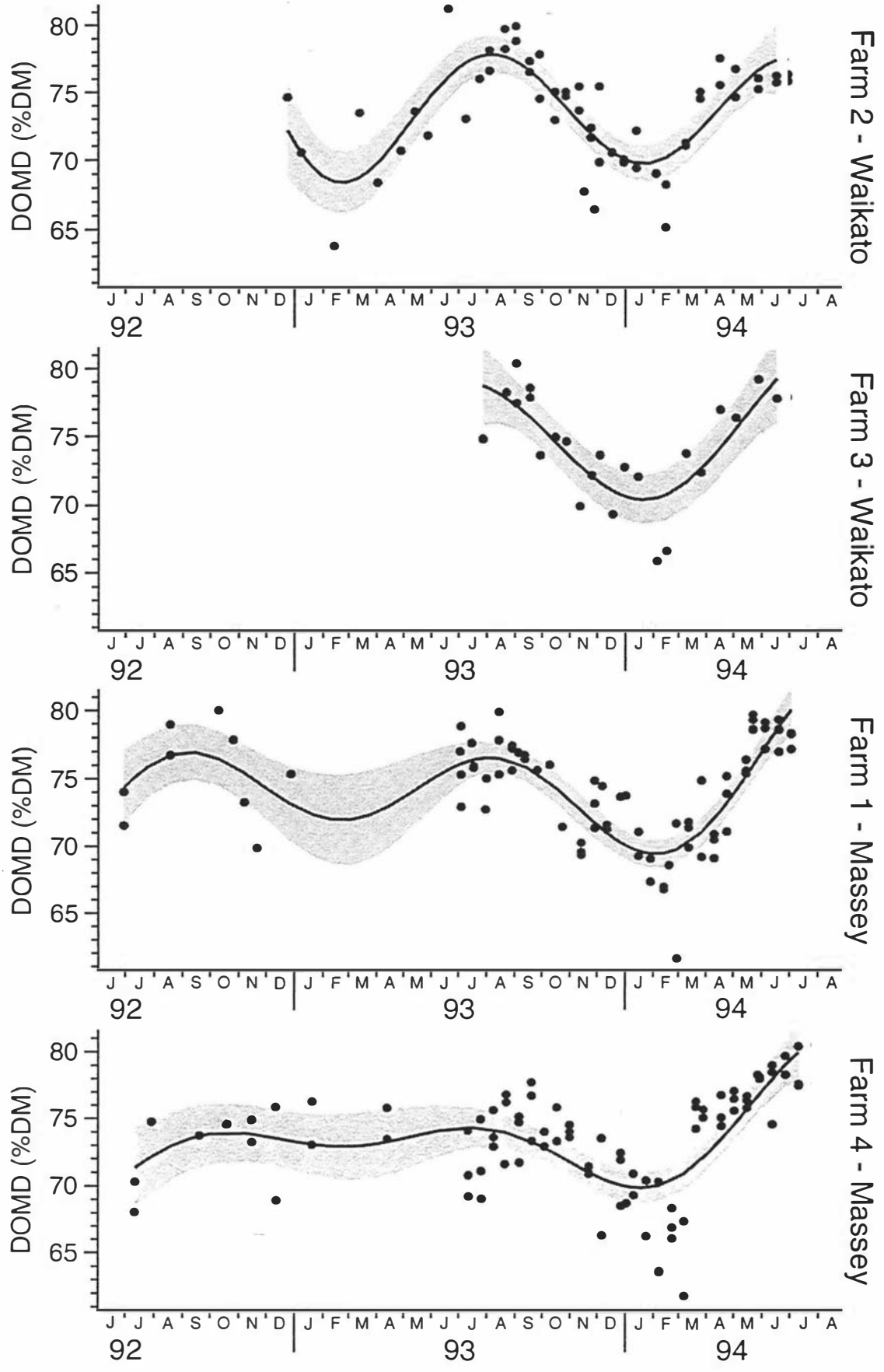


Figure 2.2 Metabolisable energy values for pasture on the four farms.

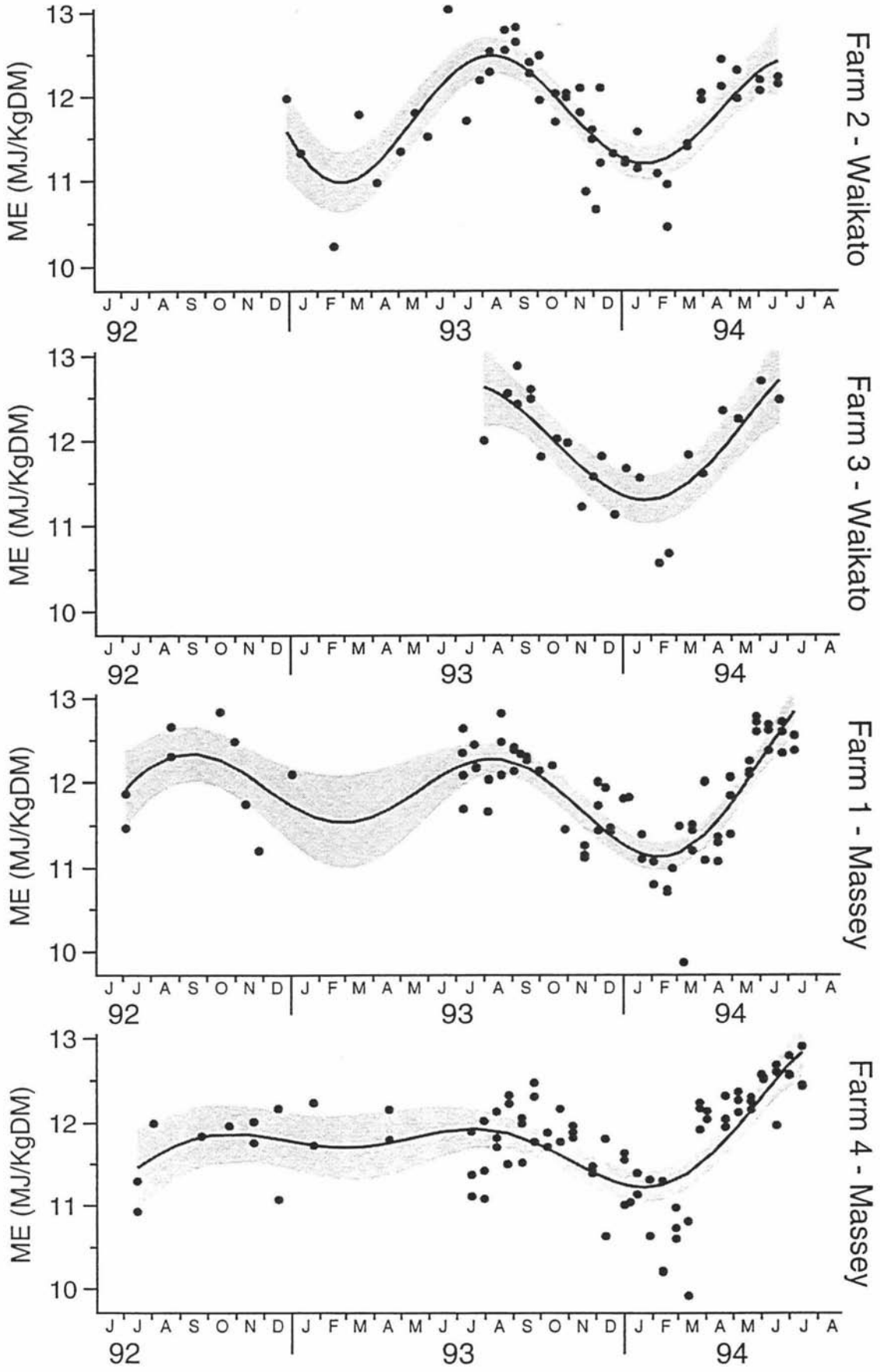
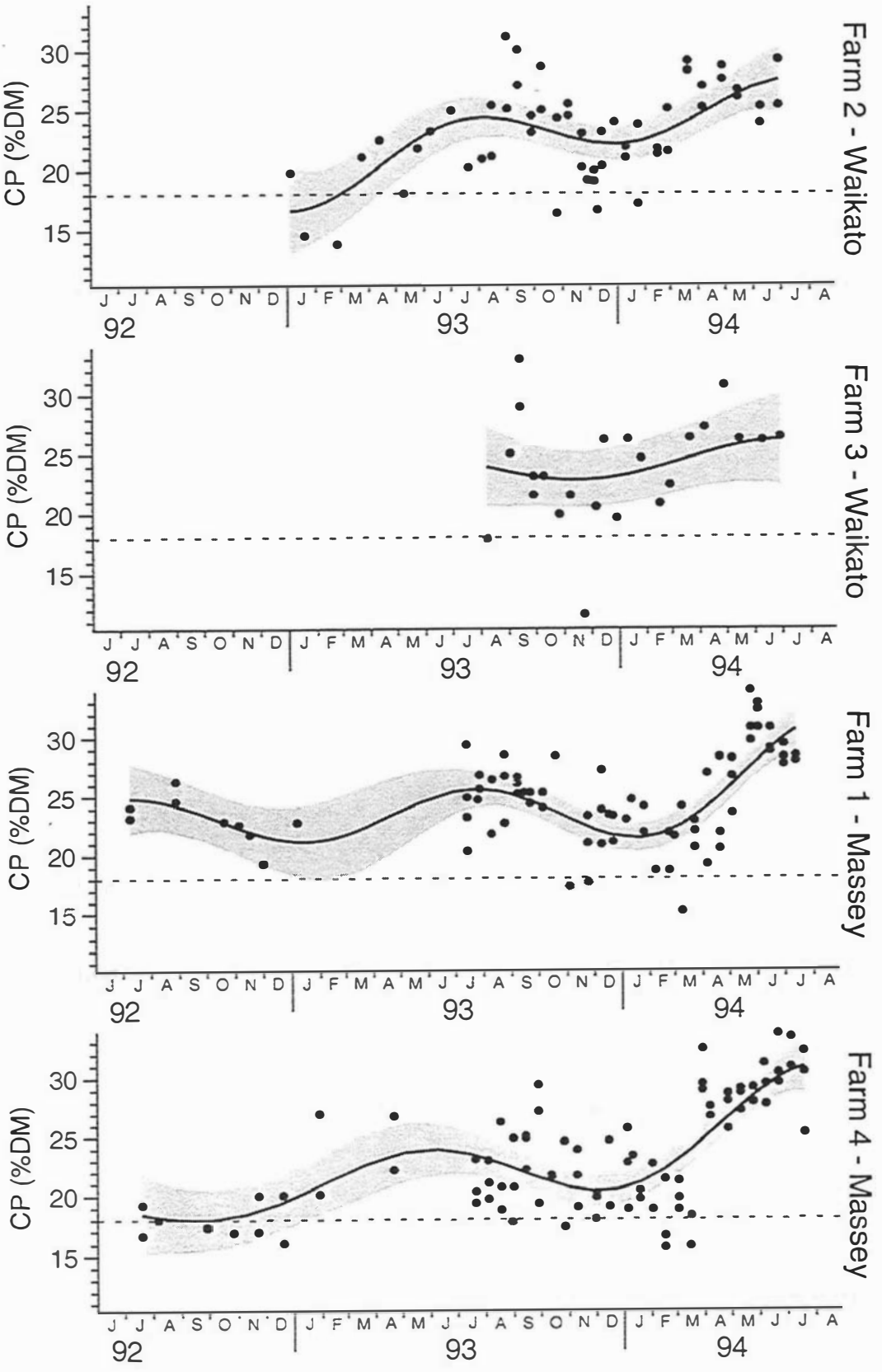


Figure 2.3 Seasonal trends for crude protein (CP) for the four farms.



Footnote: Horizontal dotted line denotes recommended CP level for 450 kg Friesian cow producing 25 litres of 4% fat milk (NRC, 1989).

2.3.4 Neutral detergent fibre (Figure 2.4)

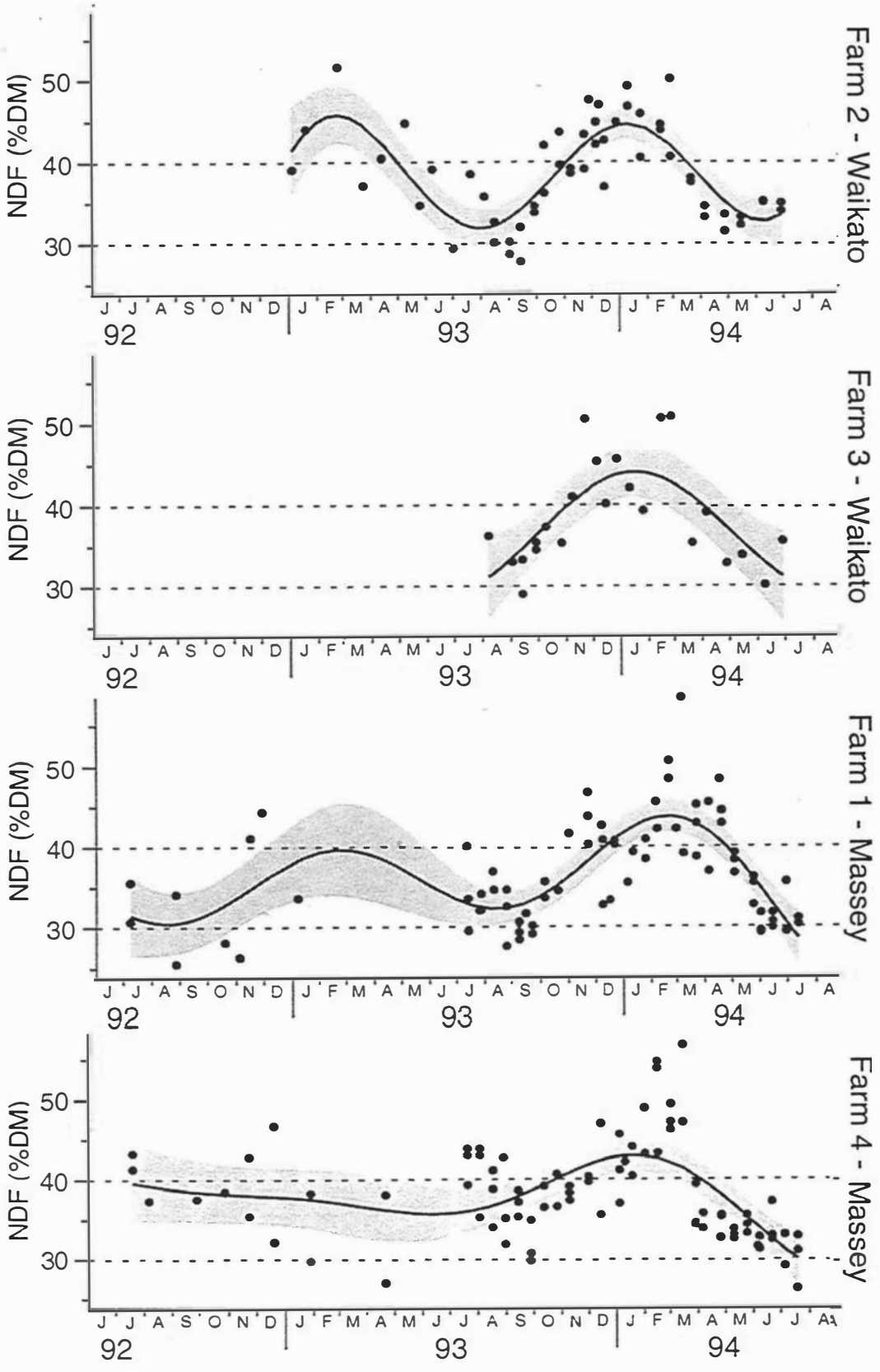
Neutral Detergent Fibre levels varied much more than any other variables with season. Although the components contributing to NDF were not measured here, NDF is chemically representing the cellulose, hemicellulose and lignin fractions combined. If the NDF levels are subtracted from the ADF levels for each sample, this will approximately represent the hemicellulose fraction of the grass. This is because the ADF represents the cellulose and lignin fractions. This would suggest that the major fibre component variation in dairy pasture through seasons is in the hemicellulose fraction (see Figure 2.5). Combination of results for all four farms and subtraction of NDF from ADF results will give an approximate value for hemicellulose in pasture. No analysis for hemicellulose occurred.

NDF is often linked negatively to dietary intake, through the slowing of digestion of more fibrous material in the diet, thus slowing passage rate in the gut and reducing appetite (Mertens, 1985). A suggested international guideline level where NDF % of the diet will start to interfere with a cow's intake is 1.4% of bodyweight as NDF (Adams, undated). The calculations in Table 2.5 suggest that NDF values will interfere with intake at around 35% NDF values or greater. Levels of NDF were over 35% in more than half of the samples measured. Maximal intake for high producing cows in the United States is attained with diet formulations near 28% NDF (Muller, 1993; Hutjens, 1995).

Table 2.5 Theoretical Calculation of excess NDF in pasture.

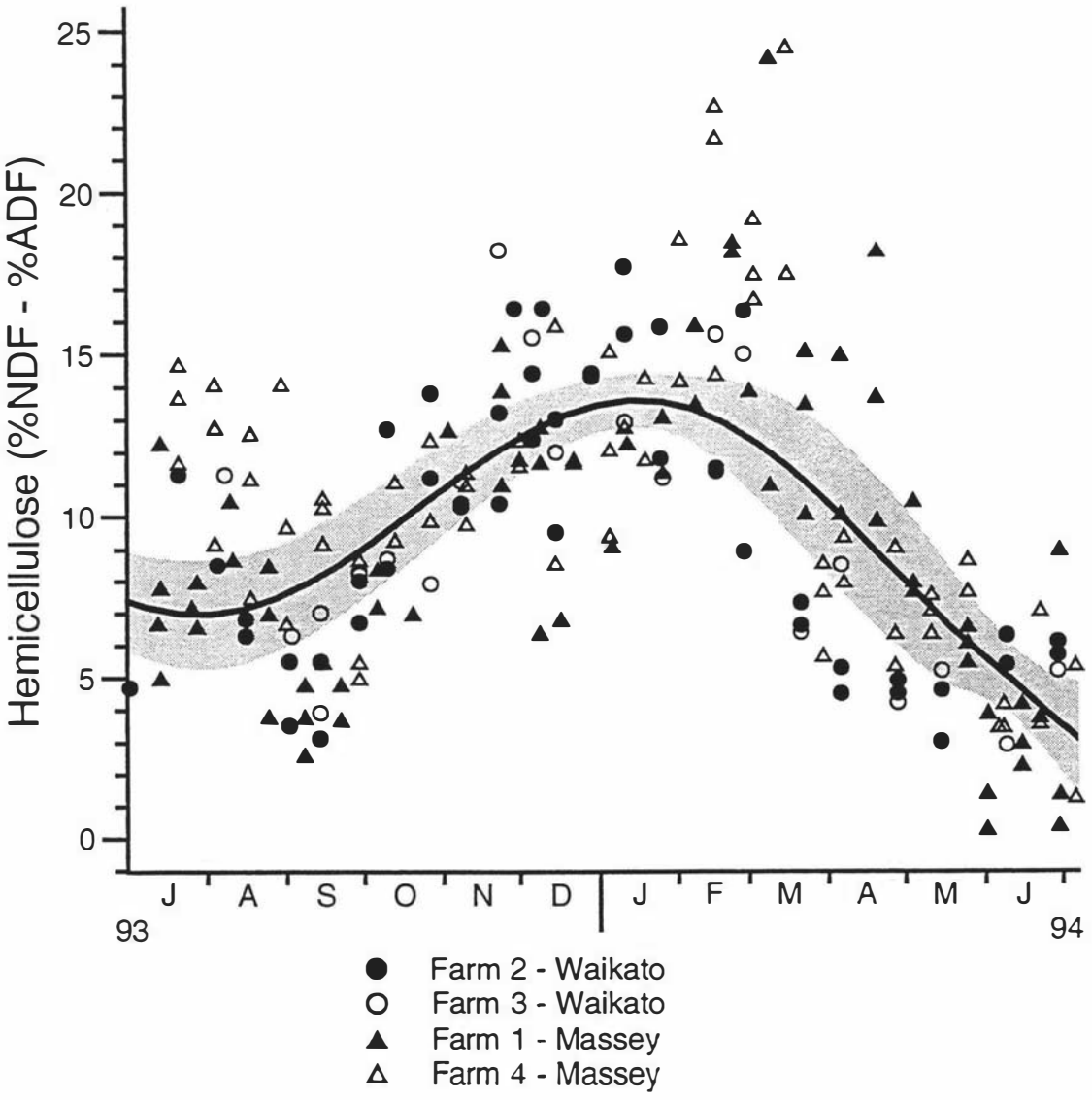
	350kg cow	400kg cow	450kg cow	500kg cow
Max. DM intake	15kg	16.5kg	18kg	20kg
1.4% Body wt.	4.9kg	5.6kg	6.3kg	7kg
30%NDF in diet	4.5kgNDF intake	4.95kgNDF intake	5.4kgNDF intake	6kg NDFintake
35%NDF in diet	5.2kgNDFintake	5.8kgNDFintake	6.3kgNDFintake	7kg NDFintake
40%NDF in diet	6kgNDFintake	6.6kgNDFintake	7.2kgNDFintake	8kg NDFintake
50%NDF in diet	7.5kgNDFintake	8.25kgNDFintake	9kgNDFintake	10kg NDFintake

Figure 2.4 Seasonal trends for neutral detergent fibre (NDF) for the four farms.



Footnote: Horizontal dotted lined lines denote the approximate lower level recommended for high producing cows (Muller, 1993) and the higher level where dry matter intake will be reduced (Mertens, 1996).

Figure 2.5 Hemicellulose (NDF - ADF) for all four farms.



Higher NDF values occur when the ryegrass is at its reproductive stage in October/November and in the summer. This is a response to increasing temperature especially (Wilson *et al.*, 1975; Fales, 1986) and increasing day length. The likely effect on milk production in NZ dairy herds is considerable, in that the cows are usually eating only pasture in October/November with no supplementation and have reached peak lactation. Maintenance of peak milk production will be difficult if pasture NDF levels are not controlled by good management practices around this time (eg. topping, ensiling surplus pasture, short rotation lengths). This could be one reason why NZ milk production curves fall off peak levels rather faster than is desirable or achievable elsewhere. Post peak decline in milk production in New Zealand is often over 10% in October and November (LIC, undated), whereas a 5% monthly decline is more common on controlled "totally mixed rations" (TMR) diets. NDF is strongly negatively associated with digestibility in the correlation matrix, and in a sense "good dairy pasture management" involves keeping adequate amounts of highly digestible pasture offered to the cows. In these data the relationship of NDF with DOMD was stronger than that of ADF suggesting that NDF would be a better predictor of digestibility than ADF.

High NDF levels in summer are likely to lead to reduced DM intakes even if cows are offered *ad lib* pasture. Cows may have difficulty maintaining weight and milking to their potential at this time because intakes are reduced, and energy density (ME value of pasture per kg DM) is also reduced.

High NDF levels in diets will favour production of high concentrations of milkfat in the milk through providing a high percentage of rumen volatile fatty acid as acetate and butyrate rather than propionate. High % milkfat is a feature of NZ grassland dairy farming.

Cows normally gain weight after peak milk production is reached (Muller, 1993). Thin cows could be described as a feature of NZ dairy farming compared to those seen internationally. The high NDF levels seen could be one factor causing this in that they limit ME intake and hence productivity and weight gain.

Addition of high NDF silage to the summer diet of high NDF pasture is unlikely to assist milk production if the cows are not underfed. This commonly occurs in the pasture-only dairy system practised here. If they are not fully fed, then addition of high NDF silage may support a lower level of production. Silage supplemented to cows for milk production should be of high quality. There are apparent responses in milk production in periods where lush (low NDF) pasture is supplemented with small amounts of hay. At first sight this appears to suggest a deficiency of NDF or ADF in the diet, but ADF and NDF levels in lush pasture are above or equal to recommended levels for high producing cows in the US. It could be that the responses seen are due to some other mechanism - for instance the "effective" fibre requirement of cows whereby cows need fibre length to assist salivation and rumen function (Muller *et al.*, 1995, Mertens, 1996).

2.3.5 Acid detergent fibre (Figure 2.6)

Acid detergent fibre varies along with NDF but to a smaller extent, with most pasture ADF values measured in the 25-30% range. This suggests that cellulose % in dairy pasture is relatively stable, with double the fluctuations occurring in NDF levels. ADF has been frequently linked to pasture digestibility values in the literature (Holland and Kezar, 1990), but the data presented here would suggest that NDF is more closely linked to digestibility ("in vitro" digestibility) than ADF. Most metabolisable energy (ME) formulae for pasture use equations with digestibility or ADF. Since ME is one of the most commonly used means of calculating feed requirements, it is possible that more precise values may be obtained with a formula based on NDF rather than ADF if digestibility values are not known.

2.3.6 Soluble carbohydrate (Figure 2.7)

Soluble carbohydrate values followed a distinct seasonal pattern, with high levels in late autumn, winter and spring and low levels in summer. This is similar to seasonal trends found in the UK (Givens *et al.*, 1993). The higher levels in late autumn and winter may

Figure 2.6 Seasonal trends for acid detergent fibre (ADF) for the four farms.

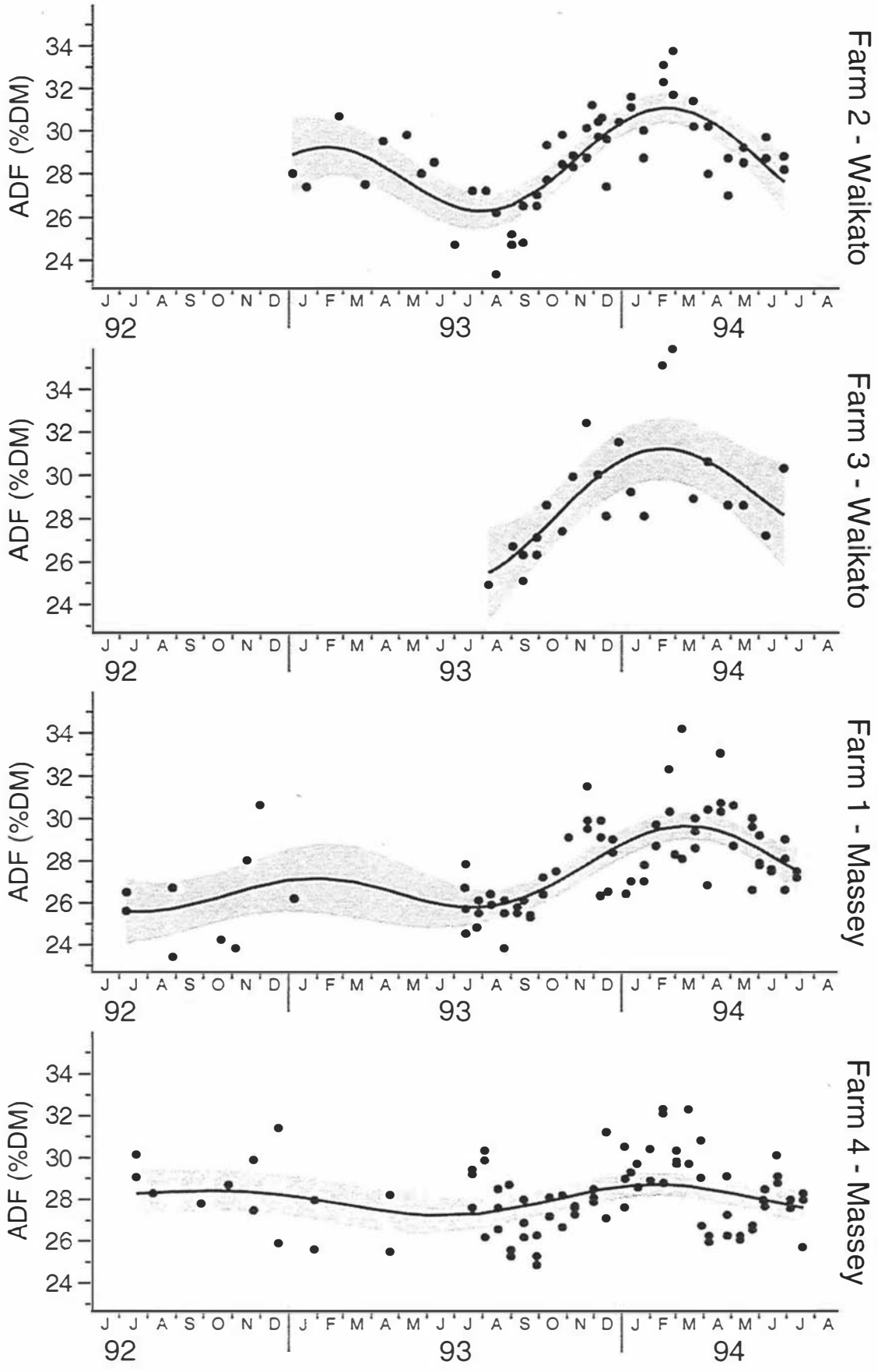
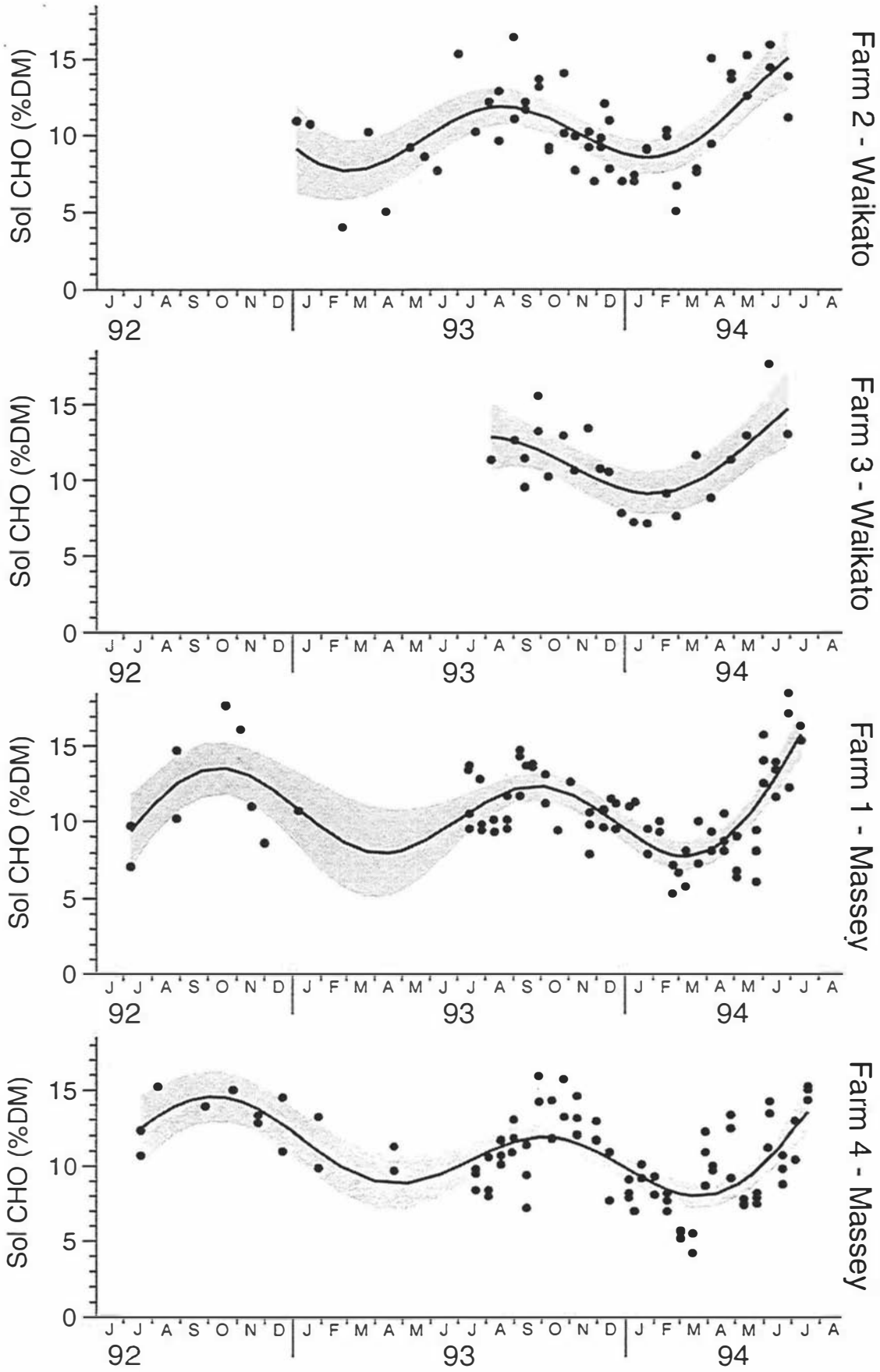


Figure 2.7 **Seasonal trends for soluble carbohydrate (SolCHO) for the four farms.**



be attributable to lower ground temperatures and longer rotation lengths. Sunlight will stimulate photosynthesis, yet growth will not occur because of low ground temperatures (Alberda, 1965). Soluble carbohydrate levels up to 38% have been recorded in these circumstances (Vartha and Bailey, 1973). Soluble carbohydrates arise as surplus energy stored that is not required for growth and plant maintenance. Lower soluble carbohydrate levels in late spring and summer probably relate to increased metabolism and respiration in plants in warm weather, and little storage of soluble carbohydrate. In addition higher temperatures stimulate the plant to produce more cell wall than cell content (Wilson *et al.*, 1975). The soluble carbohydrate analysis method used for this data may not have detected all of the soluble carbohydrate present, as analyses in previous seasons using a gas chromatography method were 3-4% higher (see results in Chapter 1).

Soluble carbohydrate levels are the most labile of the major plant nutrients in pasture, and increase by nearly 0.5% per hour during sunny days. Levels reduce markedly after several days of overcast weather and are low just after grazing as pasture regrows (Fulkerson, 1994). Although frequently less than 15% of pasture dry matter their importance in optimising rumen fermentation should not be underestimated.

2.3.7 Pectin (Figure 2.8)

Pectin levels also showed a seasonal pattern on 3 of the 4 farms, with increasing levels in summer. Since clover is known to contain considerably more pectin than ryegrass (Bailey, 1962), it is thought that increasing levels of clover in summer were the reason pectin levels rose. The pectin analysis method used is likely to have only detected 70% of the pectin present (see Appendix 1).

2.3.8 Calcium (Figure 2.9)

Calcium levels showed a seasonal pattern with higher levels in summer (especially on the two Waikato farms). It is thought that this reflects the higher clover content, as

Figure 2.8 Seasonal trends for pectin for the four farms.

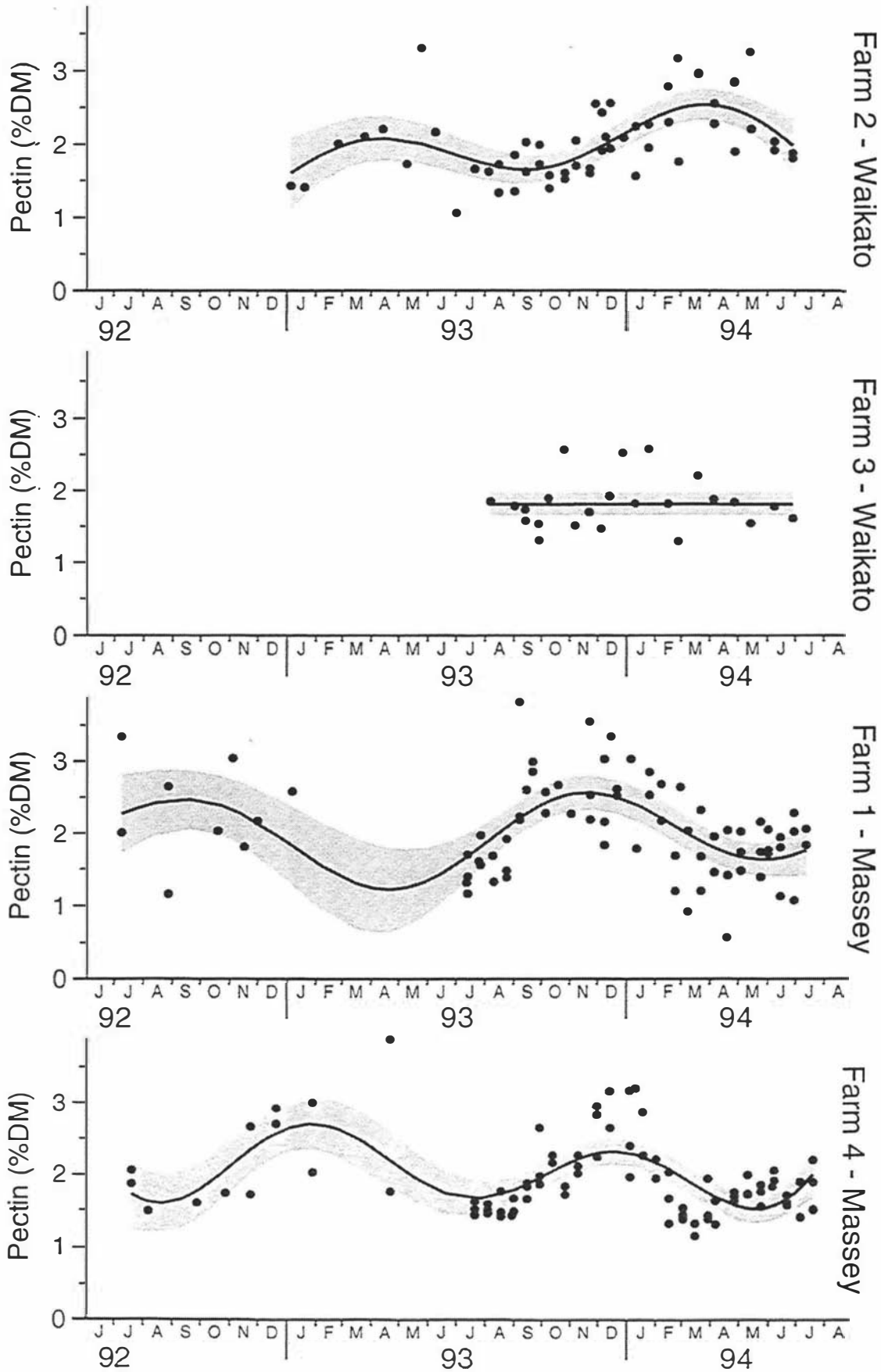
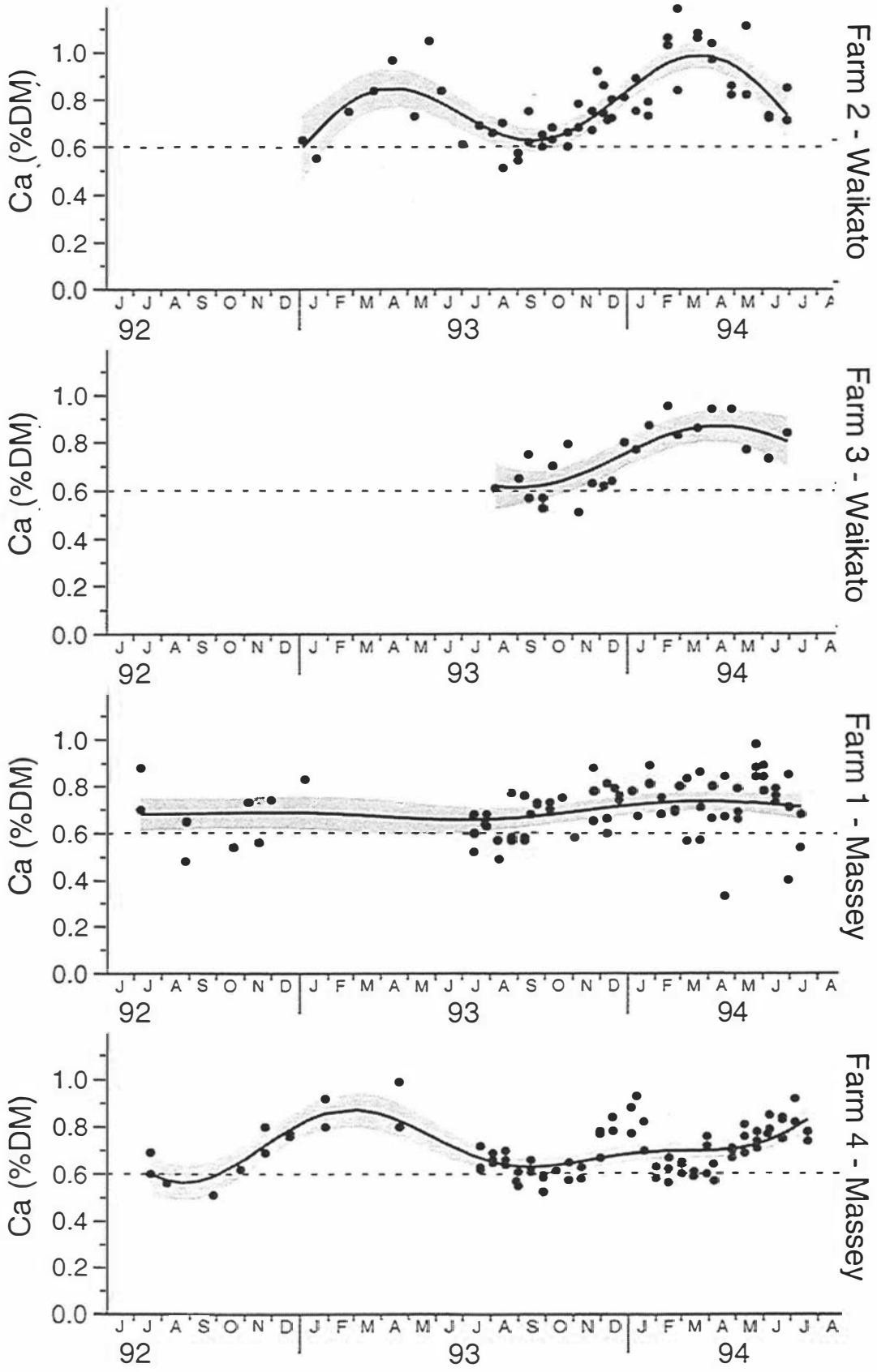


Figure 2.9 Seasonal trends for calcium for the four farms.



Footnote: Horizontal dotted line denotes recommended calcium levels for high producing cows (NRC, 1989).

clover contains more calcium (Ulyatt and Waghorn, 1993). The correlation matrix supports this (Table 2.6). Levels of calcium measured (0.5-1.0%) are adequate for moderate levels of production (25 litres of 4% fat milk), but may not be sufficient for higher levels of production (NRC, 1989). Levels are similar to those found by Smith and Cornforth (1982). Calcium application in fertiliser (superphosphate) may have affected pasture calcium levels on farm 2, which were higher.

2.3.9 Magnesium (Figure 2.10)

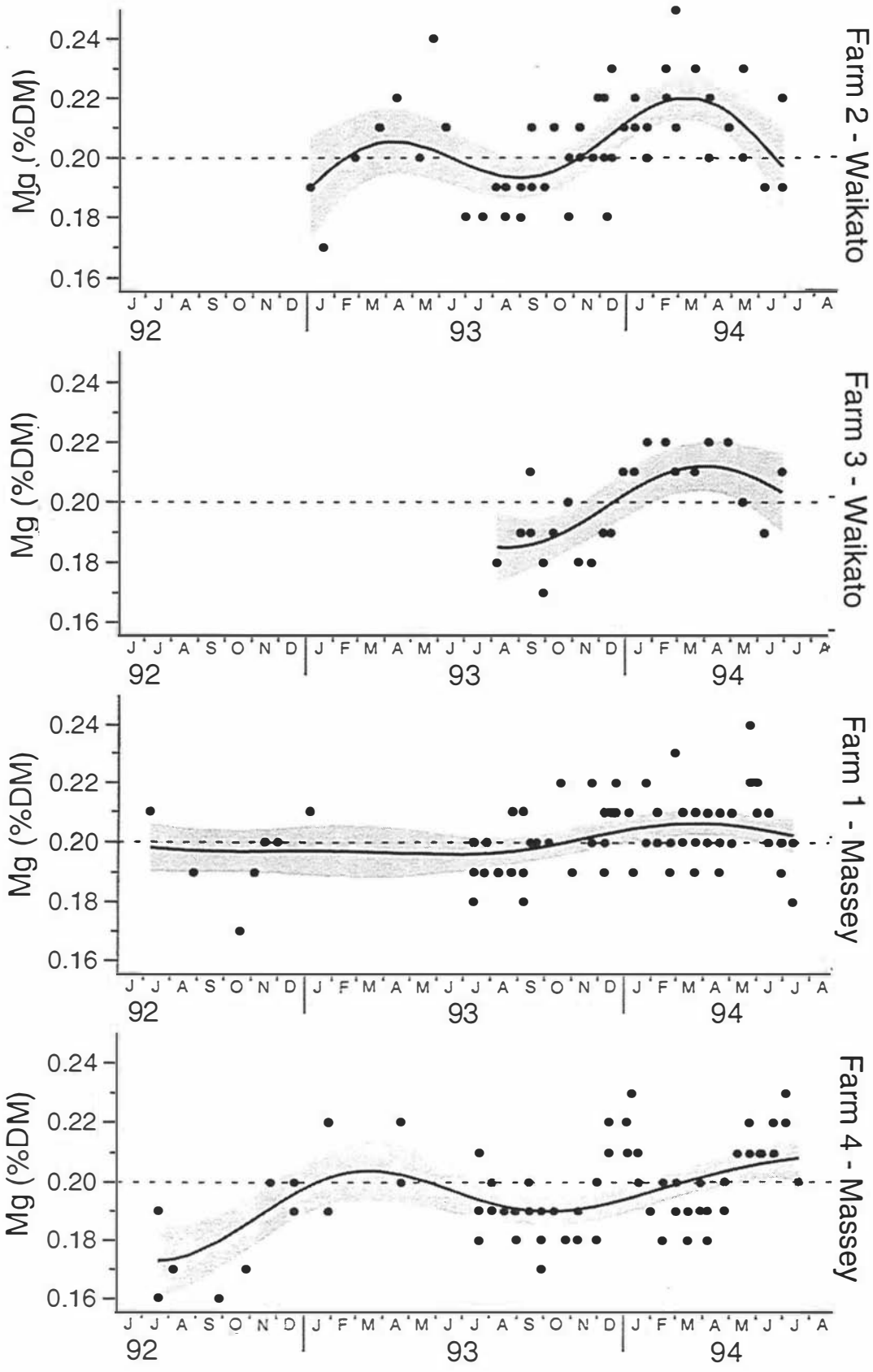
Magnesium levels were lower in winter and spring, with higher levels in summer (except Farm 4, Massey University). A large percentage of the levels were suboptimal for milk production (NRC, 1989), especially on one of the Massey farms. These results suggest that supplementation with magnesium may be needed for a longer period of the year than contemplated by most dairy farmers in NZ. Results are similar to those reported by Feyter *et al.* (1986) but lower than those reported in the more general survey of pastures by Smith and Cornforth (1982).

An added use of magnesium oxide (the most common magnesium supplement for cows) other than as a magnesium source is as a rumen buffer. Rumen pH has been shown to fall after pasture consumption to below 6.0 on some pastures and this could interfere with rumen fibre fermentation. Magnesium oxide will reduce this effect through it's buffering capacity.

2.3.10 Potassium (Figure 2.11)

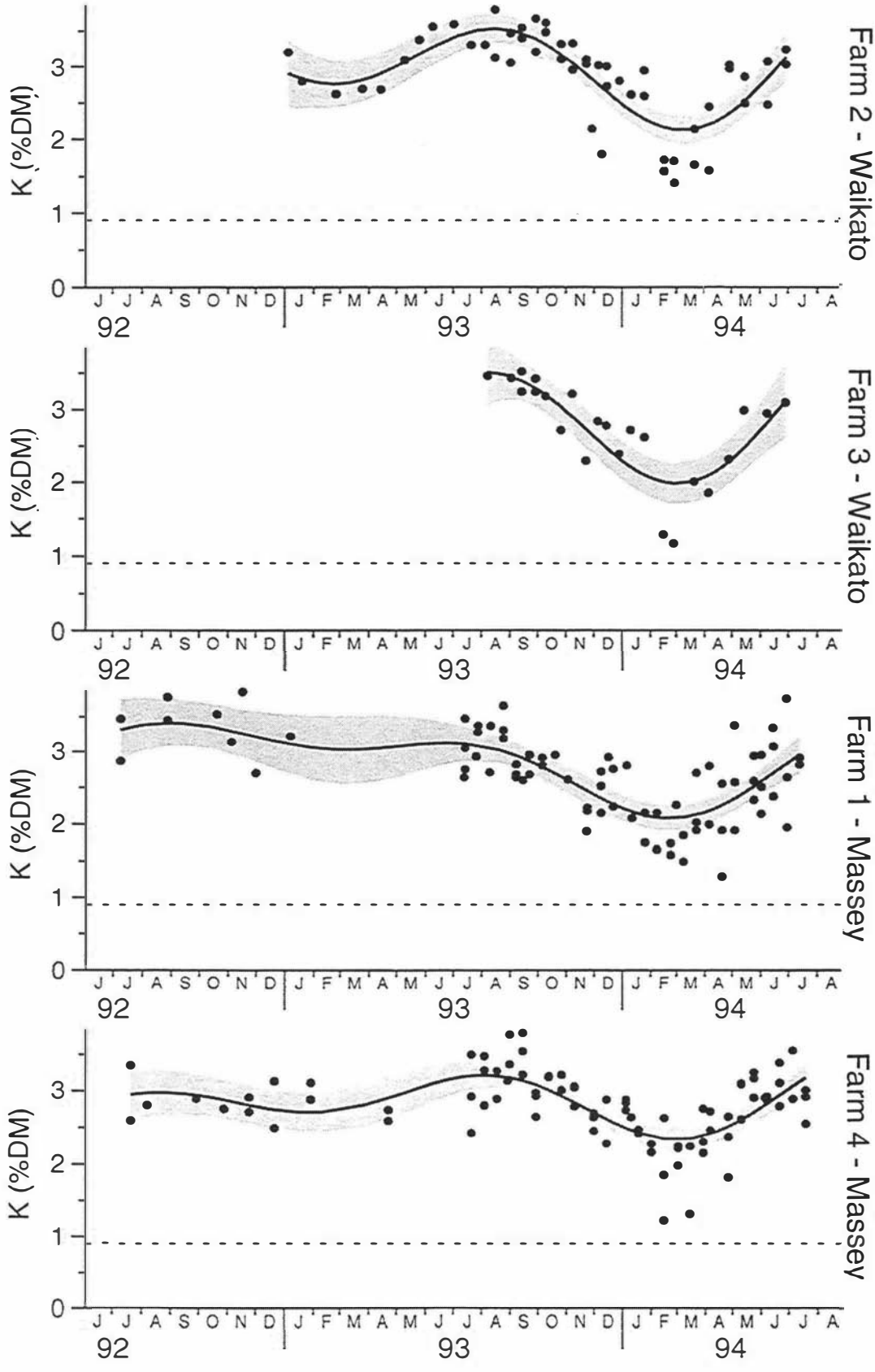
Potassium levels were well in excess of requirements for the cow and could well reduce magnesium absorption by cows (McNaught *et al.*, 1968; Feyter *et al.*, 1986). High potassium levels occurred in autumn, winter and spring, whenever higher levels of intracellular nutrients were present (CP, SOLCHO) and lower ADF and NDF levels.

Figure 2.10 Seasonal trends for magnesium for the four farms.



Footnote: Horizontal dotted line denotes recommended dietary magnesium for high producing cows.

Figure 2.11 Seasonal trends for potassium for the four farms.



Footnote: Horizontal dotted line denotes recommended dietary potassium level for high producing cows.

2.3.11 Phosphorus (Figure 2.12)

Phosphorus shows no distinct seasonal pattern. Levels measured are adequate for moderate production levels, but may not be sufficient for higher production levels (NRC, 1989). The seasonal low point in summer for phosphorus noted by Betteridge (1989) was not observed in this study, but the average level was similar to levels reported by Smith and Cornforth (1982).

2.3.12 Correlation matrix

A correlation matrix for the data is presented in Table 2.6 to illustrate interrelationships between the variables measured.

Table 2.6. Correlation Matrix for the variables measured in 237 pasture samples.

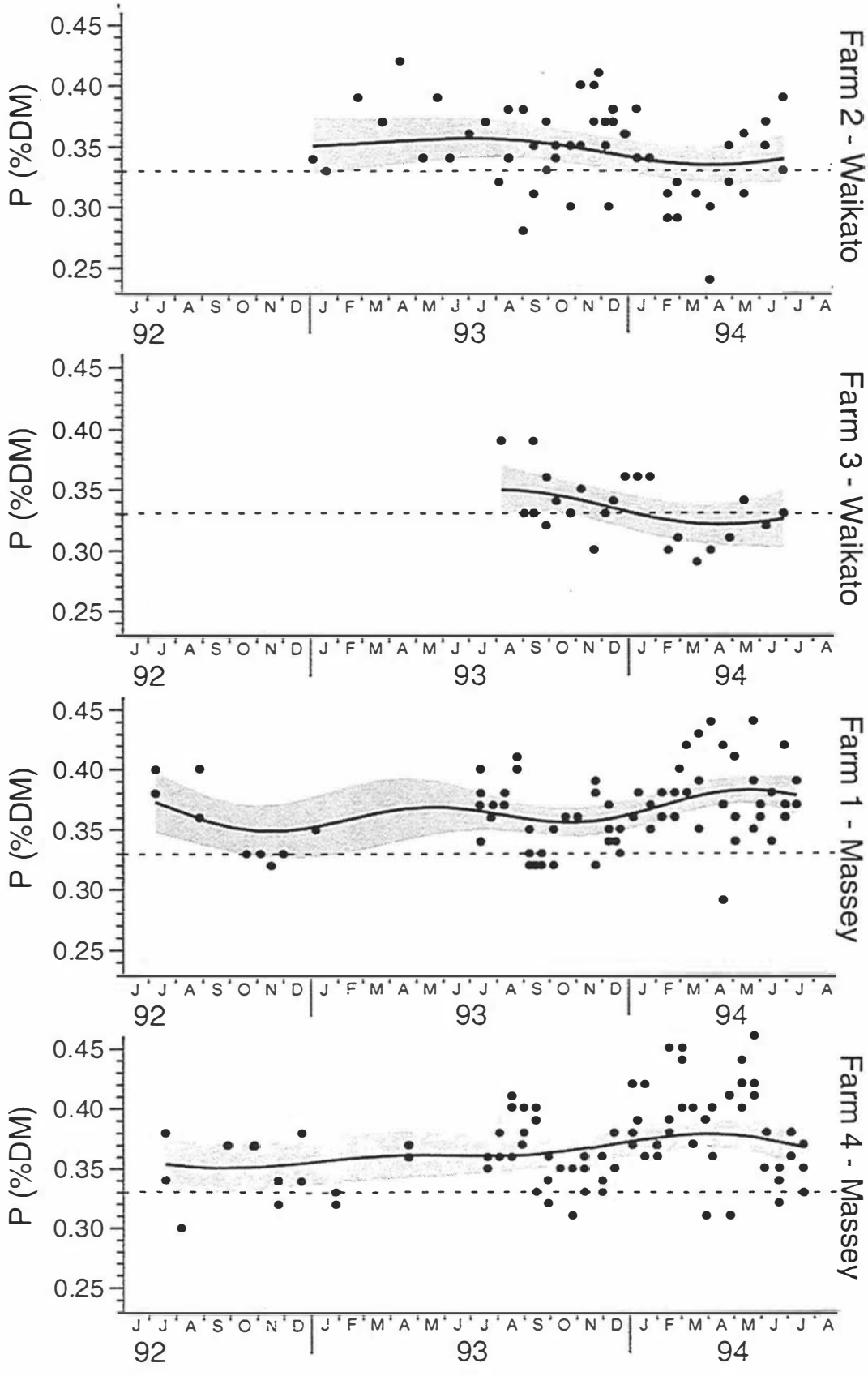
	ADF	NDF	CP	S.CHO	Pectin	Phos	K	Ca	Mg	DOMD
ADF	1									
NDF	0.85	1								
CP	-0.46	-0.7	1							
S.CHO	-0.49	-0.69	0.22	1						
Pectin	-0.22	-0.32	0.03	0.24	1					
Phos	-0.07	0.20	0	-0.57	-0.23	1				
K	-0.73	-0.65	0.30	0.43	0.02	0	1			
Ca	0.17	-0.14	0.28	-0.06	0.57	-0.19	-0.25	1		
Mg	0.18	0	0.38	-0.41	0.37	0.18	-0.2	0.78	1	
DOMD	-0.79	-0.95	0.69	0.73	0.12	-0.25	0.72	0.03	-0.08	1

Relations significant at $P < 0.05$ when r exceeds 0.19, at $P < 0.01$ when r exceeds 0.25 and at $P < 0.001$ when r exceeds 0.32.

Digestibility (DOMD) related most strongly to NDF (r = -0.95), with strong correlations with ADF, CP, SOLCHO and potassium also.

Magnesium and calcium were strongly correlated (r = 0.78). Potassium was correlated negatively with the cell wall measures (ADF, NDF).

Figure 2.12 Seasonal trends for phosphorus for the four farms.



Footnote: Horizontal dotted line denotes recommended dietary phosphorus levels for high producing cows.

SOLCHO correlated negatively with ADF, NDF, phosphorus and magnesium but positively with DOMD and potassium.

Pectin correlated positively with calcium and magnesium.

The negative correlations between the fibre nutrients (ADF and NDF) and CP or SOLCHO are similar to those seen in the data presented in Chapter 1 (Table 1.7).

2.3.13 Dietary balance in relation to the lactation curve in seasonal calving cows

Most seasonal calving dairy herds calve from July-September in New Zealand and milk till April-May. These results indicate there are several seasonal factors that discourage intake or discourage milk production throughout the lactation.

Assuming cows calve in optimal condition and dry matter supply is adequate, then early spring pasture is high in protein and possibly marginal for fibre (especially "effective fibre"). Soluble carbohydrate, or total fermentable carbohydrate is not matched well with the high protein level. Use of nitrogen fertiliser is common at this time to ensure adequate supply of dry matter for the cows, but this is likely to exacerbate the protein excess and reduce fermentable carbohydrate like soluble carbohydrate and fibre still further (see Chapter 6). Weight loss is often considerable in cows consuming pasture. As mentioned in the introduction there is evidence that urea formation in the liver requires one ammonia molecule from ammonia absorbed from the rumen and one molecule from an amino acid and cannot be formed from two rumen derived ammonia molecules (Lobley *et al.*, 1995). This could explain the apparent catabolic nature of pasture feeding.

If cows are milking especially well, then bypass protein (UDP) may limit production. Production levels over 20 litres of milk require increasing amounts of bypass protein as there is a limitation to bacterial protein supply from the rumen (Orskov *et al.*, 1981). Bypass protein was not measured in this study.

Appropriate supplements early in spring should have lower protein, possibly more "effective fibre", more soluble carbohydrate (preferably a mixture of slow and fast release carbohydrates), and could include extra fat (to raise the dietary ME concentration early in lactation) and bypass protein. Mineral levels should also be corrected to recommended levels. Maize silage would be a good base for this supplement as it combines a low CP, high starch (30% present in maize silage), and reasonable fibre.

An all pasture diet will be high in ME but is likely to encourage weight loss, and because of its variable nature is not likely to attract maximal intake for any extended period. Typically intakes reach only 3.5% of body weight with a pasture diet.

In October/November, ryegrass reaches its reproductive phase in response to longer daylength and rising temperatures. This involves rapid growth rates but also more fibrous tissue production in the plant. Unfortunately this coincides with peak milk production in seasonally calving herds. The increased fibre will reduce DM intakes because passage of digesta is slowed. In addition the energy density of pasture is reduced (as mentioned earlier), so total intake of metabolisable energy is further reduced just when cows should continue to milk at peak levels. The variable nature of pasture from day to day is also likely to encourage production decline from peak. If cows are underfed on one day they will mobilise body tissue to compensate for this, particularly higher genetic quality cows. Typically at peak, cows are at their lowest body condition and have limited ability to mobilise tissue. This probably also limits the ability of the cow to continue milking at high levels after peak milk is reached. Post peak decline of 10% of milk solids production per month is common after peak for 2-3 months. The nutritional requirement is for an energy dense supplement to maintain intake. This should contain fermentable carbohydrates and possibly bypass protein if milk production is high. Mineral levels should also be optimised.

From December onwards many districts have a dry period where ryegrass pasture composition changes to become higher in ADF and NDF, lower in protein and soluble carbohydrate. Clover in pasture will alleviate these changes and maintain quality. Protein may be suboptimal for production. The higher fibre levels will discourage high

DM intakes if adequate DM is available. In addition, readily fermentable carbohydrate in the rumen will be limiting fermentation as evidenced by the fall in soluble carbohydrate levels in summer. The nutritional requirement in summer appears to be for soluble carbohydrate or starch with some protein. The requirement for bypass protein post peak is unclear, but there is evidence that peak milk will hold better if bypass protein input is maintained (Lean, pers. comm.). Adequate mineral levels should also be maintained.

Autumn rains bring lush high protein, low fibre pasture again with similar problems to those found in spring. Additional effective fibre, reduced protein and more soluble carbohydrate would appear to be appropriate supplements. Mineral levels should also be optimised.

2.3.14 Trial design

It must be emphasised that the collection of grass samples may not have exactly mimicked grass selection by cows even though this was attempted. Cows may select a much higher protein pasture given the choice (Moran *et al.*, 1993). The sampling procedure did not always coincide precisely with grazing time, but this is not considered to have greatly affected the results.

The mineral composition analysed by NIR analysis would be expected to be less accurate than for the other nutrients measured because minerals are only measured indirectly by NIR- they do not produce an infra-red spectrum (Shenk and Westerhaus, 1994). The NIR comparison with wet chemistry values supports the accuracy of the NIR technique. In fact the NIR analyser was able to detect "outlying" wet chemistry results in this trial. The ability to process large numbers of samples quickly and cheaply may allow research into areas previously cost-prohibitive using standard wet chemistry methods. The technique should also be of practical value to farmers who will require rapid analysis to allow balancing of diets for dairy cows.

These results should help researchers define more clearly production limiting characteristics of pasture and may lead to methods of managing pasture to achieve a more desirable nutrient balance for dairy cows.

Some nutrients of potential interest in pasture were not analysed, either because NIR analysis was not available or the tests were too expensive to perform. Tests that would be of interest would include the "bypass" protein content of pastures, the non protein nitrogen component of pasture protein, partition of the fibre component of the samples into cellulose, hemicellulose and lignin. The fat/lipid component of pasture should also be measured. "Effective fibre" also needs closer definition as it applies to pasture.

2.4 CONCLUSIONS

From a nutritional viewpoint, the major issues identified from this section of the study are:

1. The excess crude protein present in pasture over and above that required for lactation and the likely energy penalty that this creates, especially in spring and autumn. This is likely to lead to increased weight loss and reduced milk production.

2. The reduction in digestibility, ME value and increase in ADF and NDF levels in October-November just as seasonally calving cows peak. This is likely to lead to reduced levels of milk production just when lactational persistence is required.

3. The low fermentable carbohydrate present in pasture (in all seasons) to maximise rumen production of bacteria for digestion further down the gastro-intestinal tract. Recommended non fibre carbohydrate (NFC = 100 - (NDF + CP + Fat + Ash)) levels in the rumen are 35% (Stokes *et al.*, 1991a). Reduced milk protein production is a likely consequence of this lack of fermentable carbohydrate.

4. The change to even less NFC in summer in pasture with increased ADF and NDF. This is likely to reduce rumen microbe supply to the small intestine further. The increased ADF and NDF are likely to limit intake in summer. More appropriate supplements at this time should contain fermentable carbohydrate. Total dietary protein could also become deficient in summer.

Clearly nutrients within pasture did vary with seasons in this study. Improved understanding of these changes could be used to improve dairy cow production through providing production limiting nutrients and enhancing cow DM intake. The economics of supplementation to balance the diet have not been clarified as yet in the New Zealand context, where milk payouts are relatively low and the cost of supplements relatively high. Even with this constraint, some byproducts and crops could possibly be used to advantage in New Zealand and to some extent already are being used to balance the pasture diet. For example, maize silage, earlage (high moisture ear corn), apple pomace, kiwifruit, avonfeed, beet pulp, fodderbeet, carrot pulp and others.

The appropriateness of measuring cow requirements using terms like ME or dry matter could be questioned on the basis of data presented in this chapter. Improved understanding of limitations to production on pasture based diets will not occur if the actual requirements are expressed in these simple terms only. The cow requires a certain amount of protein, fermentable carbohydrate and minerals, and these requirements can be broken down still further. When other feeds are added to the pasture diet, knowledge of these nutrients becomes even more important as imbalances can more easily occur. In addition, the reliance on one measurement of pasture quality (ME, which is usually derived from DOMD or ADF by formula) places undue emphasis on one laboratory test. Feed analyses are best interpreted using a combination of all the parameters measured, because many are interrelated and one tends to confirm the other. In this way, spurious results for one parameter can be discarded and better interpretation is possible.

This study and those presented in Chapter 1 have highlighted the low levels of readily fermentable carbohydrate in pasture and suggest there will be productive and reproductive advantages to cows in correcting these deficiencies in the diet. The whole

question of whether there are production responses to "balancing" a pasture diet closer to that recommended, and whether this can be profitably done needs closer examination. The following two chapters (Section 2 of the thesis) present results of supplementation trials designed to explore the above suggestions.

If attempts are to be made to alter the balance of pasture nutrients (especially CP and SOLCHO) by grazing management rather than supplementation, then further understanding of management factors influencing pasture nutrients is required. Section 3 of the thesis examines the effects of grazing and maturation of pasture on pasture nutrients (Chapter 5) and the effects of nitrogen application and the timing of nitrogen application in spring on pasture nutrients (Chapter 6).

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SECTION 2

Section 2 presents the results of two supplementation trials designed to address nutritional inadequacies in pasture.

Chapter 3

Supplementation of cows with molasses or concentrates in six commercial herds

Six commercial herds (1380 cows) were split into treated and control groups and one group supplemented with either molasses or high energy grain for 100 days. Herds were monitored for production and reproductive performance.

Chapter 4

Supplementation to "balance" nutrient intakes of cows fed pasture and maize silage

One commercial herd (240 cows) was split into treated and control groups and the treated group fed a specially formulated maize silage balancer plus maize silage and pasture whereas the control group was offered maize silage and pasture only but in isoenergetic amounts. Both herd milk production and herd reproductive performance were monitored.

CHAPTER 3

Supplementation of cows with molasses or concentrates in six commercial herds

PREFACE

The observational studies described in Chapter 1 indicated that the differences seen between herds in herd reproductive performance and milk production were associated with elevated urea levels in blood and milk, increased pasture protein (CP), and reduced pasture soluble carbohydrate (SOLCHO). In real terms the herds observed and recorded in 1990 differed in consumption of SOLCHO by 0.5 kg daily. Chapter 2 also highlighted deficiencies in a pasture only diet for rumen fermentable carbohydrate sources to match the high dietary protein in pasture at most times of the year on local dairy farms.

Bulk milk urea levels will reflect dietary protein excess in dairy herds (Moore and Varga, 1996) and some authors have suggested these levels should be reduced by addition of soluble carbohydrate or starch to the diet to assist rumen capture of CP by bacteria (Stokes *et al.*, 1991a; Obara *et al.*, 1991). Conception rate depression has been associated with elevated urea levels as mentioned in the introduction to Chapter 1, and increased anoestrus may also occur with increased urea levels due to increased negative energy balance (Moore and Varga, 1996).

The decision was made to test the response of commercial dairy herds consuming pasture to relatively long-term (86 day) supplementation with low levels (0.7-1.5 kg) of starch or soluble carbohydrate rich supplements. This would examine the hypothesis

that increasing the dietary readily fermentable carbohydrate would reduce milk urea, increase productivity, and improve herd reproductive performance.

3.1 INTRODUCTION

There appears to be very limited literature in New Zealand examining the effects of longer term concentrate supplementation on herd reproductive performance. McCallum *et al.* (1995) reported reduced anoestrus, increased conception rate and faster calving in the following season by feeding 0.5-3.5 kg DM concentrate throughout the season, as well as improved per hectare productivity, but attributed this response to improved dry matter supply. Wilson (1989) reported the results of two experiments where 2 kg concentrate (with or without protein) was fed for four weeks before mating or before calving with positive effects on submission rate, conception rate and calving spread in the subsequent season. He also found in an unpublished experiment (personal communication) that a protein deficiency in late pregnancy reduced the subsequent conception rate and milk production of heifers in early lactation. While not all results were significant, they support the use of protein containing concentrates before calving and before mating. McGowan (1981) found that anoestrus (the main reproductive problem in New Zealand dairy cows) was especially influenced by calving condition score and feeding in the weeks immediately after calving. Precalving feeding has also been shown to be important (McDougall, 1993). The depth and length of negative energy balance post-calving will affect ovulation and oestrus behaviour and are responsible for reduced conception rates when per cow productivity has increased in the US dairy system from 1951-1975 (Butler and Smith, 1989).

On balance, the literature has shown a strong influence on anoestrus from feeding levels coming up to calving, condition score at calving and feeding levels just after calving (McDougall, 1993). Feeding levels at mating time (8 weeks later) appear to be of lesser importance for anoestrus. Conception rates are also influenced by calving condition and feeding just after calving, and improved feeding near and during mating also have been shown to be important in some studies (McDougall, 1993). Herd reproductive performance is covered in more detail in the introduction to Chapter 1.

Low serum Albumin levels have been linked to increased anoestrus and conception rates by Wilson *et al.* (1985), but attempts to link measurable blood and milk parameters to fertility have not always been successful (Eddy and Ducker, 1984). Milk urea levels provide information on protein excess and the balance of protein and non structural carbohydrate in the diet (Oltner and Wiktorsson, 1983; Gustafsson, 1993; Fulkerson, 1996; Moore and Varga, 1996) and high levels have been linked to reduced conception rates.

Concentrate supplementation of high quality pasture diets has generally produced immediate responses near 0.5 litres of milk per kg of concentrate, with carryover and cumulative responses totalling near 1 litre per kg of concentrate (Broster *et al.*, 1993; Kellaway and Porta, 1993; Lean *et al.*, 1995). Substitution for pasture can be a major impediment to obtaining responses (Grainger and Matthews, 1989) and sometimes results in no immediate response at all (Kellaway and Porta, 1993). In a dairy system involving mainly pasture substitution can be a positive phenomenon in some circumstances as it may prevent overgrazing of pasture and lead to faster regrowth. Thus more pasture can be grown per hectare with supplementation in some circumstances. The experiment reported by McCallum *et al.* (1995), where 0.5-3.5 kg/cow/day high energy concentrate was fed to cows on pasture, produced responses of 136 g milk solids (fat + protein) per kg of supplement or over 1.5 litres/kg. This was a long term supplementation experiment over 2 seasons with high stocking rates which ensured high pasture utilisation and underfeeding.

Addition of small quantities of non structural carbohydrate in the form of molasses or concentrate to a pasture diet is likely to improve rumen fermentation and consequently bacterial supply to the small intestine. This is likely to lead to improved milk protein production in particular through balancing the supply of fermentable carbohydrate and protein supplied to the rumen and thus improving bacterial supply to the small intestine for absorption (Kirchgessner *et al.*, 1986; Khalili *et al.*, 1989; Obara *et al.*, 1991; Russell *et al.*, 1991; Stokes *et al.*, 1991b; Clark *et al.*, 1992; Aldrich *et al.*, 1993; Muller, 1993; Muller and Holden, 1994; Muller *et al.*, 1995). Addition of large quantities of soluble carbohydrate to cows diets, however, can be harmful through lowering rumen pH and lactic acidosis (Blood and Henderson, 1968).

3.2 METHODS

Controlled trials with sufficient numbers are difficult to achieve in a research environment, particularly for reproductive parameters and control of variables is more difficult to achieve in a commercial environment. The purpose of this trial was to measure responses to relatively low levels of carbohydrate supplements over an extended period in spring on a herd basis. These supplements were designed to lower urea levels and look for potential benefits from achieving this. Measurements were made to detect reproductive responses, milk production responses and condition score changes. Blood parameters likely to reflect nutritional change (serum albumin [Alb], beta-hydroxybutyrate [BOH] and magnesium [Mg]) were monitored and milk urea levels were also monitored in treated and control herds (see Appendix 1 for methods).

For the 1992 calving season a large field trial involving over 1,000 cows in 6 herds was designed with sufficient numbers (sufficient statistical power) to detect an improvement of more than 5% in non-return rate (conception rate) between control and treated groups. Farmers were approached that had expressed interest in helping with trial work, and were selected for their high standard of management and for the similarity of farm milk production per hectare in the previous season. Herds exceeding 160 cows were also required. The sixth herd was included for reproductive data only, as monitoring for other data (eg milk production data) was not so intense on this farm. All farms were located in the Te Awamutu area and were producing 400-550kg milkfat per hectare in the previous season and, based on previous history and veterinary practice records, would be expected to have average herd reproductive performance.

Feed companies were approached to contribute supplement at no cost to the farmer. In return, the companies could make use of the results. Cows in each herd were split into even and odd tagged cows and run separately for the duration of the trial as "control" and "treated" herds. Wherever possible the two herds were given the same allowance of pasture in the same paddock, ie. the paddock was split with an electric fence into equal portions so that each herd was grazing similar pasture at the same time. When this was not possible each herd was offered pasture of similar length in separate paddocks. Herds were milked in the same sequence as often as possible on each farm.

It was arranged with the local dairy company that separate vats would be used for treated and control herds. This allowed daily monitoring of milk production per herd and collection of a milk sample from each vat for milk urea analysis, daily milkfat, milk protein and milk lactose. With records of the number of cows milked each day it was then possible to calculate daily per cow production. Account was taken of any calf milk removed from the vat.

Seven cows in each of the treated and control herds (the same seven cows) were blood tested monthly to detect changes in blood parameters likely to alter with nutrition (Kitchenham *et al.*, 1975; Manston *et al.*, 1975; Baird, 1982; Whitaker *et al.*, 1983; Ellison, 1991; Kolver and Macmillan, 1993). Cows selected in each herd were of mixed age and breeding worth, with the same cows blood tested on each occasion. Cows were retained at morning milkings and bloods collected before 10 am, then sent to Ruakura Animal Health Laboratory for analysis using an Hitachi 911 autoanalyser.

Analysis of reproductive data was performed on the computer software programme "Seasonal dairy herd breeding record analysis - Version 2.1, 1986" developed by J.B. and S.M. Grimmett, Box 133, Matamata. [This software analyses submission rates, conception rates (NRR) and return intervals for seasonal dairy herds.] Pasture analysis methods used are outlined in Appendix 1. Dry matter % was by freeze drying, crude protein by Kjeldahl N x 6.25, neutral detergent fibre by modified Van Soest method as described by James and Theander (1981), soluble carbohydrate by Nelson's reducing sugar method, uronic acid (pectin) by serial extraction of carbohydrates method for D-galacturonic acid and digestibility (DOMD) as described by Roughan and Holland (1987).

Dry matter intakes of pasture were estimated weekly, with the assistance of another dairy consultant, using a rising plate meter. Pasture the cows were being offered was assessed for dry matter present and yesterday's residual was assessed to estimate the difference. Cows were assessed for condition score at this visit also (at least 25 cows per group, using the 1-10 LIC scale), and a pasture sample collected from the paddock that the cows were grazing. This sample was cut with shears and was representative of pasture grazed by cows on these farms and was placed in a chillibin and then in a deep-

freeze within 30 minutes of sampling. Sampling occurred on at least 8 sites in each paddock. Pasture was cut to the assessed residual level left by cows. Samples were then freeze dried (lyophilised) ready for analysis. Pasture analysis was included in this trial to provide information about dietary protein and soluble carbohydrate or starch intake, and to test the relationship of milk urea with dry matter intake, pasture protein intake and soluble carbohydrate intake suggested in data presented in Chapter 1.

In three herds half the cows were fed molasses plus pasture, with the remaining half (controls) fed only pasture. The molasses was placed in elongated troughs in the exit area from the dairy shed, so that cows would consume as much molasses as they wished immediately following milking and on their way back to pasture. In one herd (herd 4) it was necessary to initially place the molasses in containers in the paddock to gain a greater level of acceptance. It was then returned to the exit area once this had occurred. Consumption per day in all herds averaged approximately 700 ml molasses (630 g DM) per day over the trial period (see Table 3.3).

Another 3 herds were split in the same manner and received high energy pelleted concentrate (2 herds received "NRM dairy ration" and 1 herd "Tomoana Dairy Ration"). Both these concentrates contained moderate levels of protein (10-12% and 12-15% respectively) and high ME levels (11.0-12.5 MJ of ME/kg DM). These herds were fed an average of 1.5 kg wet weight (1.35 kg DM) per cow per day for the duration of the trial. The concentrate was fed once daily as soon as practicable after the morning milking (between 9.00-11.00 am). It was fed on the pasture in strips or small piles in a manner that all cows had access simultaneously. Little wastage was detected (and was estimated at less than 5%) due to the pelleted form, even though the concentrate was placed on the pasture.

Bayesian smoothing (Flexi 2.2, Upsdell and Wheeler, 1992) was used on the difference between treated and control herds on each farm for milk production data and milk urea to detect significant change over time. Where the 95% confidence limit of the combined curve does not intercept zero there was a significant difference ($P < 0.05$).

The trials began on 1st September 1992 and continued till 25th November 1992.

3.3 RESULTS

3.3.1 Summary of results

Pasture analyses for weekly samples from each farm are presented in Figures 3.1-3.5. Mean values for the whole experimental period for the variables measured are presented in Tables 3.1-3.4 and reproductive data are presented in Tables 3.7-3.9. Milk fat/cow/day and milk protein/cow/day for each herd are presented in Figures 3.6-3.15. Statistical analyses are presented graphically in Figures 3.6-3.22.

Milk urea levels were reduced by treatment in all five herds (Table 3.2). Milk production was increased by treatment in all herds except Herd 1 (Table 3.1). Molasses supplemented herds on average consumed near 700 ml molasses daily and concentrate supplemented herds 1.53 kg wet weight (Table 3.3). Condition score improved with treatment in Herds 1 and 3 (Table 3.3). These herds had the lowest milk production response (Table 3.1). All 5 herds had protein/fat ratios over 0.75, typical of Friesian herds early in lactation. Herd 5 maintained a faster rotation length between grazings (Table 3.2).

Supplemented herds had only half the number of non-pregnant cows compared to controls for the pooled results.

3.3.2 Pasture analyses

Pasture analyses from the weekly sampling are presented in Figures 3.1-3.5 for each of the five farms to demonstrate the trends in pasture nutrients through time. Pasture crude protein (CP) and digestibility (DOMD) declined from approximately mid-October, pasture neutral detergent fibre (NDF) and pasture dry matter % (DM%) increased from mid-October. Trends in pasture soluble carbohydrate (SOLCHO) cannot be discerned. Herds 4 and 5 had higher pasture CP and Herd 5 had lower pasture SOLCHO (see also Table 3.3). Herd 5 had the highest milk urea levels (Table 3.2).

Table 3.1 Mean milk production averages in the five herds-daily factory records (SEM[2]) from 1 September - 25 November.

		Protein/fat ratio	Milk litres /cow/day	Milkfat kg /cow/day	Milkprot-ein kg/c/d	Lactose kg /cow/day	Tot. solids kg/cow/d[1]
Farm 1 (220 cows, molasses)	Control (110 cows)	0.79 (0.01)	18.05 (0.22)	0.82 (0.01)	0.65 (0.01)	0.90 (0.01)	2.37 (0.03)
	Treated (110 cows)	0.79 (0.01)	17.20 (0.32)	0.8 (0.02)	0.63 (0.01)	0.83 (0.02)	2.26 (0.05)
Farm 2 (180 cows, molasses)	Control (90 cows)	0.77 (0.01)	18.44 (0.26)	0.83 (0.01)	0.64 (0.01)	0.91 (0.01)	2.38 (0.03)
	Treated (90 cows)	0.77 (0.01)	19.24 (0.21)	0.87 (0.01)	0.67 (0.01)	0.95 (0.01)	2.49 (0.03)
Farm 3 (160 cows, meal)	Control (80 cows)	0.78 (0.04)	18.00 (0.25)	0.79 (0.01)	0.62 (0.01)	0.90 (0.01)	2.31 (0.02)
	Treated (80 cows)	0.80 (0.05)	18.52 (0.21)	0.81 (0.01)	0.65 (0.01)	0.92 (0.01)	2.38 (0.04)
Farm 4 (450 cows, molasses)	Control (225 cows)	0.76 (0.04)	-	0.83 (0.01)	0.63 (0.01)	0.86 (0.01)	2.32 (0.03)
	Treated (225 cows)	0.75 (0.03)	-	0.85 (0.01)	0.64 (0.01)	0.88 (0.01)	2.37 (0.02)
Farm 5 (200 cows, meal)	Control (100 cows)	0.78 (0.00)	-	0.88 (0.01)	0.69 (0.00)	0.95 (0.01)	2.52 (0.02)
	Treated (100 cows)	0.78 (0.01)	-	0.93 (0.01)	0.73 (0.01)	1.00 (0.01)	2.66 (0.02)

[1]Total solids includes milk protein, milk fat and milk lactose in this case.
[2]SEM were derived from herd values over weeks from weekly mean production.

Table 3.2 Mean milk urea, supplement, condition score and rotation length in the 5 herds (SEM[1])

		Supplement	Rotation Length (average)	Milk urea (bulk vat)	Condition Score
Farm 1	Control		23.5	5.51 (0.18)	4.07 (0.06)
	Treated	713ml Molasses	23.5	4.74 (0.14)	4.24 (0.04)
Farm 2	Control		25.2	6.48 (0.10)	4.41 (0.06)
	Treated	596ml Molasses	25.2	6.05 (0.11)	4.40 (0.04)
Farm 3	Control		25.1	6.22 (0.14)	4.25 (0.05)
	Treated	1.53kg Concentrate	25.1	5.69 (0.13)	4.35 (0.06)
Farm 4	Control		22.2	6.04 (0.18)	4.52 (0.02)
	Treated	771ml Molasses	22.2	5.61 (0.17)	4.53 (0.06)
Farm 5	Control		17.0	7.74 (0.11)	4.56 (0.04)
	Treated	1.54kg Concentrate	17.0	7.16 (0.10)	4.56 (0.05)

[1]SEM derived from weekly mean values per herd.

Table 3.3 Average pasture measurements or concentrations for the five trial farms (SEM).

	Herbage Mass[1] kgDM/ha	Dry matter Intake[1] kg	Pasture % CP	Pasture % NDF	Pasture % SOLCHO	Pasture% Uronic Acid	Pasture % Dry Matter
Farm 1	3117 (132)	17.4 (1.0)	17.8 (0.82)	39.5 (1.67)	12.4 (0.9)	2.0 (0.1)	16.4 (0.45)
Farm 2	2777 (158)	16.2 (1.16)	19.2 (1.05)	39.8 (1.4)	9.8 (0.42)	2.0 (0.13)	17.0 (0.7)
Farm 3	2646 (124)	15.8 (1.32)	19.8 (0.75)	37.4 (0.7)	10.3 (0.62)	2.5 (0.19)	14.4 (1.15)
Farm 4	2664 (99)	17.8 (0.58)	21.5 (0.61)	37.7 (0.87)	10.6 (0.54)	2.0 (0.16)	16.5 (0.65)
Farm 5	2830 (175)	20.9 (0.71)	22.8 (1.13)	38.3 (1.27)	8.5 (0.5)	2.1 (0.12)	15.5 (0.50)

[1]Average herbage mass offered to cows and dry matter intakes suggest that Herd 1 was offered similar amounts of pasture to the other herds. This was not the case, as Herd 1 was offered lesser amounts early in the trial period and more later in the trial period. This herd was also considerably thinner than other herds in the trial (see condition score, Table 3.3).
[2]SEM derived from weekly mean values per herd.

Figure 3.1 **Dry matter % for weekly samples from the five farms versus time (1 September-25 November).**

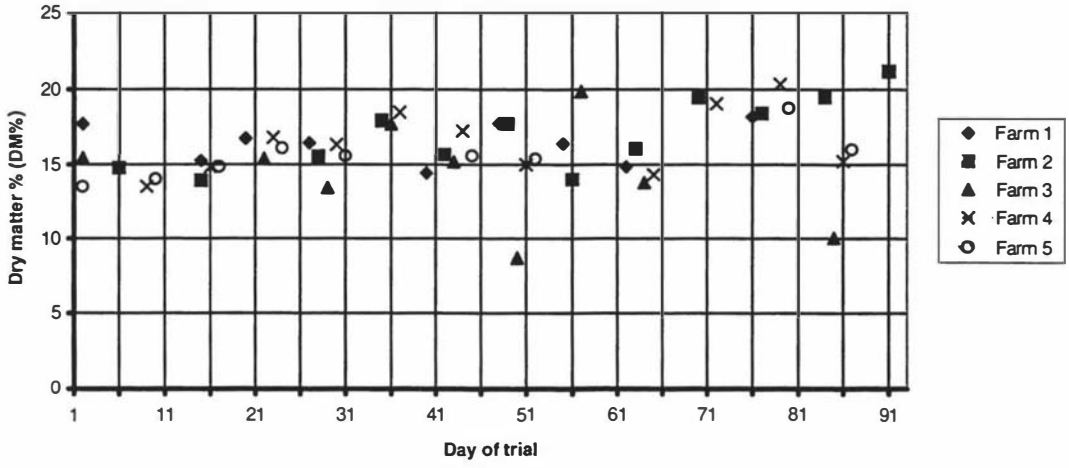


Figure 3.2 **Digestibility (DOMD) for weekly samples from the five farms (1 September-25 November).**

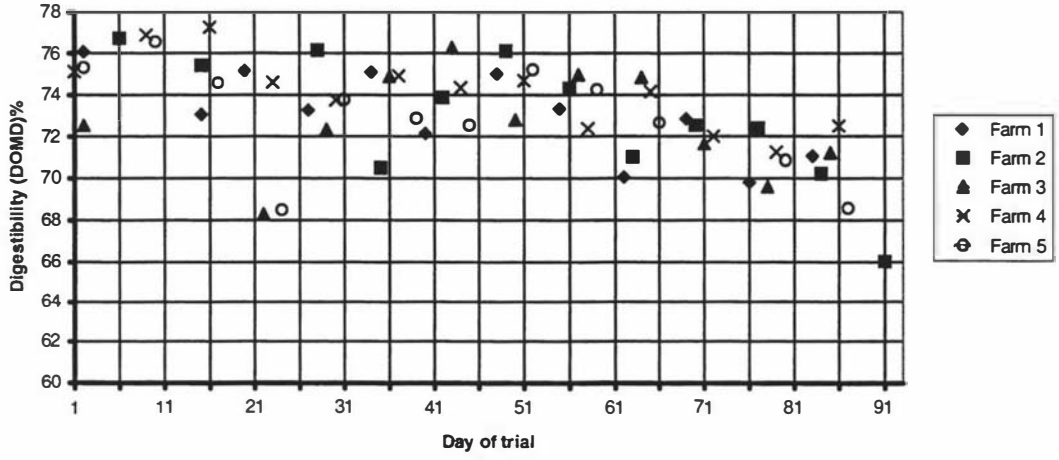


Figure 3.3 **Crude protein concentration for weekly samples from the five farms (1 September-25 November).**

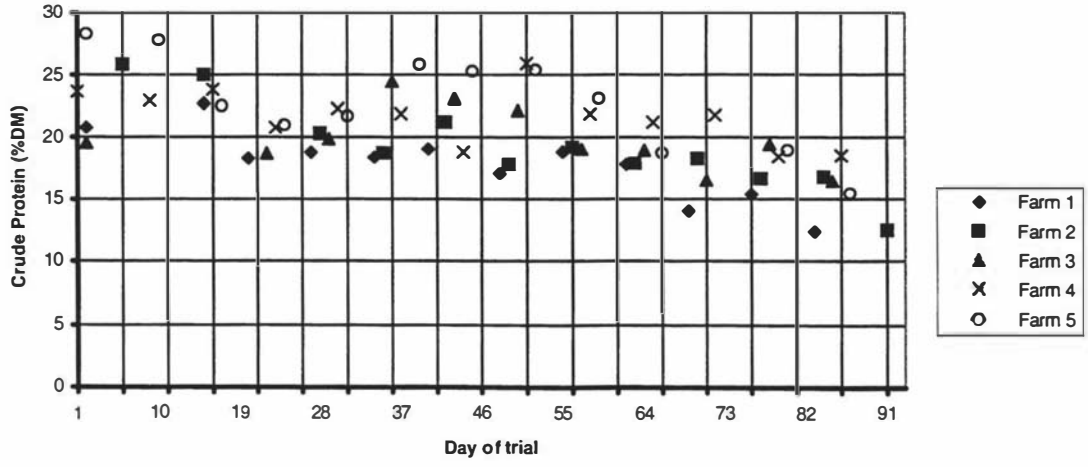


Figure 3.4 NDF concentrations for weekly samples from the five farms (1 September-25 November).

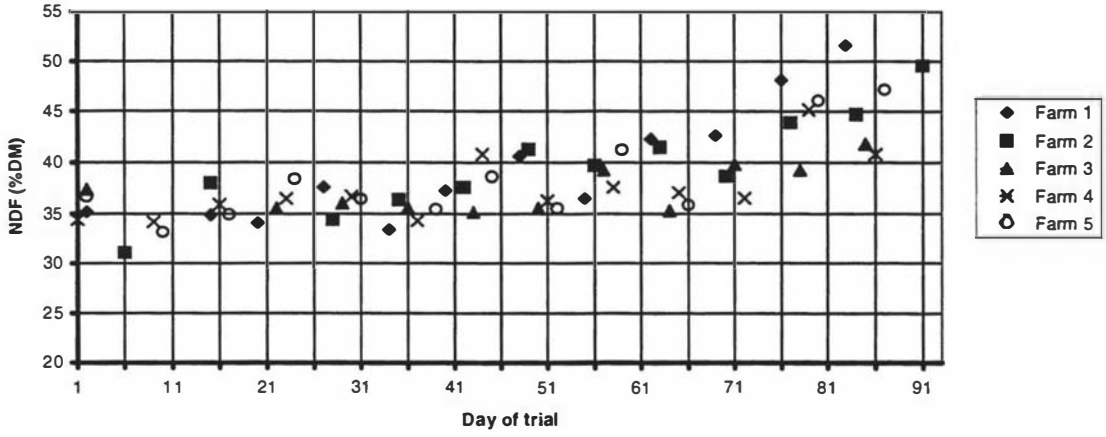
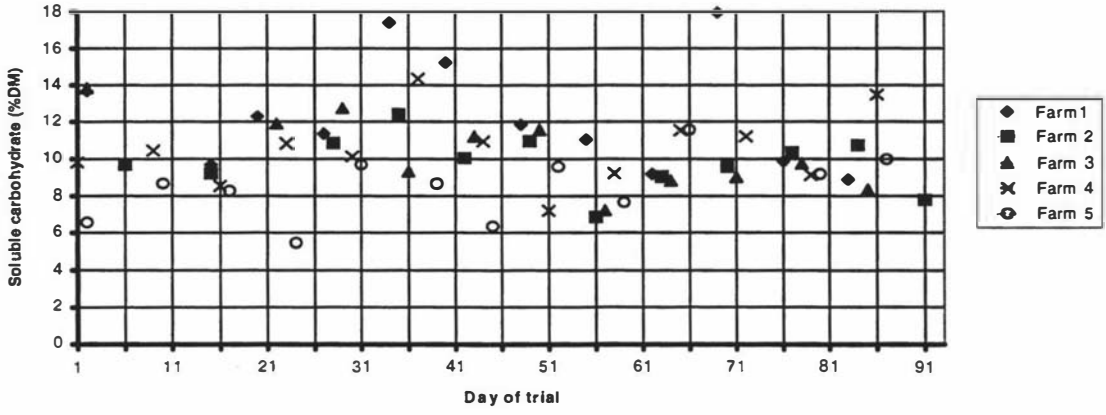


Figure 3.5 Soluble carbohydrate concentration for weekly samples from five farms (1 September-25 November).



3.3.3 Milk production

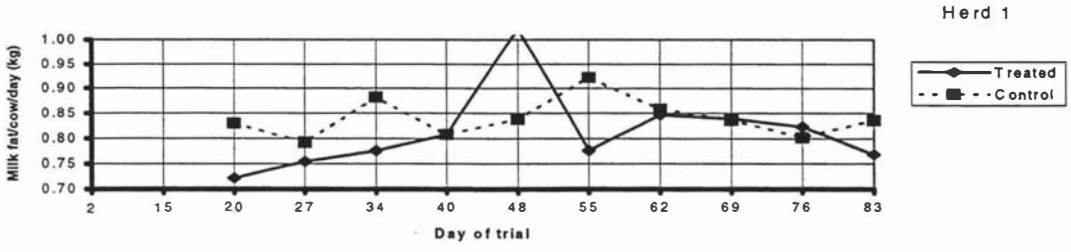
Milk production responses are presented in Table 3.1 and Figures 3.6-3.15. Per cow milk fat and milk protein production were reasonably similar in all herds, with herd 5 the highest and herd 1 the lowest.

For all milk production data the difference between control and treated herds daily production per cow (meaned for each week) has been used for statistical purposes to assess the significance of the results. The differences were significant at the 5% level where the 95% confidence interval did not intercept zero (Figures 3.16-3.22). Pooling of all treated and control herds for statistical assessment was justified because herds selected were similar for production/ha and for herd reproductive performance in

previous seasons, herds were all located within a 30 km radius and were likely to have encountered similar weather conditions, supplements although not identical were all readily fermentable carbohydrate sources in low amounts, and cow numbers would only be sufficient for statistical purposes for reproductive results if all results were combined. The trials were specifically designed with sufficient numbers to achieve power in the reproductive data. Milkfat/cow/day (Figure 3.17) production was on average significantly increased by supplementation ($P < 0.05$) from mid-October to late November. Milk protein/cow/day (Figure 3.18) was significantly improved ($P < 0.05$) from late September to late November, milk lactose/cow/day (Figure 3.19) was significantly improved ($P < 0.05$) only for a short period in early November, milk solids (fat + protein + lactose)/cow/day (Figure 3.20) was significantly improved ($P < 0.05$) from mid-October to late November, and milk protein/milk fat ratio (Figure 3.21) was significantly improved from mid September to mid October. Responses in milk litres/cow/day did not reach significance (Figure 3.16).

Supplementation began in early September yet most of the milk production responses did not occur till October/November. Milk production responses were generally less than one litre (4% fat corrected milk)/cow/day per kg DM of supplement in the October/November period (Fig. 3.20). Herds 2 (molasses) and 5 (concentrate) showed the greatest production responses, and Herd 1 (molasses) had a negative production response.

Figure 3.6 Milkfat/cow/day for Herd 1 versus time (1 September-25 November)[1].



[1]Herd numbers were altering daily in September and accurate herd numbers took 1-3 weeks to establish.

Figure 3.7 **Milkfat/cow/day for Herd 2 versus time (1 September-25 November).**

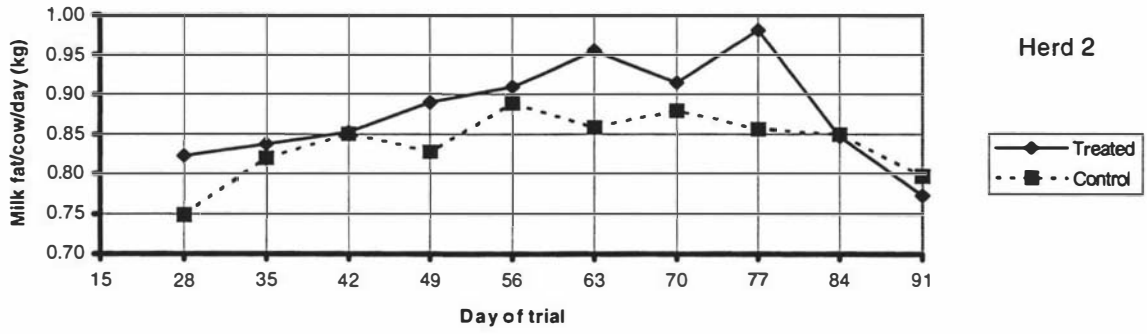


Figure 3.8 **Milkfat/cow/day for Herd 3 versus time (1 September-25 November).**

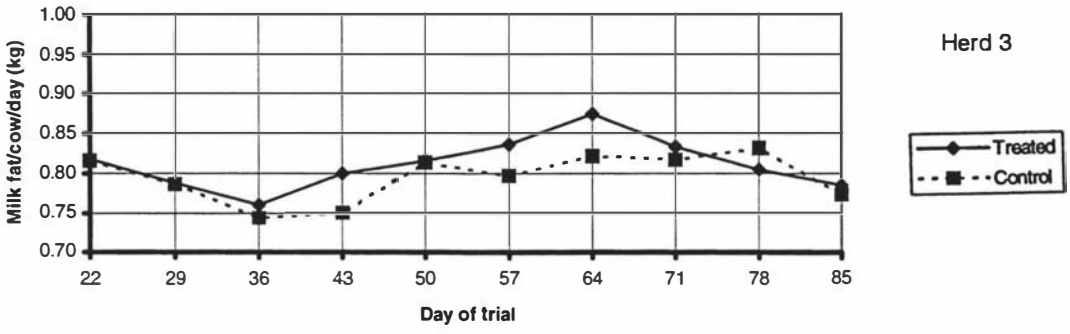


Figure 3.9 **Milkfat/cow/day for Herd 4 versus time (1 September-25 November).**

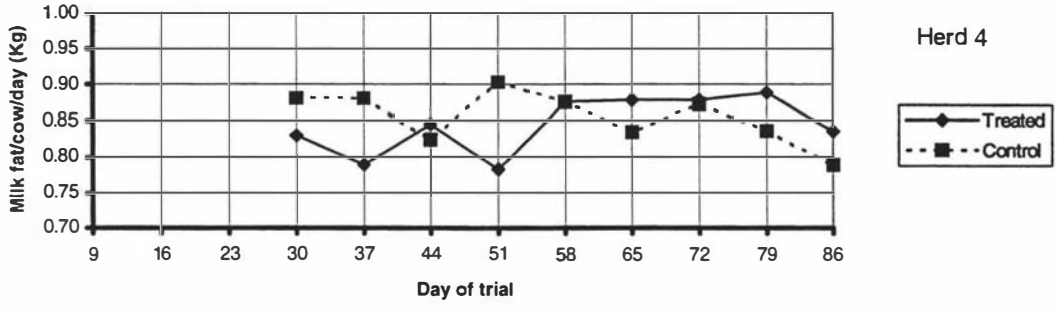


Figure 3.10 **Milkfat/cow/day for Herd 5 versus time (1 September-25 November).**

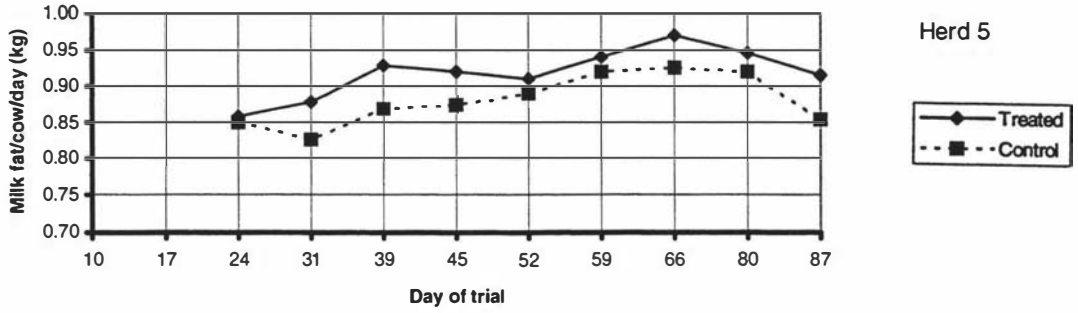


Figure 3.11 **Milk protein/cow/day for Herd 1 versus time (1 September-25 November).**

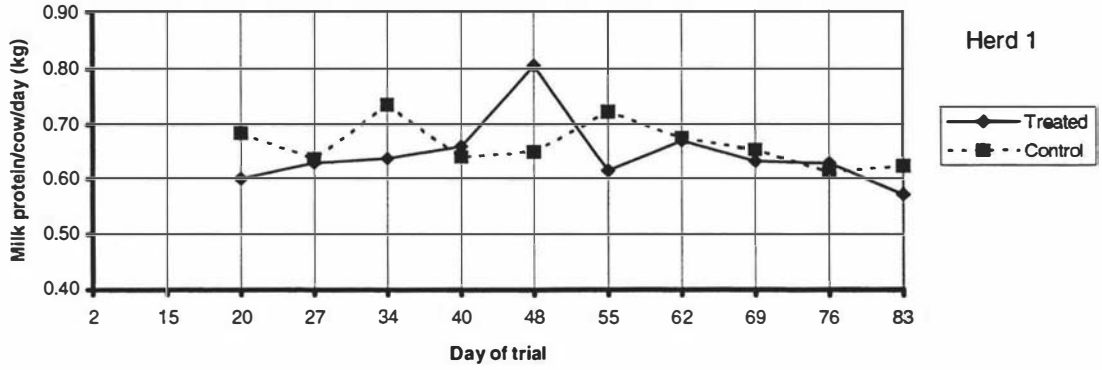


Figure 3.12 **Milk protein/cow/day for Herd 2 versus time (1 September-25 November).**

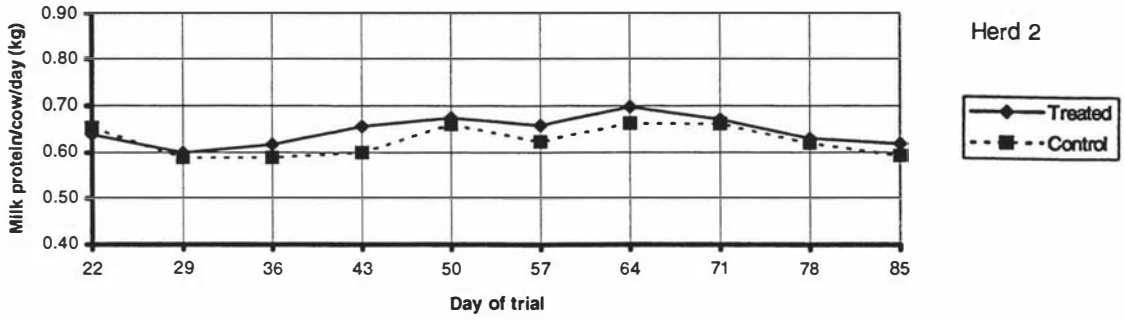


Figure 3.13 Milk protein/cow/day for Herd 3 versus time (1 September-25 November).

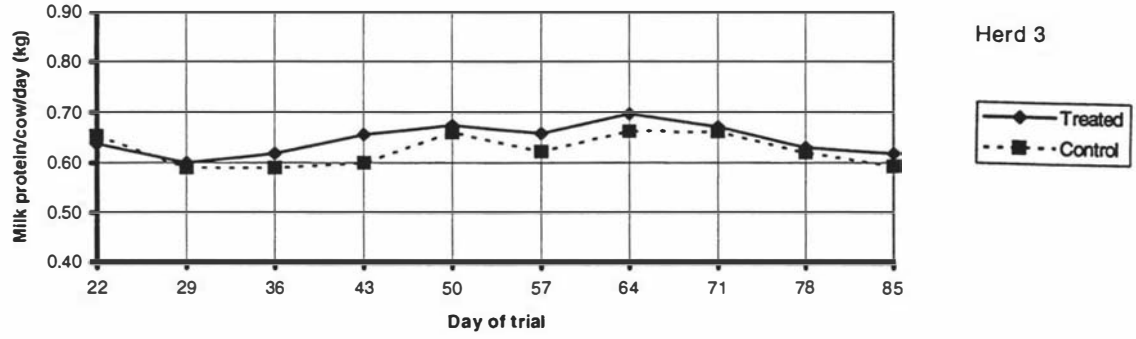


Figure 3.14 Milk protein/cow/day for Herd 4 versus time (1 September-25 November).

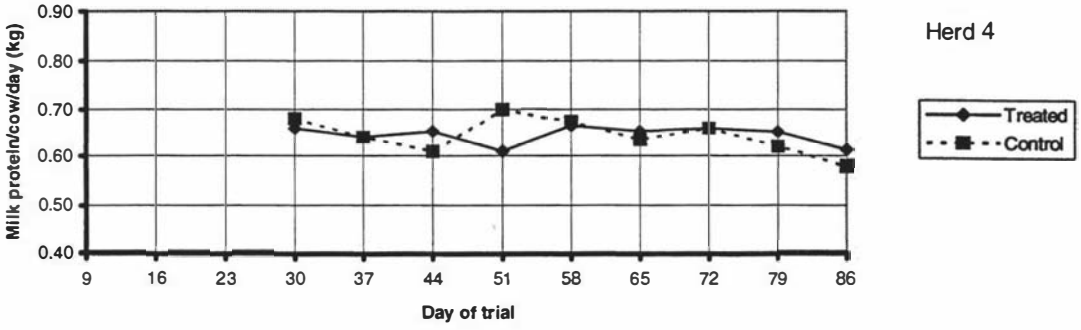


Figure 3.15 Milk protein/cow/day for Herd 5 versus time (1 September-25 November).

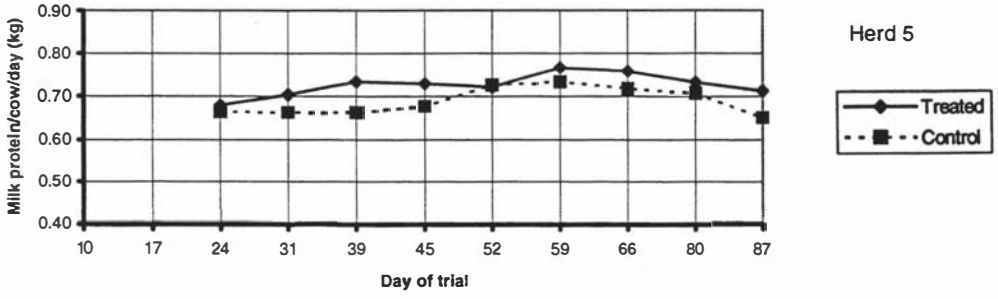
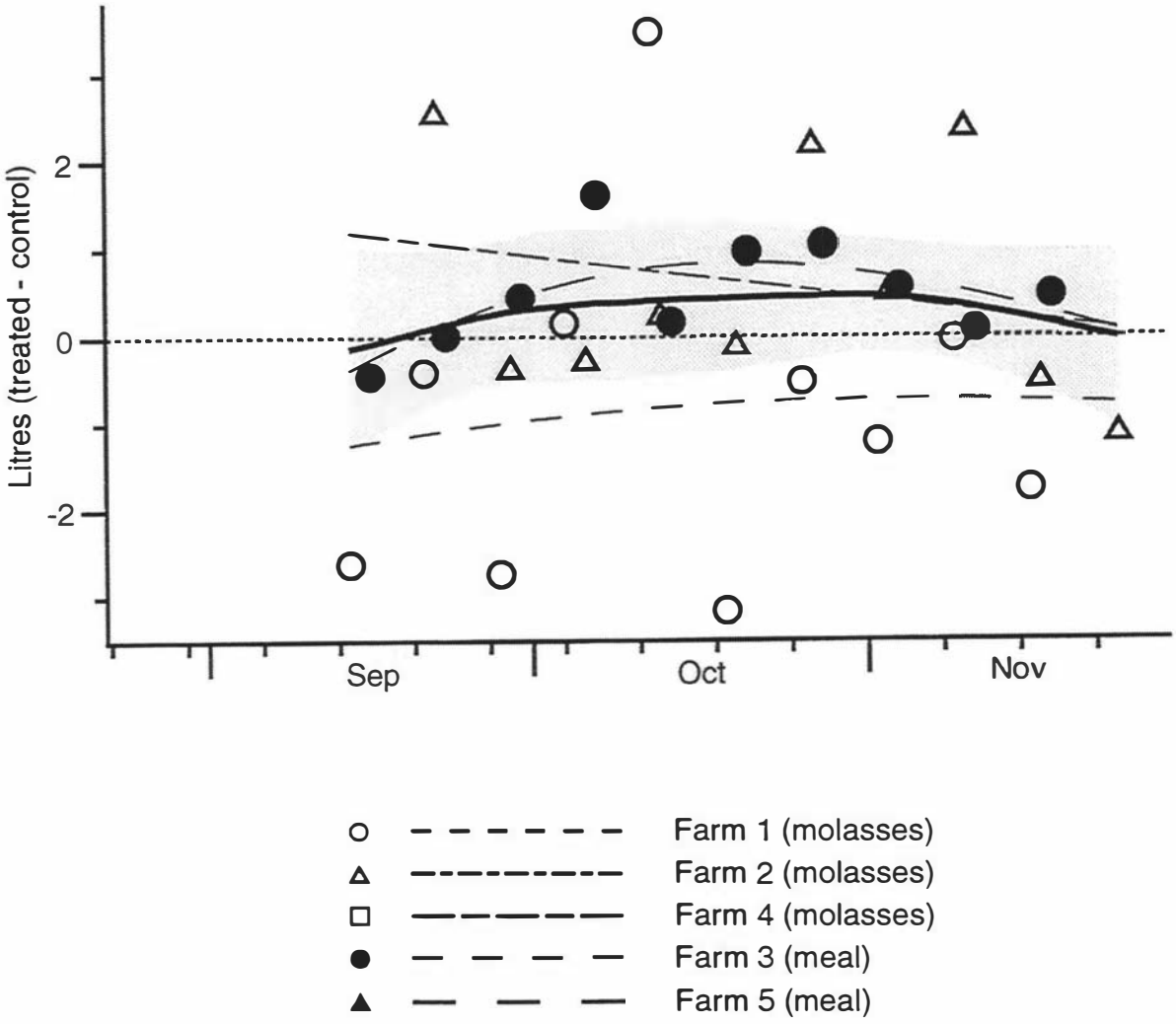


Figure 3.16 Difference in per cow litres milk/day between treated and control herds versus time[1,2,3,4].



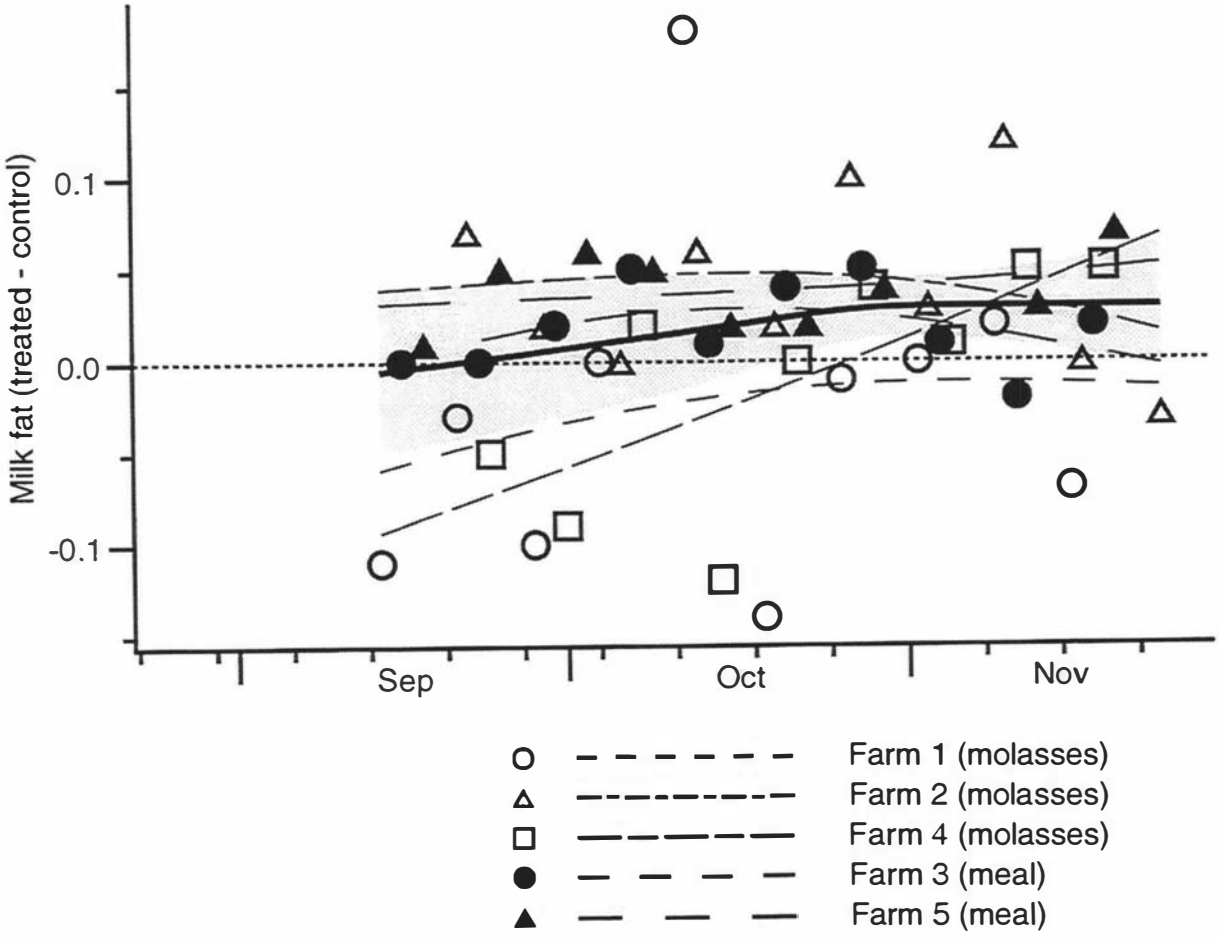
[1]Each data point represents the difference between weekly means (treated minus control).

[2]Each finer line represents the fitted regression line (using Flexi 2.2) of differences over time.

[3]The solid smoothed curve combines results from the five herds.

[4]Differences significant at $P < 0.05$ where the 95% confidence interval does not intercept zero.

Figure 3.17 Difference in per cow milk fat/day (kg) between treated and control herds versus time.



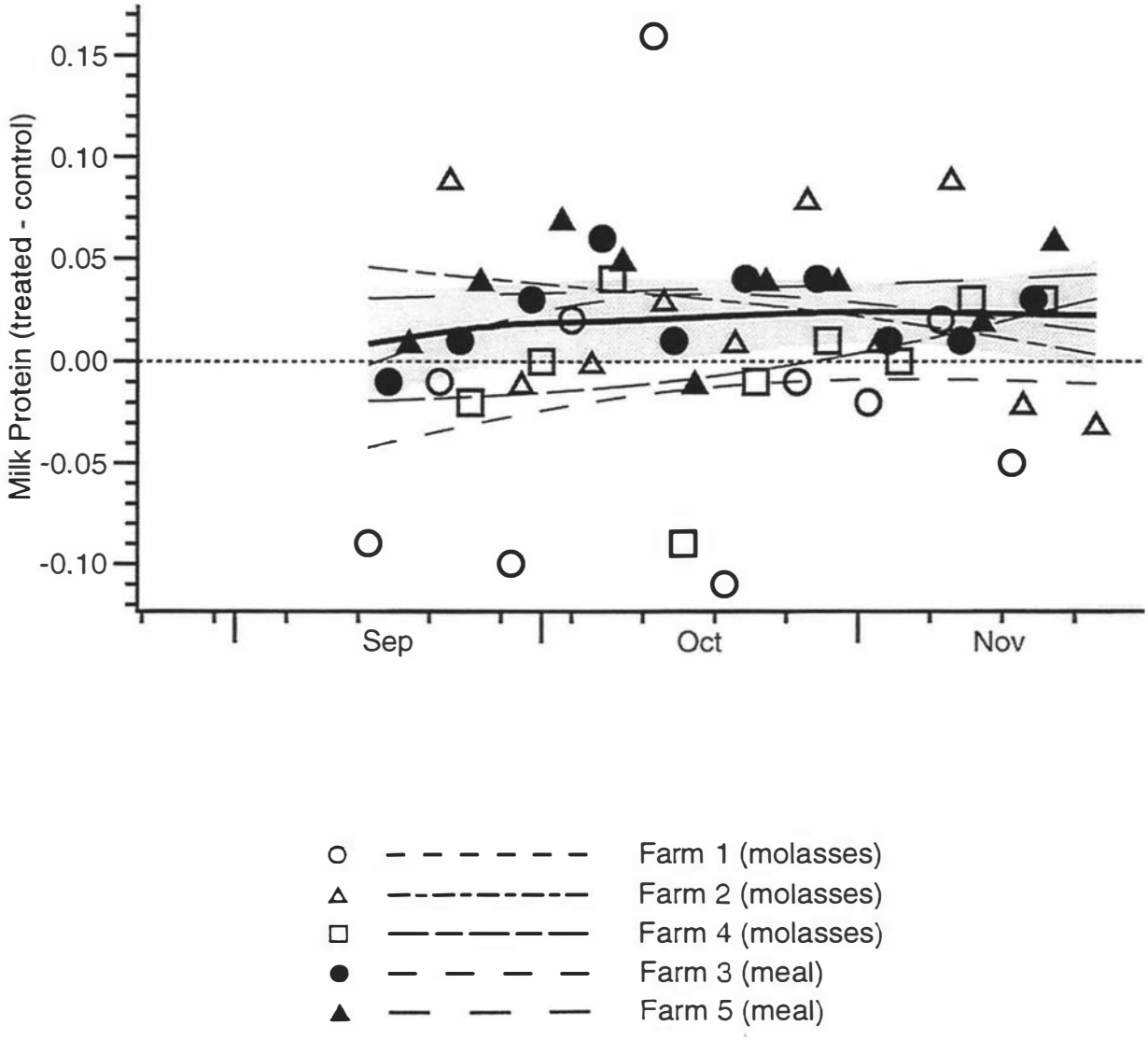
[1]Each data point represents the difference between weekly means (treated minus control).

[2]Each finer line represents the fitted regression line (using Flexi 2.2) of differences over time.

[3]The solid smoothed curve combines results from the five herds.

[4]Differences significant at $P < 0.05$ where the 95% confidence interval does not intercept zero.

Figure 3.18 Difference in per cow milk protein/day (kg) between treated and control herds versus time.



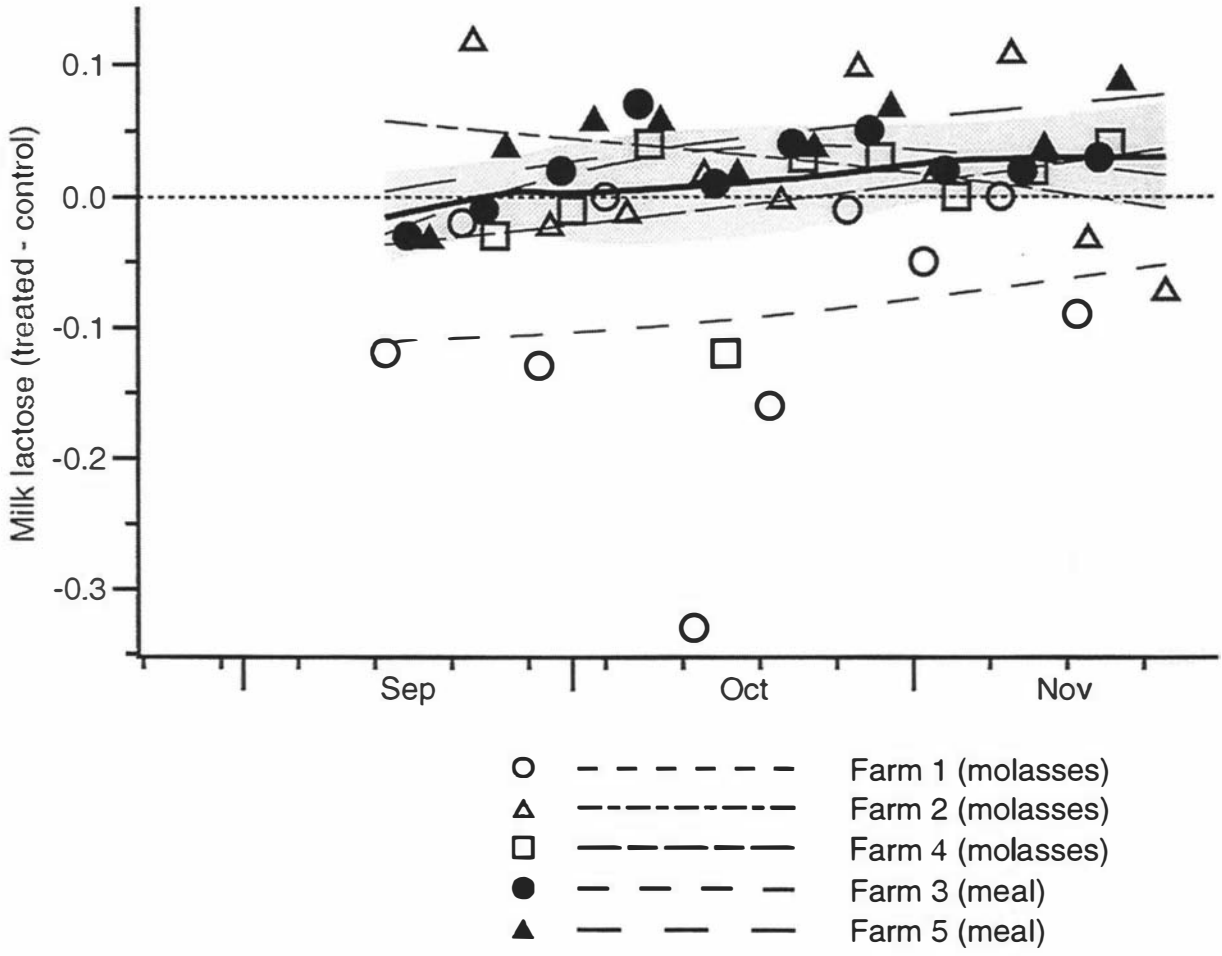
[1]Each data point represents the difference between weekly means (treated minus control).

[2]Each finer line represents the fitted regression line (using Flexi 2.2) of differences over time.

[3]The solid smoothed curve combines results from the five herds.

[4]Differences significant at $P < 0.05$ where the 95% confidence interval does not intercept zero.

Figure 3.19 Difference in per cow milk lactose/day (kg) between treated and control herds versus time.



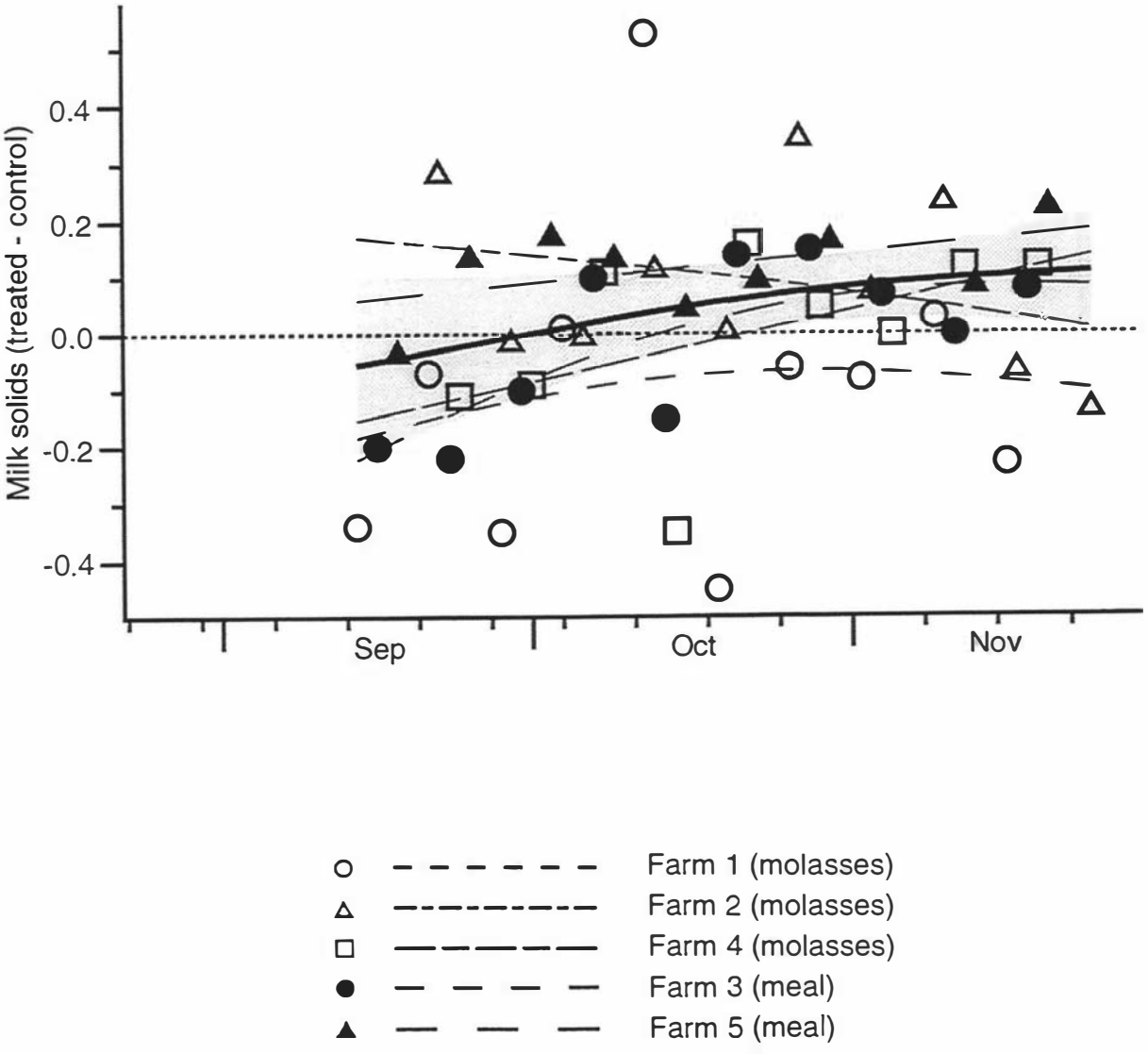
[1]Each data point represents the difference between weekly means (treated minus control).

[2]Each finer line represents the fitted regression line (using Flexi 2.2) of differences over time.

[3]The solid smoothed curve combines results from the five herds.

[4]Differences significant at $P < 0.05$ where the 95% confidence interval does not intercept zero.

Figure 3.20 Difference in per cow milk solids (kg milk fat, plus kg milk protein and milk lactose) between treated and control herds versus time.



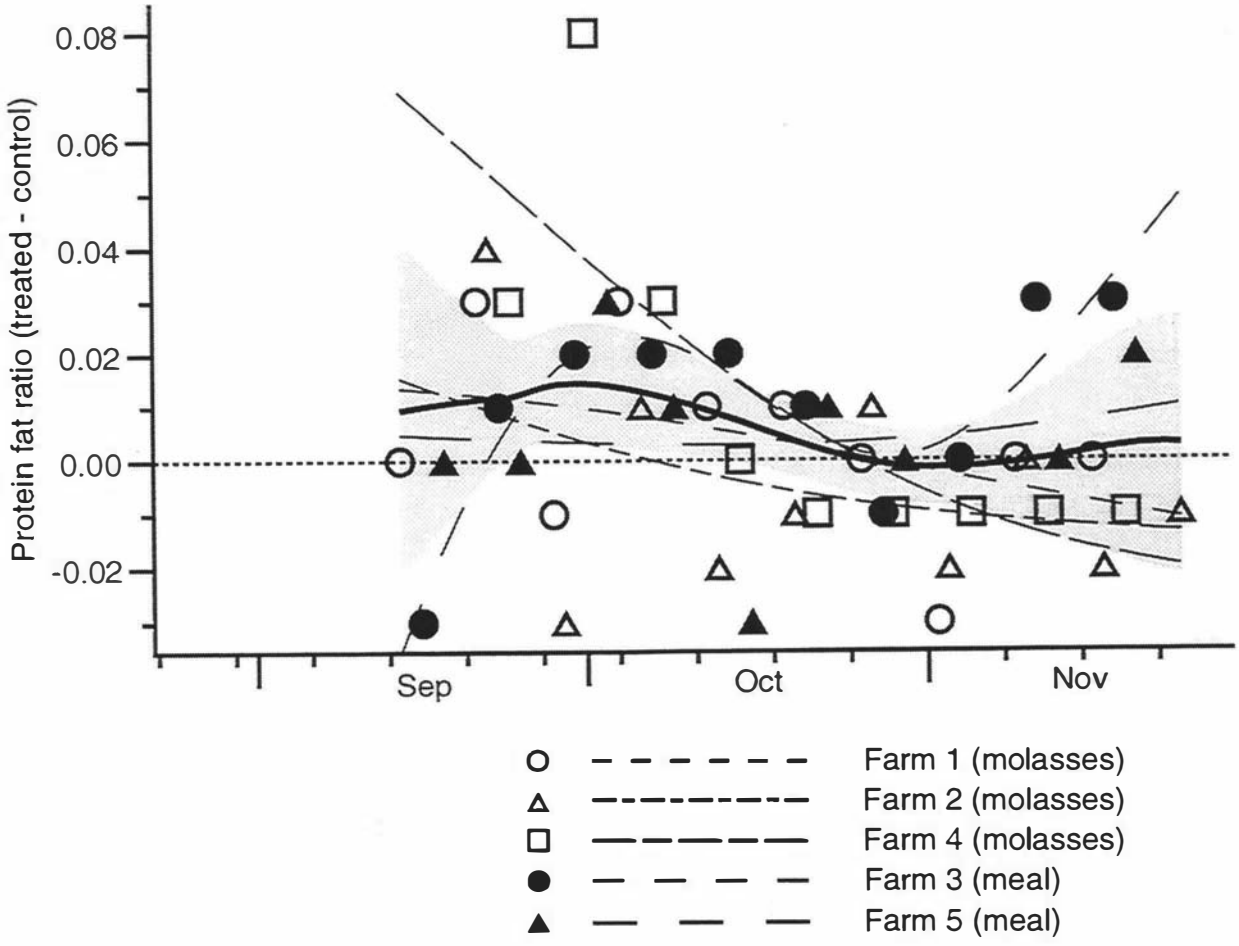
[1]Each data point represents the difference between weekly means (treated minus control).

[2]Each finer line represents the fitted regression line (using Flexi 2.2) of differences over time.

[3]The solid smoothed curve combines results from the five herds.

[4]Differences significant at $P < 0.05$ where the 95% confidence interval does not intercept zero.

Figure 3.21 Difference in per cow protein/fat ratio between treated and control herds versus time.



[1]Each data point represents the difference between weekly means (treated minus control).

[2]Each finer line represents the fitted regression line (using Flexi 2.2) of differences over time.

[3]The solid smoothed curve combines results from the five herds.

[4]Differences significant at $P < 0.05$ where the 95% confidence interval does not intercept zero.

3.3.4 Blood analyses and milk urea results

Serum magnesium (Mg), albumin (Alb) and beta-hydroxybutyrate (BOH) were monitored monthly on seven cows from each of the treated and control herds on each property. A pre-trial blood sample was collected from these cows also to assist with interpretation. Occasionally individual cows were not retained at morning milking for blood sampling (see Table 3.4).

Mean BOH levels were higher in August in both control and treated pre-tests (0.48 mmol/l and 0.57 mmol/l, respectively), then reduced to 0.39 mmol/l and 0.36 mmol/l after one month, then 0.33mmol/l and 0.34 mmol/l after 2 months and finally 0.53 mmol/l and 0.53 mmol/l after 3 months respectively. No significant differences were detected between treated and control herds using a T-test for BOH.

No significant differences were detected between treated and control herds for serum Mg using a T- test on the monthly blood results. Mean serum Mg gradually increased every month in treated and control herds from 0.75 mmol/l (pre-test), 0.81 mmol/l, 0.83 mmol/l to 0.85 mmol/l respectively (results not presented in detail).

Albumin levels improved with supplementation in most of the herds (Table 3.4), but differences were not significant using a T-test. For brevity only Albumin results are tabulated.

Milk urea was consistently reduced by treatment in all herds and on average the difference was statistically significant ($P < 0.05$). Individual herd results are presented in Figures 3.22-3.26 and statistical analysis is presented pictorially in Figure 3.27. Milk urea levels were highest in Herd 5 (Table 3.2).

Table 3.4 Mean values for Albumin (SEM).

Albumin (g/l)		Pre-test (31/8)	After 1 month	After 2 months	After 3 months
Farm 1	Control	30.5(n=6) (0.99)	31.7 (n=6) (0.61)	32.2 (n=6) (0.61)	32.9 (n=7) (0.40)
	Treated	29.9 (n=7) (0.86)	32.6 (n=5) (0.81)	33.0 (n=7) (0.65)	34.1 (n=7) (0.88)
Farm 2	Control	32.9 (n=7) (0.8)	32.2 (n=6) (0.8)	33.1 (n=7) (0.46)	-
	Treated	33.3 (n=7) (0.36)	33.4 (n=7) (0.2)	34.2 (n=6) (0.6)	-
Farm 3	Control	30.0 (n=7) (0.72)	32.8 (n=5) (1.16)	33.4 (n=7) (0.65)	-
	Treated	29.4 (n=7) (0.97)	30.7 (n=6) (0.33)	31.6 (n=7) (0.6)	-
Farm 4	Control	30.6 (n=7) (0.81)	32.1(n=7) (0.46)	33.3 (n=7) (0.47)	32.0 (n=5) (1.34)
	Treated	30.9 (n=7) (0.83)	33.0 (n=7) (0.85)	34.0 (n=6) (0.86)	32.5 (n=6) (0.89)
Farm 5	Control	33.7 (n=7) (0.52)	32.4 (n=7) (0.81)	32.0 (n=7) (0.62)	33.3 (n=7) (0.68)
	Treated	32.9 (n=7) (1.0)	32.1 (n=7) (0.77)	31.4 (n=7) (0.43)	32.9 (n=7) (0.8)

Figure 3.22 Weekly mean milk urea for Herd 1 versus time (1 September-25 November).

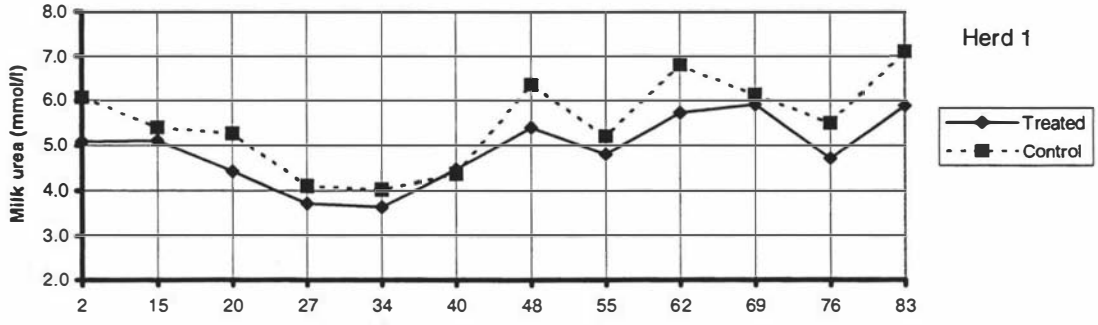


Figure 3.23 **Weekly mean milk urea for Herd 2 versus time (1 September- 25 November).**

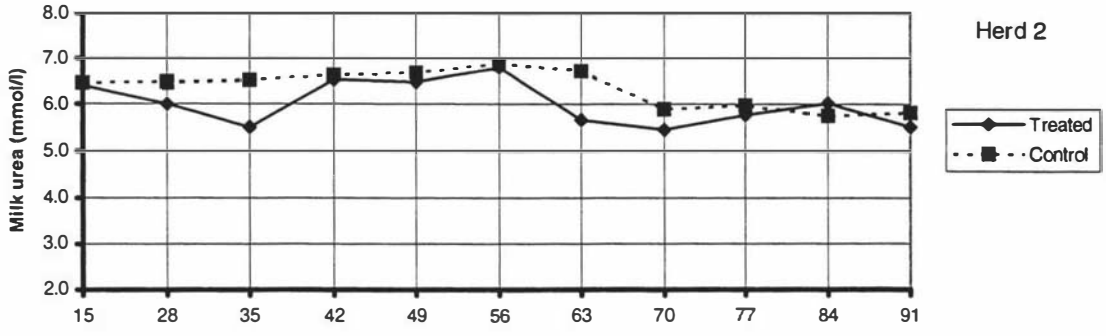


Figure 3.24 **Weekly mean milk urea for Herd 3 versus time (1 September - 25 November).**

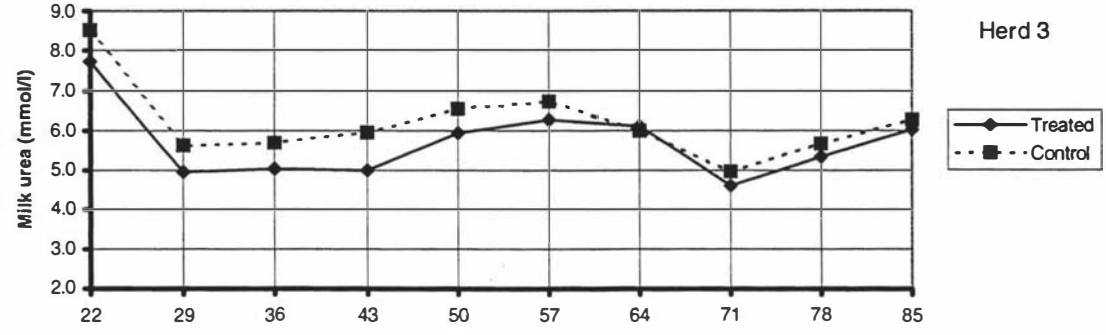


Figure 3.25 **Weekly mean milk urea for Herd 4 versus time (1 September - 25 November).**

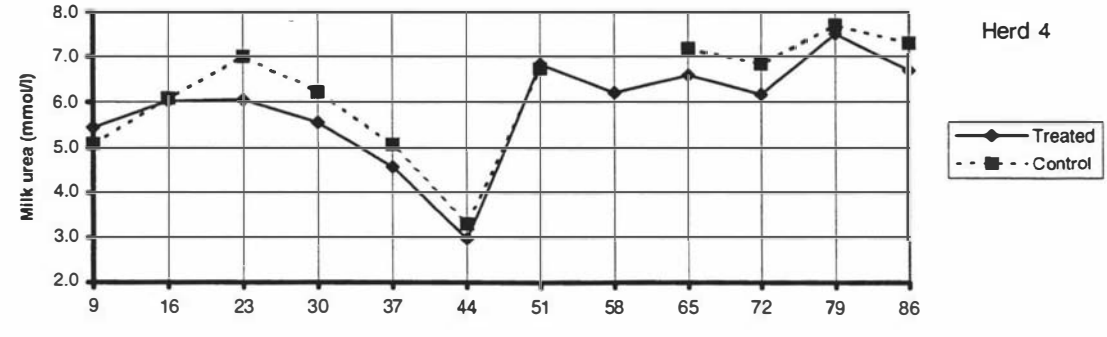
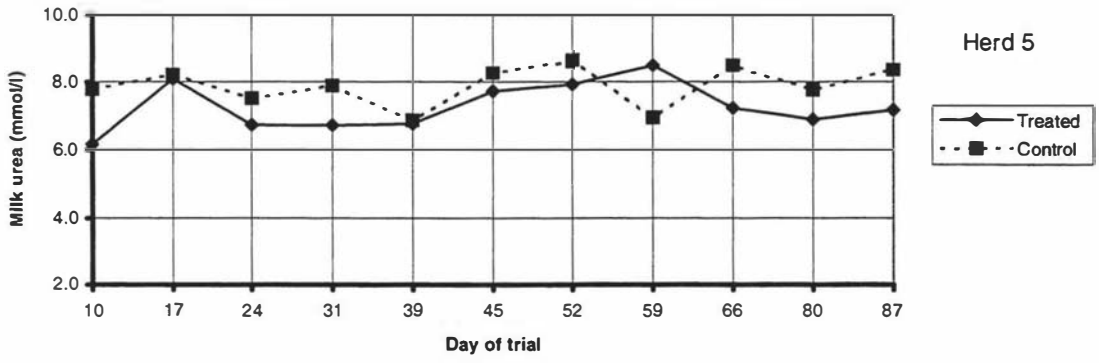


Figure 3.26 Weekly mean milk urea for Herd 5 versus time (1 September - 25 November).



3.3.5 Correlations between urea, pasture nutrients and intake

A correlation matrix (over time) was used initially to examine the interrelationships between dietary characteristics and milk urea levels (Table 3.5). Indices chosen were those theoretically related to milk urea levels. Regression equations were then developed using Excel 5.0 to test the predictive value of milk urea for dietary characteristics based on the relationship between pasture CP, pasture SOLCHO and urea suggested in Chapter 1 (Table 3.6). The strongest relationships in the correlation matrix (apart from those that are a function of the other, eg CP vs CPxDMI) were between the (bulk) milk urea results and CP% in the diet, dry matter intake and SOLCHO in the diet.

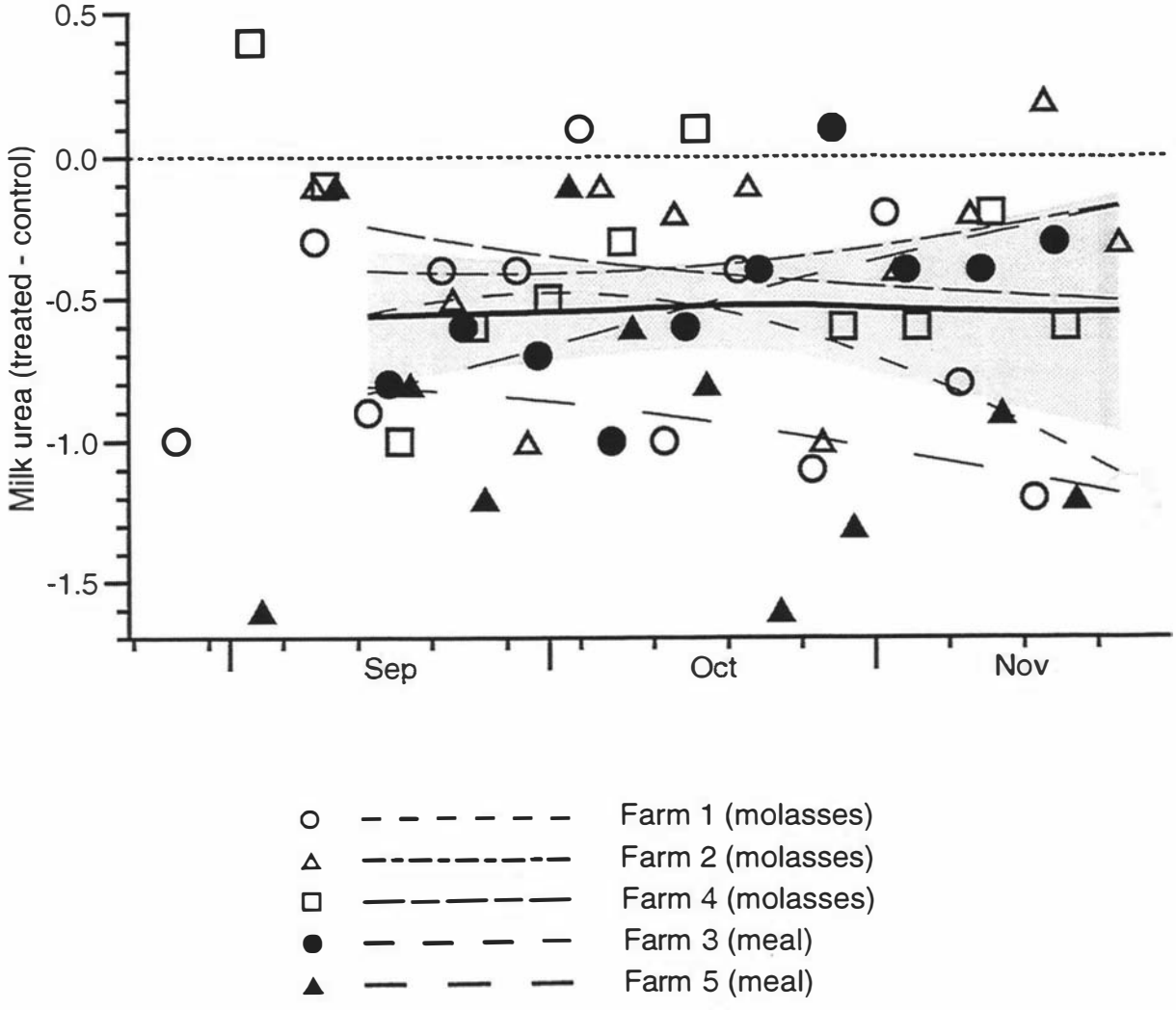
Table 3.5 Correlation matrix of chosen indices related to milk urea[2]

	Day No	*urea*	*DMI*	*CP*	*CPxDMI*	*CP/ S.CHO*	*CPxDMI/ S.CHO*	*%S.CHO*
Day No	1							
Urea	0.13	1						
DMI	0.54	0.34	1					
CP	-0.59	0.26	-0.17	1				
CPxDMI	0.02	0.47	0.70	0.57	1			
CP/S.CHO	-0.19	0.44	0.14	0.67	0.58	1		
CPxDMI/ S.CHO[1]	0.05	0.52	0.55	0.47	0.80	0.89	1	
%S.CHO	-0.19	-0.42	-0.30	-0.21	-0.39	-0.79	-0.77	1

[1]Protein % in diet x Dry matter intake divided by soluble carbohydrate %

[2]Correlations significant at $P < 0.05$ when greater than 0.205, $P < 0.01$ when greater than 0.27, and $P < 0.001$ when greater than 0.34

Figure 3.27 Difference in bulk vat milk urea (mmol/l) between treated and control herds versus time.



[1]Each data point represents the difference between weekly means (treated minus control).
[2]Each finer line represents the fitted regression line (using Flexi 2.2) of differences over time.
[3]The solid smoothed curve combines results from the five herds.
[4]Differences significant at $P < 0.05$ where the 95% confidence interval does not intercept zero.

The ability for bulk milk urea level to be used to predict either intake or CP% in the diet was examined by calculating regression equations from the data available (Table 3.6). Only weak relationships were found between CP, SOLCHO, DMI and urea and even when some intake data was removed. Milk urea was of limited use in predicting DMI, CP% or SOLCHO% in pasture or ratios of these variables.

Table 3.6 Regression equations for the relationship between dietary parameters and milk urea.

Regression equations for dietary parameters available predicted using milk urea[1]	Regression equation (s.e.)	Adjusted R^2 (residual s.e.)	Significance of relationship of parameter with urea
CP% x DMI	= 104.6 + 39.9 x urea (31.7) (5.1)	0.38 (59.8)	***
(CP% x DMI)/SOLCHO%	= -7.73 + 6.36 x urea (4.85) (0.78)	0.41 (9.2)	***
CP% / SOLCHO%	= 0.30 + 0.226 x urea (0.26) (0.04)	0.23 (0.49)	***
Log CP% x DMI/Log SOLCHO%	= 0.30 + 0.226 x urea (1.98) (0.32)	0.42 (3.74)	***

[1]12 of 111 values were removed from the dataset to obtain these figures. These were removed because they fell well outside the norm for one parameter measured (DMI). Considering the potential variability of parameters measured in pasture and in particular estimated DM intake this was considered justifiable, eg in some instances DMI was estimated at 20-22 kg from 450 kg cows producing 0.8 kg milk fat.

The strongest of the regression equations using milk urea levels was that equation which would predict dietary protein excess ameliorated by SOLCHO, ie Pasture (CP x DMI)/Pasture SOLCHO. This fits well with current understanding of milk urea (Moore and Varga, 1996) and rumen function.

3.3.6 Reproductive results

All cows in each herd were manually palpated for pregnancy, with the exception of the 450 cow herd in which 70 cows from each group considered to be possible empty cows were manually palpated (these were cows that were mated late, that may possibly have

returned on heat or that otherwise were considered at risk). Submission rate is defined as "% of the herd to be mated that is presented for service by artificial breeding for a specified period" - the period in this case was 4 weeks.

A significantly lower ($P < 0.05$) number of cows were "empty" at the end of the mating season between the combined "treated" herds compared with the "control" herds (see Tables 3.7 and 3.8). The bulls were removed from herds in late January. There was no significant difference in submission rate between these groups, and no significant difference in services per cow bred. Although not significant, there were more "late returns" (cows returning to service greater than 40 days after first mating) in the control group, especially Herds 1 and 3.

Table 3.7 Reproductive data for each herd.

		Submission rate (4 wks)	% returns to service over 49 days	Services/cow bred	Non return rate (60d)	Cows not pregnant at end season
Farm1 (n=220)	Treated	90%	1%	1.69	56%	4
	Control	96%	10%	1.44	68%	6
Farm 2 (n=180)	Treated	97%	11%	1.36	75%	2
	Control	95%	9%	1.54	66%	4
Farm 3 (n=160)	Treated	96%	7%	1.56	66%	1
	Control	93%	22%	1.50	73%	3
Farm 4 (n=450)	Treated	86%	7%	1.27	81%	6
	Control	88%	0%	1.32	76%	13
Farm 5 (n=200)	Treated	97%	22%	1.26	83%	3
	Control	95%	15%	1.15	89%	2
Farm 6 (n=170)	Treated	88%	6%	1.38	73%	2
	Control	91%	3%	1.66	61%	5

Table 3.8 Reproductive performance in six[1] supplemented herds versus control herds.

	"Supplemented" Herds	"Control" Herds
"Empty" Cows at end of mating season	18 cows[2] (2.7%)	33 cows (5%)
Submission rate to AB	85.6%	88%
Services per cow bred	1.41	1.44
Comments	-	More "Late Returns" over 40 days (see Table 3.9)

[1]Data for a sixth herd meal supplemented was available, but data from this herd was not available for pasture, blood samples and urea levels.
[2]Difference significant at the 5% level using a generalised linear model with binomial error function logit link.

The effect on predicted calving spread for the following season was also analysed as this was a potential response to supplementation (Table 3.9). All herds were combined for this calculation.

Table 3.9 Predicted calving spread for "Supplemented" herds and "Control" herds to low level carbohydrate supplementation in spring[1]

Predicted Calving Spread-1993	"Supplemented" Herds	"Control" Herds
Calved by 2 weeks	42.5%	39%
Calved by 4 weeks	70.5%	70.6%
Calved by 6 weeks	84.8%	82.5%
Calved by 8 weeks	90.3%	86.8%
Calved by 9 weeks+	99%	98.1%

[1]Analysis performed using computer software "Seasonal Dairy Herd Breeding Record Analysis Version 2.1,1986".

No significant differences were present between groups.

3.4 DISCUSSION

The appropriateness of combining results from 6 farms with 3 different supplement types (molasses and 2 brands of concentrate) may be questioned. In all cases, however, the supplement was a low level of supplementary fermentable carbohydrate and large cow numbers were required to identify small but economically important responses.

3.4.1 Pasture analyses

Pasture protein and digestibility declined and NDF levels increased on all farms in early/mid October whatever management practices were or whether feed supply was in excess of demand. This is similar to data from other studies presented in this thesis (Chapter 1 and 2), and suggests that environmental conditions determine this change in ryegrass/white clover pastures at the same time each year (Wilson *et al.*, 1975). The production decline noted in most herds at this time is likely determined by the above changes in pasture nutrients. The decline in digestibility is likely to lead to reduced DM intake in October and hence lower milk production. Reduced DM intakes with lower digestibility pasture are a well established phenomenon (Hodgson, 1977; Horn *et al.*, 1979; Wilson *et al.*, 1995). The DM% of pasture gradually rose during the trial. This would be expected to have a positive effect on intake (John and Ulyatt, 1987), which may counteract the increased NDF which would tend to reduce intake (Mertens, 1985). Pasture CP declined in October/November to levels that were only marginally adequate for high productivity (NRC, 1989). High pasture CP and reduced pasture SOLCHO occurred on Farm 5. This herd had the highest milk urea levels, the highest milk production and the shortest rotation length between grazings.

3.4.2 Milk production response

Milk production responses to supplementation were generally small and variable between herds. Positive responses (less than 1 litre per cow, and 0.6 litres/kg supplement) were distinctly higher in two of the herds (Herds 2 and 5), and only for part of the supplementation period. Better production responses occurred in the better conditioned herds (Herds 2 and 5). The negative response of herd 1 is of particular interest. This herd calved in poorer condition than the others and the cows appear to have partitioned the supplement towards weight gain because condition score improved the most in the treated group on this farm. The lower albumin levels in Herd 1 confirm they were in lower condition before the trial started. Pasture on farm 1 tended to have lower CP levels. Addition of a low protein supplement (molasses) may have lowered total dietary protein levels below optimal levels (17-19%, NRC) and thus encouraged weight gain rather than production. Soluble carbohydrate levels in pasture from farm 1 were also higher which may have assisted the partitioning to weight gain. Assuming DMI estimates were correct, a negative production response implies the supplement somehow upset intake or rumen fermentation. Rapid release of soluble carbohydrate in the rumen could lower pH via lactic acid formation and this may then interfere with rumen fermentation, as pH in the rumen above 6.0 is favoured by fibre digesting bacteria. The higher SOLCHO in pasture grazed by herd 1 may have contributed to an acidosis caused by supplementation with a highly degradable soluble carbohydrate like molasses.

A similar but lesser weight gain partitioning occurred in herd 3, which was also relatively light before the trial started. This herd had a low response to supplementation.

The highest production response was obtained mainly in October/November well after the start of the trial. The reasons for this are unclear but partitioning to weight gain before a milk response could occur was a possible reason. Another is the lower quality pasture available in October/November (see Figs 3.1-3.5), which would have restricted potential intake of metabolisable energy. Addition of concentrate at this stage would have raised potential ME intake in the face of ample pasture because ME concentration

of the concentrate was higher than pasture. Another possible reason is that rumen pH could have been lower in September due to lower fibre levels, and thus interfered with responses.

This late response to treatment is similar to the "cumulative response" referred to by Kellaway and Porta (1993), and may be due to an improvement in condition, rumen adjustment to the new diet, improved cover of pasture from substitution which allowed better feeding later, a reduced substitution rate as the trial progressed or a dietary quality issue as mentioned in the previous paragraph. Better responses to supplementation of cows on pasture were obtained by Salam *et al.* (1996) in November than early October, which is similar to that found in this trial. "Carryover" responses to supplementation after the trial period were not examined in this trial, but have produced as much response as the trial period itself in some trials (Kellaway and Porta, 1993).

The results suggest that milk production responses to supplementation of a pasture diet with moderate amounts of carbohydrate in early lactation are more likely to occur in better conditioned cows and after 1 month of supplementation and when pasture quality is poorer.

The trial design did not involve cow weighing or sufficiently accurate estimates of dry matter intake to detect differences between the groups on each farm. Substitution of 0.4-1.35 kg carbohydrate supplement per cow for the same amount of pasture (the highest likely substitution rate would be 1:1) would have resulted in 50-100 kg DM/ha less pasture eaten each day. The DM detection methods available in this trial did not have sufficient accuracy to detect this degree of change with certainty. Milk production measurement after the supplementation was complete may also have been helpful to measure carryover effects on production which can be more than half of the total milk response (Kellaway and Porta, 1993). Carryover effects into the subsequent lactation were detected by Wilson (1989) through improved conception and hence more milk production. This effect was not seen in this trial.

3.4.3 Milk urea levels

Milk urea levels averaged between 5 and 7.5 mmol/l in the 5 herds during the trial. These levels are higher than recommended (Gustafsson, 1993; Moore and Varga, 1996; Semptey and De Vischer, undated) for optimal rumen function (recommended levels near 4-4.5 mmol/l urea) and indicate dietary rumen degraded protein excess (Fulkerson, 1996; Moore and Varga, 1996). The levels found are higher than those reported to produce reduced conception rates (Ropstad and Refsdahl, 1987; Ferguson *et al.*, 1993). Ferguson *et al.* (1988) found reduced conception rates occurring over 7 mmol urea/l (≡20 mg/dl serum urea nitrogen). Evidence from this trial is equivocal on the cause, but significantly reduced "empty" cows were found, eg Herd 5 had low empty cow rates but higher milk urea levels.

Milk urea levels were reduced with supplementation and the mean values were significantly different between treated and control groups (Fig. 3.27). A lower milk urea level is likely to reflect reduced nitrogen excess in the rumen (Oltner and Wiktorsson, 1983; Kirchgessner *et al.*, 1986; Gustafsson, 1993; Fulkerson, 1996; Moore and Varga, 1996) and increased capture of excess dietary protein from the pasture diet. Rumen fermentation should be improved by addition of carbohydrates to a pasture diet (Stokes *et al.*, 1991b; Muller *et al.*, 1995). The milk production and reproductive responses noted in this trial could have come from improved rumen function and the reduced urea levels rather than increased dry matter intake. The lowered milk urea levels with supplementation suggest that this was occurring, but relative improvements in protein production over fat production would also be expected from improved carbohydrate supply to the rumen. This only occurred at a significant level in late September and early October in some herds.

The predicted milk production penalty using the equation produced by Danfaer *et al.* (1980) for a 23% crude protein diet as opposed to an 18% crude protein diet would be approximately 1.5 litres of 4% fat milk. Assuming that the DM intakes in this trial were not altered by the treatment (this assumes 1:1 substitution) then the dietary intake of protein by the cow would have decreased by approximately one percentage unit (Spartan dairy ration balancer, undated), which could provide 0.3 litres of 4% fat milk.

This is close to the level of responses found in this trial, ie. thus it is possible that the production responses came from changes in dietary balance rather than increases in intake of metabolisable energy. Moore and Varga (1996) state that the penalty for excess dietary protein is 0.2 Mcal of net energy/100 g excess protein or near 1.3 MJ of ME/100 g excess protein, which is similar to the predictions of Danfaer *et al.* (1980). Potential errors in DMI mean no conclusion can be reached on the source of milk production responses.

The milk urea reduction with treatment was of a similar magnitude whatever the level or type of supplement. This may suggest a limit to manipulation of urea levels by inclusion of carbohydrate sources into the diet.

The potential for milk urea to be used to predict pasture crude protein % or vice versa was examined by developing the correlation matrix and the regression equations. The best relationship existed for DMI (dry matter intake) multiplied by CP% (crude protein % in the diet) with an adjustment for SOLCHO% (soluble carbohydrate %) in the diet. This is logical in the light of current understanding of rumen fermentation, in that protein excess in the rumen will be determined by all of those factors combined (Stokes *et al.*, 1991b; Moore and Varga, 1996).

Dry matter intakes using pre and post grazing measurements with a rising plate meter can be subject to error, and pasture sampled may not have reflected exactly what the cows ate. These two factors may have led to inaccuracies in the regressions developed. Milk urea is also known to vary with other factors (diurnal pattern, protein catabolism, parity, breed, stage of lactation, mastitis; Gustafsson, 1993) and any of these may also have reduced the accuracy of the prediction. Apart from the selection of groups (odd and even tag numbers) and the large number of cows involved, no attempt was made to account for these factors.

3.4.4 Blood parameters

Blood parameters measured (Alb, Mg, BOH) showed no significant changes with treatment using T-tests. Herds with higher Alb levels at the start of the trial (and higher condition scores) had the better milk production response. This would suggest that more cow condition early in lactation is beneficial to peak production, which is consistent with standard management recommendations. It is also beneficial to fertility. Magnesium and BOH levels fell within normal reference ranges for these parameters in blood. Albumin is a relatively stable blood parameter that reflects protein status in cows, and BOH reflects fat tissue mobilisation for energy supply. The lower Alb levels in cows on Farm 1 are consistent with lower body condition as detected visually.

3.4.5 Reproductive response

Improved herd reproductive efficiency occurred in the supplemented herds with half the level of non pregnant cows found at the end of the mating season compared to control herds. The reason for the improved fertility is not obvious from the data recorded, and is especially influenced by the result in herd 4 (the larger herd). The result is nevertheless statistically significant at $P < 0.05$. There were no significant differences in submission rate or non-return rate between treated and control herds. Most of the reproductive data is based on observation of returns to "heat". Farmers often become less vigilant once AB mating is complete and bulls are run with the herd. It is possible some late returns were not seen, which would support the argument that control cows had more late returns. Condition score changes favouring the treated groups in two of the herds suggest that increased partitioning to weight gain was occurring (weights were not recorded in this trial). Subsequent experience by the author involving regular weighing would suggest that condition score changes tend to be underestimated by many people within the dairy industry, including consultants, based on comparison of weighing and condition scoring. In retrospect, it is likely that the condition scores estimated in this trial were greater than actually recorded if it is accepted that one condition score equals near 30 kg body weight (20-35 kg/condition score in Holmes and Wilson, 1984).

Improvements in condition score are likely to improve fertility if weight gain was occurring approaching and during mating (McClure, 1961; King, 1968; Butler and Smith, 1989). There were only small differences in predicted calving patterns for the next season.

3.4.6 Herd 1

Negative milk production responses to supplementation occurred in herd 1, yet reproductive responses were positive. Low cow condition at trial start and condition score gain during the trial were features of supplementation on this farm. Partitioning to weight gain rather than production seems to have occurred. This may be a "survival" response to the low condition, or possibly due to suboptimal crude protein levels in the diet for lactation which would tend to promote condition gain and good reproduction instead of milk production. Alternately, the molasses may have upset rumen fermentation, although positive supplement responses to molasses occurred in the other two herds at similar levels of consumption.

3.4.7 Herd 5

Milk solids production per cow was highest in Herd 5, milk urea levels were highest (mean urea 7.16 and 7.74 mmol/l in treated and control herds), pasture CP was high and pasture SOLCHO was low on Farm 5. At first sight there does not appear to have been a relationship between production and urea levels as suggested in Chapter 1, although the relationship between urea and pasture CP and SOLCHO was apparent. Cows consuming high amounts of dry matter that are excessive in CP will have a higher dietary protein excess and hence higher milk urea. The shorter rotation length may explain the reason for the higher pasture CP and lower SOLCHO as pasture does not start to accumulate SOLCHO until after the 1st leaf emerges, which would have allowed less time with a fast rotation (Fulkerson, 1996).

3.5 CONCLUSIONS

The level of supplementation in this experiment was insufficient to significantly alter dietary protein levels from those offered in the pasture alone, but did increase dietary non-structural carbohydrate levels by 2-5%. Considerably more than 1.5 kg of low protein concentrate would be required to change dietary protein and carbohydrate levels sufficiently if 15-20 kg pasture with a 25% CP level is being consumed. This was beyond the scope of this particular trial.

Nevertheless significant production and reproductive responses occurred in this trial, but it is unclear whether these came from improved dry matter intake or improved dietary balance, or both. The better response later in the trial period is of interest, and may warrant further investigation. Addition of a rumen buffer may have improved responses to supplement early in the trial. In practice, concentrate supplements are often stopped when it is perceived that adequate pasture dry matter is available. These results indicate this may not be the correct policy. Continuation of supplementation through the reproductive phase of ryegrass growth in October/November while pasture is of lower digestibility may assist with maintenance of peak lactation until higher quality pasture returns with the arrival of higher % clover and more vegetative ryegrass.

Milk urea appears to be a useful tool to predict dietary protein excess, but does not directly reflect dietary protein % because DM intakes and pasture SOLCHO must also be taken into account. More precise measurement of diet and intake would be required to accurately define the relationship between urea, Pasture CP, Pasture SOLCHO and DMI.

The economic benefit of the responses measured could not be clearly ascertained from the data measured, but was apparently positive on 2 or 3 of the farms and negative on others based on the reproductive and milk production responses measured in this trial, current milk values of 30c/l of milk and Pregnant vs Empty cow values of $1000 vs $250. The poorest milk production response (in fact a negative effect of supplementation) occurred in the thinnest herd (Farm 1) and the better responses in milk production occurred in the better conditioned herds (Farm 2 and 5). This would

suggest that some of the supplement was partitioned to body maintenance or weight gain and to ensure reproductive success in the lighter herds. Condition score changes in herds 1 and 3 tend to confirm this. Production responses to supplementation have been reported to be greater in high BI cows underfed in spring (Penno and Carruthers, 1995) - this does not appear to be the case in this trial where the better fed cows responded better to supplementation. Economic responses to supplementation need to be assessed over longer periods, including whole and subsequent lactations, including the period after supplementation is complete and need to include effects on cow weight and condition which can result in mobilisation of body reserves after the trial period has ended (Kellaway and Porta, 1993).

Reproductive benefits need to be included in economic evaluations of supplementation with concentrates. In this trial the value of the improved reproduction equalled the value of the improved milk production based on current prices for culls and milking cows, and the costs of treating anoestrus cows. Although significant, the results should be interpreted with care because the large Herd 4 influenced the significance of the results considerably. It should also be noted that not all of Herd 4 were pregnancy tested because of numbers involved, but the selected cows did contain all the empty cows when subsequently confirmed with the herd owner. If reproductive responses can be predicted with some certainty it would be helpful in assessing the economics of supplementation. This trial goes some way to assisting prediction of likely reproductive responses in average herds, but needs to be extrapolated with caution as many factors affect reproductive performance in dairy herds. Despite this few other trials have examined reproductive benefits of supplements to a pasture diet in this manner in the New Zealand context and with this number of cows.

It was not clear from the results of the trials presented in this chapter whether improved dietary balance or altered DM intakes provided the responses to supplementation with readily fermentable carbohydrate. Supplementing the pasture diet with readily fermentable carbohydrate sources to a level closer to that recommended (NRC, 1989; Stokes *et al.*, 1991b; Muller, 1993) has not markedly enhanced cow performance in spring but has produced measurable responses. The nature of production limiting nutrients in a pasture only diet appears to be more complex than simply a deficiency of

fermentable carbohydrate levels. The following chapter (Chapter 4) describes a trial where dry matter intakes, more complete dietary balance and cow weights were monitored in more detail in an attempt to separate the issues of dietary balance and dry matter intake as major influences on milk production.

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CHAPTER 4

Supplementation to "balance" nutrient intakes of cows fed pasture and maize silage

PREFACE

The supplementation trial presented in Chapter 3 examined supplementation of dairy cows on pasture with limited quantities of readily fermentable carbohydrate sources (molasses or high energy concentrate), but did not attempt to supplement or "balance" the cow's diet with more of all the known potential production limiting nutrients (NRC, 1989; Muller, 1993; Ulyatt and Waghorn, 1993; Muller *et al.*, 1995). Milk production will only be as high as the first limiting nutrient (Lean *et al.*, 1995), and this will frequently be metabolisable energy (ME), unless the cow draws from reserves as is the case immediately after calving when energy demand exceeds intake (Muller, 1993). The question, once lack of dry matter (lack of ME) available does not become the production limiting nutrient, is then which nutrients do limit milk production in a pasture based dairy system?

The trial results reported in this chapter are from a commercial dairy herd where comparison was made between a "balanced diet", formulated according to dietary recommendations from dairy nutritionists and computer ration balancing programmes, with an isoenergetic diet that was not specifically balanced. Pasture was supplemented with maize silage in both control and treated groups. The aim was to separate the issues of dietary balance and dry matter intake as they affect milk production and fertility.

4.1 INTRODUCTION

Often maize silage supplementation of pasture (two thirds pasture, one third maize silage) without further balancing does not result in higher per cow production than a pasture only diet offered in the same quantities (eg. Hutton, 1975; Holden *et al.*, 1994), but better cow condition is maintained and better cow reproductive performance usually results. However, other trials have reported improvements in production when limited quantities of maize silage supplemented specific levels of clover (Stockdale, 1994; Stockdale and Dellow, 1995). The low responses are perhaps not surprising because the energy density of maize silage is less than pasture (usually near 10 MJ of ME/kg DM vs 11.5-12 MJ of ME/kg DM in pasture) and hence for the same DM intake, consumed ME will be less. The lower crude protein levels in maize silage (8%) and increased levels of starch (near 30% in maize silage) should help balance excess crude protein (CP) and readily fermentable carbohydrate (RFC) deficiency in a pasture diet. Other factors affecting responses to maize silage will include the degree of substitution that occurs, and possibly mineral deficiencies exacerbated by the inclusion of maize silage (eg magnesium, calcium, phosphorus and sodium).

Cows fed fresh pasture do not receive the perfect diet for production as mentioned in the introductions to the first 3 Chapters in this thesis (Sections 1.1.2 and 2.1). Crude protein (CP) recommendations in NRC for high producing cows are near 18-19%, with up to 35% of this crude protein recommended to be "bypass" protein (also called UDP, RUDP or UIP) (NRC, 1989). Pasture is frequently well above this level for crude protein, sometimes exceeding 30% CP (Moller, 1991; Muller, 1993). The crude protein level in pasture depends largely on the maturity of pasture eaten, nitrogen application, species of pasture eaten and season (Holmes, 1989; Ulyatt and Waghorn, 1993; Moller, 1995; Moller, 1996a). Fresh, highly digestible ryegrass pasture is essential to maintain cow intakes, and is always likely to be above dietary recommendations in CP concentration (see Chapter 2). Rumen undegraded protein (UDP) content of pasture has been estimated between 35% and 15% of total CP (Ulyatt *et al.*, 1988; NRC, 1989; Muller, 1993), with fresh immature ryegrass pasture more likely to have less UDP than mature pasture (Van Vuuren *et al.*, 1991; Muller, 1993). Production limiting amino acids within the UDP are likely to be of particular importance (Muller, 1993; Rulquin

and Verite, 1993; Schwab, 1993). Methionine and lysine are the most likely amino acids to be deficient on pasture diets, particularly at higher production levels, but others have been mentioned also. However, Salam *et al.* (1996) found no response to addition of protected methionine to a pasture diet. Orskov *et al.* (1981) found that the rumen could produce enough bacterial protein for absorption at the small intestine to produce near 20 litres of milk. Above this some bypass protein is needed, with higher production requiring proportionately more.

Readily fermentable carbohydrate (soluble carbohydrates, pectin) levels in pasture are often below dietary recommendations (NRC, 1989; Stokes *et al.*, 1991b; Muller, 1993; Ulyatt and Waghorn, 1993; Hutjens, 1995) especially for high levels of production. Optimal rumen fermentation for bacterial growth will occur with non-structural carbohydrate (NSC) levels near 30-35% according to Stokes *et al.* (1991). Low NSC in pasture occurs especially when it is growing rapidly, shortly after grazing, in summer, after N application (Bailey, 1962; Smith, 1973; Buxton and Fales, 1994; Fulkerson, 1994; Moller, 1996; and Chapters 1, 2, 3 and 5 in this thesis) and after prolonged mild overcast weather with limited sunlight (Fulkerson, pers. comm.). Typically, soluble carbohydrate (SOLCHO) in spring pasture is 5-15% of total DM but occasionally can reach 30-40% in cold clear weather where photosynthesis continues but growth is slowed (Smith, 1973; Vartha and Bailey, 1973; Vartha and Bailey, 1980; Nosberger, 1993). Pectin can be included as a fermentable carbohydrate (although technically it is part of the cell wall fraction) and typically is present at 2-5% of DM of mixed ryegrass/clover pasture depending on clover content, which has more pectin (Ulyatt and Waghorn, 1993). Starch levels only amount to 1-3% in ryegrass/white clover pasture (Fulkerson, 1996). The combination of SOLCHO, pectin and starch is referred to as readily fermentable carbohydrate (RFC). Addition of maize silage to the pasture diet improves the RFC content, especially with starch. The RFC content of a well eared maize silage crop is near 30%.

As explained in the introduction to Chapter 1 and 2, and repeated briefly here, the consequences of insufficient RFC and excessive CP become apparent in the rumen where rumen bacteria require fermentable energy to "capture" the high levels of rumen degradable protein (RDP) present in pasture. Pasture RDP is broken down to ammonia

in the rumen before being reassimilated into bacterial protein which is then digested and absorbed in the small intestine. Excess ammonia that is not captured is absorbed through the rumen wall into the bloodstream and is converted to urea in the liver, which is then mainly excreted in the urine (Danfaer *et al.*, 1980; Beever, 1993; Nolan, 1993; Moore and Varga, 1996). This is an energy expensive process (particularly conversion from ammonia to urea) (Oldham, 1984). Each 100 g of excess protein above requirements costs the cow near 1.3 MJ of ME (Moore and Varga, 1996). This energy is then not available for production or is removed from body energy reserves (especially fat). There is some evidence also that one of the ammonia molecules required to form the urea must come from circulating amino acid or presumably body tissue - both can not come from excess ammonia absorbed (Lobley *et al.*, 1995). This may also add to the catabolic effect of excess dietary CP. Reduced conception rates have been noted in many studies where excess RDP causes elevations in blood or milk urea levels in cows (Jordan and Swanson, 1979; Ropstad and Refsdahl, 1987; Ferguson *et al.*, 1988; Gustaffson, 1993), but not in all. Addition of maize silage to pasture diet will reduce dietary protein closer to recommended levels, as maize silage contains only 7-8% protein (NRC, 1989).

The "effective" fibre ("scratch factor") content of fresh pasture has received more attention recently (Muller *et al.*, 1995; Kolver, 1996; Mertens, 1996). It is felt in some quarters that the elasticity of fresh pasture is too high and it does not stimulate good rumen function, salivation and mat formation in the rumen. This is borne out by the observation that relatively low % of cows are ruminating at any one time when grazing fresh pasture. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) levels appear adequate in pasture (NRC, 1989), but effective fibre may not be. The moisture content of pasture can be 90%, and is frequently near 85%. Clear evidence is lacking, but restriction in potential intake of DM seems likely in pasture due to moisture content (John and Ulyatt, 1987; Ulyatt and Waghorn, 1993). DM intakes of 3.5-4% of body weight are often regarded as maximum on ryegrass/clover pasture only diets, yet 4-5% is frequently achieved on totally mixed rations (TMR) (Hutjens, 1995). Maize silage contains near 25% ADF and hence does not alter dietary ADF significantly when added to a pasture diet. It may not perform any significant "scratch factor" effect either, as it is chopped fine to allow compaction in the ensiling process.

Supplementation trials with concentrates added to the pasture diet have typically yielded considerably less than 1 litre of milk per kg of supplement, yet theoretically 2 litre per kg of supplement should be possible if no energy goes to weight gain (Muller, 1993; Lean *et al.*, 1995). Reasons for poor results could reflect deficiencies in design of trials which are often short term and do not take account of weight gain or production responses after the supplementation period (Lean *et al.*, 1995). Dietary balance has often not been considered and concentrate simply regarded as extra dry matter offered - exceptions are those cited by Wilson (Wilson and Moller, 1993), Kellaway and Porta (1993) and Salam *et al.* (1996). Another factor in supplement responses will be "substitution" where less pasture DM is frequently consumed when concentrate is added to the diet (Grainger and Matthews, 1989; Carruthers and Penno, 1995). Substitution occurs with all supplements and is greater at higher intakes. Some concentrates will show more substitution than others. In particular, addition of bypass protein sources to the concentrate may reduce the extent of substitution (Kellaway and Porta, 1993). Supplements that balance the diet closer to that recommended are likely to lead to increased DM intake (Hutjens and Sapienza, 1993; Lean *et al.*, 1995) and consequently reduced substitution. At times, however, substitution can be used to advantage to avoid overgrazing of pasture, thus enhancing the pasture recovery through leaving residuals at higher levels that will encourage faster regrowth (Brougham, 1956, 1958, 1959). If substitution results in available pasture not being consumed and then senescing and decaying, then supplementation is simply replacing a less expensive food (pasture) with a more expensive food (concentrate) and is unlikely to be economic.

Dairy nutrition specialists overseas refer to the "1 in 200" rule where every litre higher peak yield per cow achieved translates into the opportunity for 200 litres more production from the same cow over the rest of the lactation (Broster and Broster, 1984). There is argument that this does not apply in New Zealand because cows often encounter a dry summer and are underfed at this stage so production then reduces to a base level and does not recover. To some extent this is true, in that a period of underfeeding will shorten opportunity for a carryover effect, and this can easily occur in a pasture only dairy system with daily variation in quantity and quality of pasture.

Dairy cows in the seasonal calving system practised in New Zealand are usually reaching peak production in October, just at the stage that perennial ryegrass is reaching its reproductive stage in response to rising temperature and increasing daylength. Reduction in digestibility and consequently potential DM intake is inevitable because more than 50% of growth in the reproductive stage is cell wall (NDF) and temperature is the main instigator (Buxton and Fales, 1994; Nelson and Moser, 1994; Chapter 2 in this thesis). Higher percentages of clover in mixed ryegrass/clover swards will help maintain digestibility and potential intake. Reduction of per cow production off peak will often exceed 10% per month (LIC, undated) whereas 2-3% is achievable on controlled diets like TMR (Muller, 1993; Mahanna, undated).

The characteristics of a recommended early lactation TMR diet could be compared with a typical pasture diet or pasture/maize silage diet as follows (Table 4.1).

Table 4.1 Differences in pasture, pasture/maize silage and recommended early lactation diets[1].

	Typical Spring Pasture Diet	Pasture 65% / Maize silage 35% Diet	Recommended Early Lactation Diet*
Crude Protein%	25-30%	18-19%	18-20%
UDP% (% of C.P.)	20%	21%	30-35%
RDP% (% of C.P.)	80%	79%	60-65%
NSC (non structural carbohydrate)%	14-20%	20-25%	30%+
Sol. Carbohydrates%	10-15%	16-20%	30-35%
Fat%	4%	3.9%	7% max
NDF%	32-35%	38%	>28% but < 45%
ADF%	20-24%	25%	>20%
Dry Matter%	15%	21%	>20%
Metabolisable Energy (MJ of ME/kg DM)	>11.5	>11.3	>11
Magnesium %	0.18%	0.18%	>0.2%
Calcium %	0.5%	0.45%	>0.4%
Phosphorus %	0.3-0.4%	0.33%	>0.25%
Sodium %	0.15%	0.11%	>0.15%
Potassium %	3%	2.3%	>1%

[1]Sources include NRC (1989), Muller (1993), Hutjens (1995), Spartan dairy ration balancer and other chapters in this thesis. UDP=rumen undegraded protein, RDP=rumen degraded protein, NDF=neutral detergent fibre, ADF=acid detergent fibre, NSC=non-structural carbohydrate (= 100 - (NDF + CP + Ash + Fat)).

Maize silage addition to the fresh pasture diet will reduce dietary protein, increase starch, increase DM%, increase ADF slightly, and reduce dietary K, Na, P, Ca and Mg.

It was decided to test the responses of dairy cows in commercial herds consuming pasture plus maize silage to a more "balanced" ration at peak lactation. Permission was obtained from the manufacturers to trial a newly formulated concentrate ration ("NRM Maize Silage Balancer") which would address most of the apparent deficiencies in the pasture/maize silage diet. The concentrate contained increased amounts of UDP, fat, calcium, magnesium and phosphorus as well as a high ME value (13.5 MJ of ME /kg DM).

4.2 METHODS

The trial was designed to test the response in milk production and herd fertility to achieving dietary balance in cows consuming a mix of pasture and maize silage. Cows in both groups were offered the same amount of metabolisable energy and dry matter for a 6 week period in spring. The only difference was in the nutrients supplied in the "balancer" concentrate.

A commercial dairy herd of 240 high BI (Breeding Index 130) Jersey cows was split into two equal groups on 5th August 1993 based on odd and even tag numbers. The herd was located in the south Waikato district. Cows were in good condition for calving - condition score 4.8 (LIC scale 1-10). The farmer was chosen for his willingness to participate, for his ability to carry out the measurements required and possession of a high skill level in managing dairy pasture. The trial design is illustrated in Fig 4.1 and dietary differences in Table 4.2.

Figure 4.1 Trial Design.

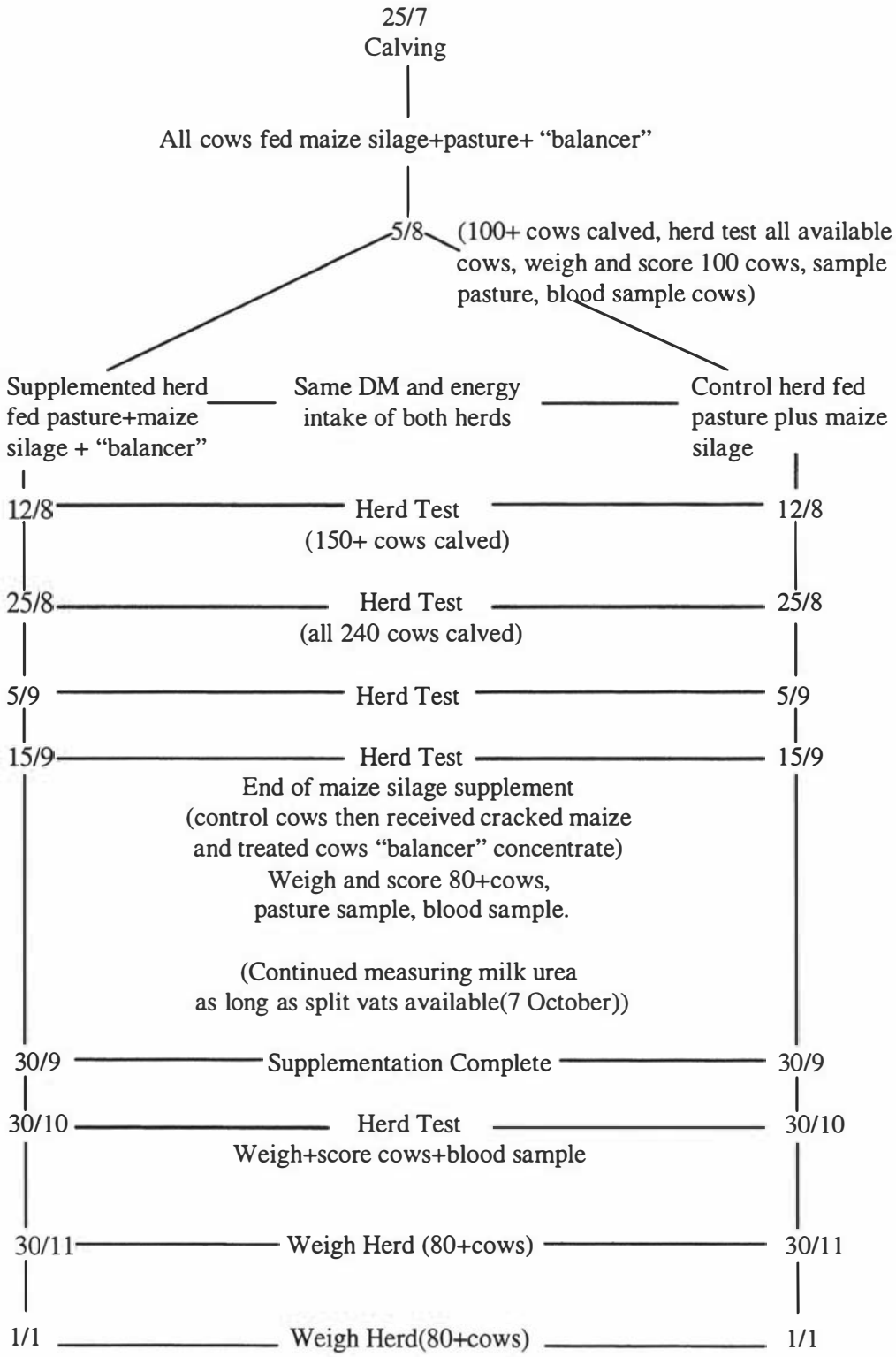


Table 4.2 "Assessed" diets of supplemented and control herds.

	Supplemented herd (ME value in brackets)	Control herd (ME value in brackets)
Maize silage (analysis: ME10.4MJ/kgDM,CP 6.5%, Dry matter 35%, UDP%of CP50% (assumed))	3 kg DM (31.2 MJ)	4.5 kg DM (46.8 MJ)
Pasture (analysis: ME11.5MJ/kgDM,CP 23%, Dry matter 15%, UDP% of CP 20% (assumed))	10 kg DM (115 MJ)	10 kg DM (115 MJ)
Concentrate (analysis: ME13.5MJ/kgDM, CP24%, Dry matter 85%, UDP60% (assumed))	1.3 kg DM (17. 5MJ)	-
Total Dry Matter offered to cows/day (Total ME offered to cows/day)	14.3 kg DM (163.7 MJ)	14.5 kg DM (161.8 MJ)
Crude Protein% in diet	19.6%	17.9%
Undegraded Protein% in diet (est[1])	5.2%	4.2%
Crude Fibre in diet (est[1])	21%	22%
Soluble Carbohydrate, Starch and Pectin in diet (est[1])	20%	17.6%
Dry Matter% of diet	26%	19%

[1]These diets are based on the analyses available and where no value was available, standard values from NRC or other sources. Commercial feed testing laboratories analysed the pasture, maize silage and concentrate. The concentrate contained additional calcium, phosphorus, sodium and magnesium.

Both herds received supplementary magnesium oxide drenched each morning in the dairy shed (25 g MgO).

Even numbered tag cows became the supplemented group and the odd numbered tag cows the control group. From the calving start date of 25 July maize silage (3 kg) and "balancing" concentrate (1 kg) were fed with pasture to allow rumen microflora adjustment to the trial diet.

The farm was split into two farmlets based on paddock numbers (odd and even), so that one group grazed even numbered paddocks and the other group grazed only odd numbered paddocks. This split occurred on 5 August. The cows were milked in the same sequence every day.

Diets were designed to be isoenergetic, with approximately 10 kg DM pasture intake to both groups. This was assessed using a capacitance meter. The control group were fed 4.5 kg DM maize silage and 10 kg DM pasture from 5 August till 13 September. From 13 September till 30 September the cows were fed 2 kg DM cracked maize plus pasture only, because there was no maize silage left by the 13 September, and the cracked maize grain was chosen because it was the closest dietary ingredient available commercially, and this was fed till the end of the trial supplement period on 30 September. The supplemented group were fed 3 kg DM maize silage and 10 kg DM pasture. In addition they were fed with the maize silage 1.3 kg DM "balancer" concentrate from 5 August to 13 September. From 13 September till 30 September the cows were fed pasture and 2 kg "balancer" concentrate only, as the maize silage available was all used by this time. Supplements were fed in the paddock using a side-delivery silage wagon. Wastage was assessed as minimal (< 5%).

Two pasture samples were collected from the farm and analysed for pasture nutrients at commercial laboratories (one at the start of the trial and the other in mid-September). The maize silage was sampled and analysed for nutrient content only once. The analysis of nutrients in the "balancing' concentrate was provided by the company manufacturing the product.

Average farm pasture cover was measured at the start of the trial on 3 August, again on 19 August and again on 20 September. This was done using a capacitance meter and measuring each paddock on the farm. All paddocks were measured, but unfortunately all individual paddock records were not retained, making statistical comparison of pasture cover data impossible.

Plate 4.1 Maize silage with concentrate being fed in paddock along fenceline.

Cows from each group were weighed on five occasions using electronic scales, twice during the supplementation period and on three later occasions. At least 40 cows in each group were weighed on each occasion. At the first three weighings condition score was also assessed.

Blood samples were collected from the same ten cows between 9.00-11.00 am in each group on three occasions (5 August, 17 September and 2 November) and analysed for serum albumin (Alb), serum beta-hydroxybutyrate (BOH), serum magnesium (Mg), selenium (Se) and ferroxidase (Fx). These tests were performed to detect change due to plane of nutrition and to check on the possibility that trace element deficiencies were interfering with the trial (see Appendix 1 for detail on test procedures).

Milk was placed in separate vats for each treatment group. This involved a short delay between milking of each group as the milking machines were emptied of milk before

the next group could be milked. The daily milk dockets provided one measure of differences in productivity between the two treatment groups. These milk production figures could not be tested directly for significance because individual cow production was not measured, but the daily difference between groups was analysed using Flexi 2.2 (Upsdell and Wheeler, 1992). The differences between herds were significant at the 5% level where the 83% confidence bands did not overlap for the two herds. This 83% confidence band was chosen to allow pictorial representation of significance at $P < 0.05$. Six whole herd milk production tests were performed on individual cows by the Livestock Improvement Corporation Herd Testing Service. These results were also used and presented in Figure 4.3.

Milk samples were collected daily or every second day from each vat and analysed for milk urea levels. This was done to monitor the likely excess rumen degraded protein in the diet in both groups (Moore and Varga, 1996).

Reproductive analysis was performed using the Dairyman (Hayes and Morris, 1996) programme for each group at the end of the mating season. This was done to detect any differences in submission rate, conception rate and "empty" rate.

Condition scores were assessed as recommended by the Livestock Improvement Corporation Consulting Officers network (scale 1-10).

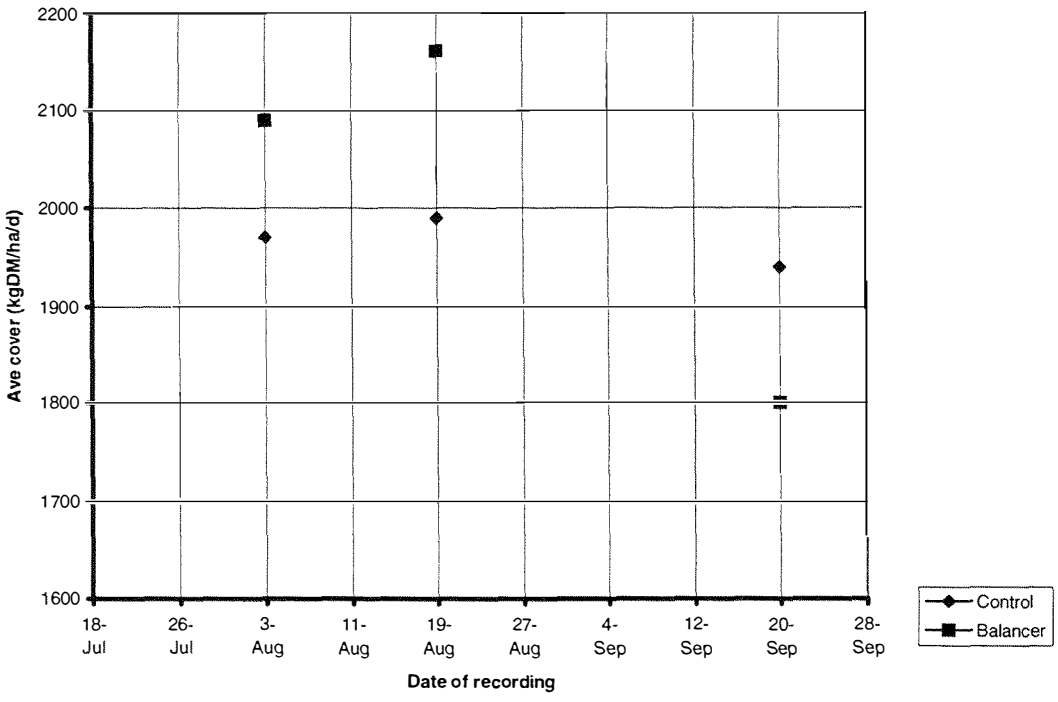
The trial design was modified slightly on 13 September because no maize silage remained and pasture cover was low. This could potentially have negated any possible carryover effects on production which the trial was designed to detect, so additional concentrate was added to the diet of both groups. In the case of the control group this was 2 kg cracked maize grain in place of the maize silage from 13 September till 30 September plus pasture. After 30 September the cows remained on their separate farmlets but were fed only pasture. The herds remained separate till 30 October and were then joined into one herd again.

4.3 RESULTS

Pasture cover averages are presented in Figure 4.2 for the two farmlets. All paddocks on the farm were measured.

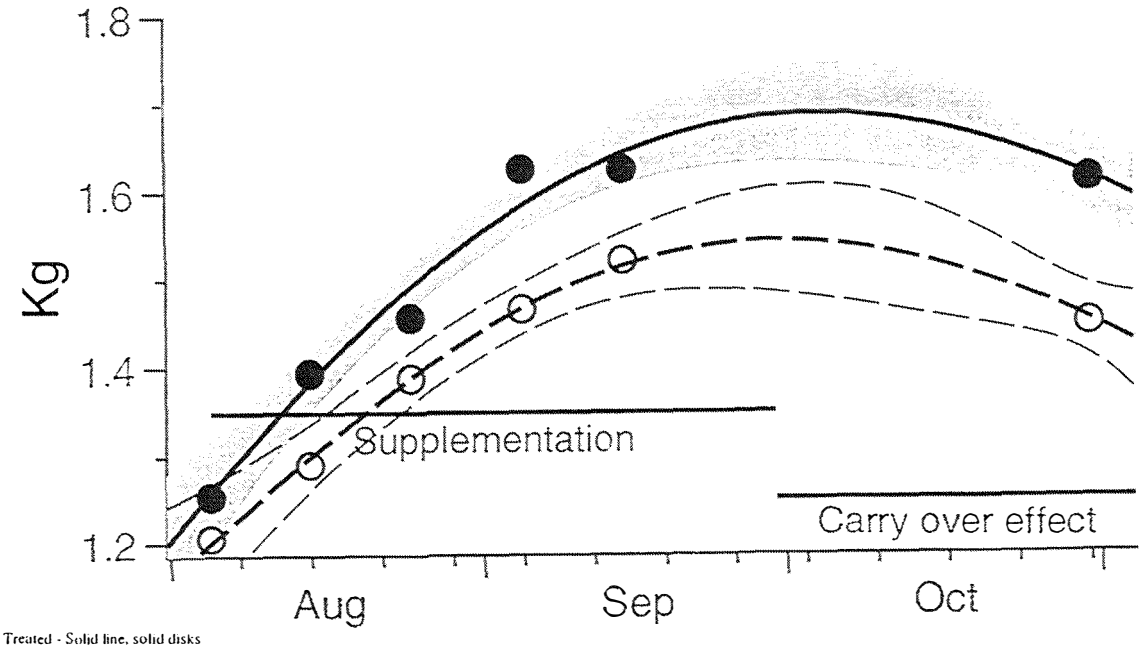
Average cover was higher in the supplemented farmlet at the start of the trial simply because of the selection method for paddocks (odd and even paddock numbers), but by the end of the trial was lower than the control farmlet. Daily post-grazing residual measurements confirmed that the supplemented cows were consuming more pasture. This increased consumption meant the supplemented herd had to go onto a faster rotation midway through the supplementation period to avoid being underfed on pasture, which was being maintained at near 10 kg DM intake per cow per day.

Figure 4.2 Average Pasture Cover for each farmlet.



Milk production responses are illustrated in Figure 4.3, where daily milksolids per cow are presented graphically through time. Both herd test data (points) and daily dairy company production results (smoothed line) are presented in the same figure. Statistical significance at the 5% level is shown where the 83% confidence bands do not overlap on the graph. Statistical smoothing using Flexi 2.0 was used (Upsdell, 1994).

Figure 4.3 Milk solids per cow per day.



Treated - Solid line, solid disks
Control - Dashed line, hollow circles
Shaded, dashed regions are the 83% confidence intervals for treated, control herds.
Treatments are significantly different at the 5% level where they do not overlap.

A significant milk production response occurred during the supplementation period and there was a large and significant "carryover" effect after the period of supplementation.

The weights measured in both groups are represented in Figure 4.4 and Table 4.3. Only a mean value was available for the last two weighings. A t-test did not reveal any differences between the groups at 5 or 10% significance at any weighings. Both herds gained weight during the trial period and afterwards as well. Although not statistically significant, the supplemented cows appeared to be gaining less weight during the latter part of the supplement period.

Figure 4.4 Liveweight change in the two trial herds.

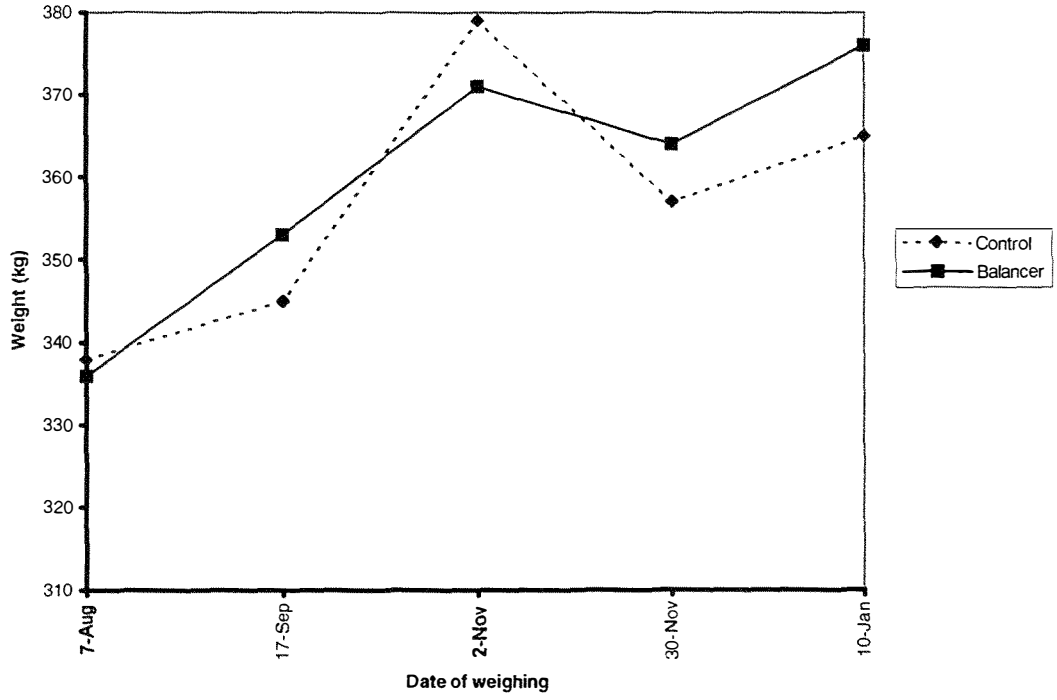


Table 4.3 Average weights (kg) and condition scores of a sample of 50 cows from each herd.

	Control herd liveweight (kg)	Control herd condition score	Supplemented herd liveweight (kg)	Supplemented herd condition score
7th August 93	336(SEM5.09)	4.6	338(SEM8.2)	4.7
17th September 93	353(SEM7.61)	4.8	345(SEM7.13)	4.8
2nd November 93	371(SEM7.16)	5.0	379(SEM7.67)	5.0
30th November 93	357[1]	-	364[1]	-
10th January 93	365[1]	-	376[1]	-

[1]Only mean values available

Reproductive performance of the two herds is presented in Table 4.4.

Table 4.4 Reproductive performance in supplemented and control groups.

	Control Herd	Supplemented herd
Submission Rate to AB after 4 weeks	95%	94%
Non-return rate 42 days	59%	59%
"Empty cows" at end of season	7/120(5.8%), P< 0.1	3/120(2.5%)

Submission rates to AB (Artificial Breeding) at 4 weeks into mating were almost identical and non-return rates after 42 days of mating were identical. "Empty" cow percentages tended to be lower in the supplemented cows ($P < 0.1$). Statistical analysis was done using a generalised linear model with binomial error function logit link.

Blood analysis results are presented in Table 4.5.

Selenium (Gpx), magnesium (Mg) and copper (Fx) levels were well within acceptable ranges for healthy dairy cows and are not considered to have influenced the trial results. Albumin levels were high at the start of the trial, fell initially then increased during the trial. There is again a suggestion in the albumin levels (as with the liveweights: Table 4.3) that more tissue mobilisation occurred in the supplemented group during the supplement period and that this more than compensated after supplementation was complete when weight gain increased at a faster rate in the supplemented group. These changes were small, however and T-Tests indicated no significant difference.

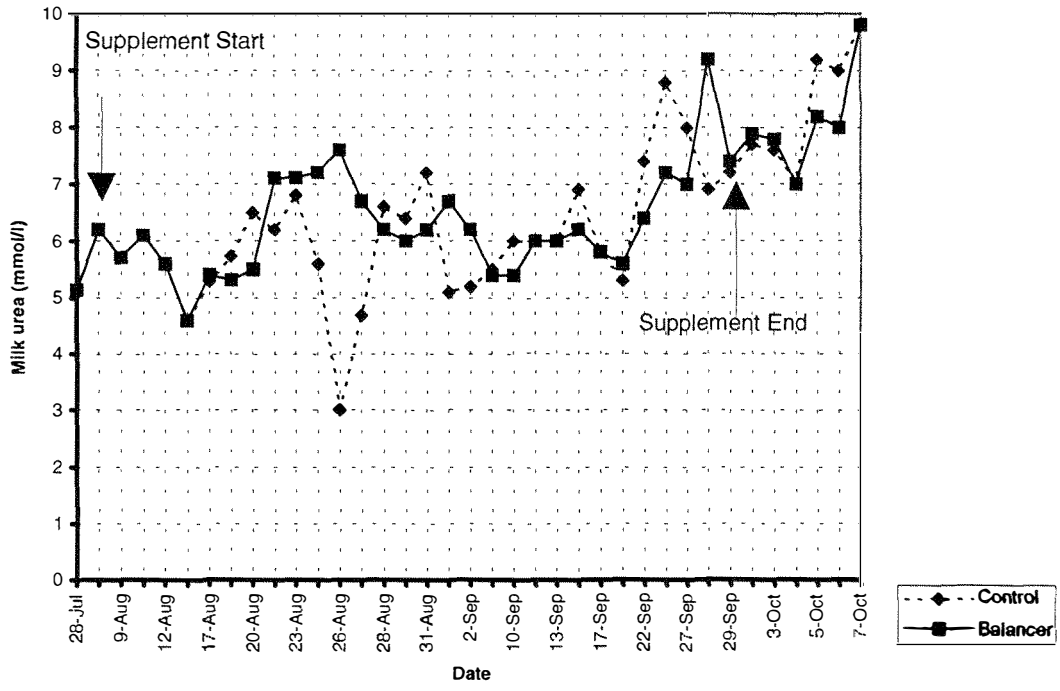
Table 4.5 Blood parameters in supplemented and control groups on 3 consecutive samplings from the same cows (Std).

		17 Aug 93	17 Sep 93	2 Nov 93
Albumin (g/l)	Control (N = 10)	33.2 (std 2.37)	32.5 (std 2.84)	33.3 (std 3.08)
	Supplemented (N = 10)	34.4 (std 2.55)	33.2 (std 1.81)	33.8 (std 2.44)
BOH (mmol/l)	Control (N = 10)	0.39 (std 0.10)	0.35 (std 0.05)	0.49 (std 0.11)
	Supplemented (N = 10)	0.42 (std 0.06)	0.36 (std 0.05)	0.53 (std 0.11)
Magnesium (mmol/l)	Control (N = 10)	-	0.89 (std 0.18)	0.78 (std 0.20)
	Supplemented (N = 10)	-	0.84 (std 0.16)	0.71 (std 0.14)
Selenium(nm/l)	Control (N = 3)	866 (std 30)	1002 (std 69)	710 (std 77)
	Supplemented (N = 3)	670 (std 144)	1025 (std 95)	796 (std 66)
Ferroxidase (U/l @ 37oC)	Control (N = 4)	29.2 (std 6.5)	28.25 (std 3.6)	28.2 (std 5.2)
	Supplemented (N = 4)	36.8 (std 4.4)	27.75 (std 6.3)	27.4 (std 4.5)

Ketone levels (BOH) were relatively low in all measurements and no differences between groups were noted with a T-test.

Milk urea levels are presented in Figure 4.5 for the supplemented and control groups. No significant differences between groups were seen. The short period of decline in the supplemented herd in late August is unexplained. A steady elevation in urea levels in both groups can be seen from early September in both groups.

Figure 4.5 Milk urea levels in supplemented and control herds before during and after the supplementation period.



4.4 DISCUSSION AND CONCLUSIONS

A significant milk production response occurred in this trial (Figure 4.3). The supplemented group of cows and the control group were offered the same amount of metabolisable energy during the supplement period as far as it was possible to achieve on grazed pasture. The milk production response appears to have come from increased appetite and possibly increased tissue mobilisation (Fig. 4.2 and Fig. 4.4). This can be seen from the reduction in pasture cover that occurred on the supplement farmlet compared to the control farmlet. Unfortunately individual paddock measurements were not retained so standard errors could not be estimated.

This increased appetite necessitated a change in experimental design because the supplemented cows were leaving lower residual levels after grazing. When the first

grazing rotation was completed part way through the trial insufficient pasture was available on the supplemented farmlet to feed the cows fully. It was decided to shorten the rotation length for this herd in an attempt to maintain similar amounts of pasture offered to both groups. This action was considered justifiable as rapid pasture growth could be anticipated through warmer spring weather.

Although the measurements of weight differences between the two groups and serum albumin levels between the two groups are not statistically significant, they appear to suggest that increased tissue mobilisation was occurring in the supplemented group. Increased appetite and increased tissue mobilisation are both known to occur with supplementation of cows with increased amounts of UDP (Kellaway and Porta, 1993). The large "carryover" effect on milk production after supplementation was complete is consistent with nutritional principles of feeding dairy cows (the "1 in 200" rule) (Fig. 4.3).

The supplemented group produced 100 kg milk solids more per hectare than the control group by the end of the milking season despite the two herds being joined together again soon after the completion of the spring trial period. This equates approximately to the "1 in 200" response. This response would be less likely to occur if the cows were not well fed post-peak, ie. a dry summer could potentially reduce this response if cows were fed grass or conserved grass only at less than optimal rates. The higher urea levels in both control and supplemented groups in October probably indicate increased DM intakes and hence higher urea levels due to increased excess rumen degraded protein in the rumen (Fig. 4.5). This is also probably the reason that the treated herd had slightly higher urea levels as their intake was increased (Moore and Varga, 1996; Trevaskis, 1996).

At the time of mating to AB (Artificial Breeding) there were no differences in commonly measured reproductive performance parameters between groups. Submission rates and non return rates were almost identical. Cows not pregnant at the end of the mating season (mating finished mid-January 1994) were significantly different at the 10% level, with less empty cows in the supplemented herd. The results for both herds were well above the national average, which is 7% of cows empty each year in seasonal

calving herds. The reason why the number of empty cows is less in the supplemented group is unclear, but may relate to the increased weight gain noted in the post supplementation period. This could be expected to improve conception rates (non return rates) because the weight gain was occurring during the mating period in October/November and a rising plane of nutrition is thought to favour fertility (Butler and Smith, 1989). Increased appetite again appears to be the reason why this has occurred because these cows were producing better also. Milk urea levels were not measured during mating, because it was not possible to separate the milk from each herd at this stage of the trial. High urea levels have been shown in some studies to reduce conception rates (Jordan and Swanson, 1979; Ropstad and Refsdahl, 1987; Ferguson *et al.*, 1988; Gustafsson, 1993). These may have been involved here, but samples up till just before AB start showed higher urea levels in the supplemented group which had higher fertility.

The increases in body weight that occurred in both herds in August, September and October would probably not be considered normal at this time of the year on dairy farms, as cows would be expected to mobilise tissue post calving (Muller, 1993; Hutjens, 1995). The weight increases indicate good levels of intake and feeding in excess of requirements for maintenance and production. They also suggest that further productivity gains are possible because full mobilisation is not occurring early in lactation. Similar weight gains in spring were observed by Salam *et al.* (1996). It could be argued that the increased weight measured from July till October was not in fact weight gain but increased gut fill. The increased milk urea levels in September/October suggest increased DM intake in both groups, and since the diet was mainly pasture (which has only 15% dry matter) this seems a strong possibility.

Blood parameters measured did not indicate any significant differences between the two groups. Levels for selenium, copper, magnesium, ketones (BOH), and albumin were all within normal reference ranges for dairy cattle and none are thought to have influenced the responses seen.

Milk urea levels measured from the separate vats were almost the same for each herd until mid September, when the supplemented group developed slightly higher levels.

These differences continued past the end of September when supplementation was completed, suggesting that the differences related to differences in the type of pasture grazed or higher DM intakes of a high CP diet (Oltner and Wiktorrson, 1983; Kirchgessner *et al.*, 1986; Semptey and De Vischer, undated). Milk urea levels are known to reflect the degree of excess rumen degraded protein (RDP) present in the cows diet (Fulkerson, 1996; Moore and Varga, 1996). Cows in the supplemented herd would have been grazing shorter, less mature pasture after the first few weeks of the trial, which could be expected to have a higher level of crude protein (Holmes, 1989). The rise in milk urea levels in mid-September in both herds reflects both increased intake of a diet that contains excess protein above requirements and could also indicate shorter, more rapidly growing pasture with higher CP levels and lower SOLCHO levels. No effects on fertility were noted from milk urea levels well above 7 mmol/l in October.

The milk production responses seen in this trial occurred with no additional metabolisable energy offered to the cows as supplement, but appear to have come from an increased consumption of pasture. The changes in pasture cover from 19 August to 20 September would suggest that approximately 400 kg DM extra pasture was eaten by the supplemented herd daily. By calculation, this would equate to 3.3 kg DM extra per cow daily. This has produced an immediate response in milk production of approximately 0.15 kg milk solids per cow. This level of response does not account fully for the extra pasture that has apparently been eaten, ie. approximately 40 MJ of ME extra apparently eaten as pasture but only milk with an ME value of 12 MJ approximately produced. Whilst there could be errors in the method of pasture measurement (capacitance meter), it seems unlikely that this could account for all the discrepancy of ME apparently used for production.

The milk production response has also occurred because of a difference in the type of nutrients offered between the two groups which has altered cow appetite, because dry matter offered and ME offered were similar in both groups. In retrospect, more pasture samples would have improved the reliability of dietary balance assessments. Whilst the amount of soluble carbohydrate offered in the supplemented herd would have increased slightly and theoretically this could increase the production of bacterial protein in the rumen and enhance production by increasing the absorption of digested bacterial protein

in the small intestine, this seems unlikely. The milk urea levels were not different in the two groups initially, which indicates that excess RDP levels were probably similar. This would suggest that similar bacterial growths in the rumen occurred on both diets. Fibre levels in the supplemented herd would have been slightly lower than in the control herd diet, but both levels were close to ideal for early lactation and are not considered to have influenced the results. Crude protein levels in the diet were higher in the supplemented herd, but both levels were close to the recommended levels in NRC and were not considered to have influenced the results.

Maize silage is known to be relatively short of methionine and lysine for optimal milk production, especially lysine (Schwabb, 1993; Rulquin and Verite, 1993; Broderick, 1994). Pasture is thought to be marginal in methionine levels for optimal production. These two amino acids are likely to be the most production limiting on this type of diet, particularly at higher production levels. Increased supply of these amino acids as UDP, in particular, is likely to enhance appetite because a balanced diet close to the cow's requirements will lead to increased appetite (Hutjens, 1995; Lean *et al.*, 1995). This appears to be the most likely reason why increased production has occurred in this trial, because the concentrate used in this trial contained enhanced levels of these amino acids (I. Lean, pers. comm.).

This trial has shown no "substitution" effect to the "balancing" concentrate, and in fact the converse appears to have occurred. Supplemented cows consumed more pasture, as evidenced by the lower average pasture cover in the supplemented herd and milk production levels. A suggested mechanism for this enhanced appetite is that the concentrate provided a production limiting nutrient to the udder (possibly lysine), which then produced more milk. The increased milk production then created a demand for increased DM consumption, and the cow consumed more if it was available. This is consistent with the low substitution levels reported in supplement trials where more UDP is supplied to the diet (Kellaway and Porta, 1993). In future, close attention to the formulation of the concentrates used may provide the opportunity for enhanced production from pasture supplemented with maize silage and concentrates without reduced consumption of pasture occurring.

Further trials are needed to confirm some of the findings in this trial, because they could have significance for potential milk production from pasture/maize silage based diets in New Zealand. The observation that supplemented cows consumed more pasture needs to be repeated, because higher DM intakes from the same cow would benefit our dairy system in that increased production per hectare would be possible without increasing the maintenance requirement, ie. the same number of cows could produce more, or less cows could produce the same amount of milk. Precise measurement of intake of cows grazing pasture is notoriously difficult. The trial could be criticised for lack of precision in the area of DM intake estimates, but supporting information from blood chemistry, weights and production tend to confirm the findings of increased appetite in the "balanced" herd. Chromium or alkane markers used on selected cows or stall feeding cows cut pasture would be needed to measure cow intakes more accurately. Indoor trials may distort "substitution" effects, however.

On the basis of these results, it appears that improved responses to supplementation of dairy cows on pasture are possible if attention is paid to the "balance" of dietary ingredients. Whilst maize silage supplementation of pasture addresses many of the balance deficiencies in a pasture only diet (Sapienza, 1993), addition of further nutrients has provided additional production improvement.

ACKNOWLEDGEMENTS

This trial was performed with the assistance of several people. In particular, the farm sharemilkers M. and K. Jamieson, and B.J. Mackay assisted with weighing and condition scoring, downloads of LIC milk production data from the DairyMan computer programme and some of the calculations. The NZ Dairy Group assisted with provision of a second vat so that separate sampling could occur. Dr M. Upsdell, Statistician, AgResearch Ruakura assisted with statistical analysis and preparation of some of the graphics.

SECTION 3

Factors affecting pasture nutrient composition were examined in this section. The final chapter then combines the findings in the various chapters into an integrating discussion with concluding comments.

Chapter 5

Nutrient changes in pasture as it matures and after grazing

Changes in pasture nutrients after grazing and with maturation were studied using caged and grazed sites on two dairy farms.

Chapter 6

Effects of nitrogen fertiliser (urea) on pasture nutrient composition - results of a replicated split plot trial

The effect of different rates of nitrogen application and the timing of these applications in the winter/spring period on nutrient composition of pasture were examined.

Chapter 7

Final Discussion

This chapter combines the results presented in the previous chapters, discusses the significance of the results and makes concluding comments.

CHAPTER 5

Nutrient changes in pasture as it matures and after grazing

PREFACE

This chapter is an initial evaluation of variation in pasture nutrients with maturity and grazing. Lack of replication and standardisation of sites precludes any formal statistical assessment of the findings. These data have been presented because little comparable information on New Zealand pasture exists and it helps with background information on aspects of the variation in pasture nutrients of relevance to the thesis.

This first chapter in Section 3 of the thesis returns to develop findings presented in Section 1. Factors influencing changes in pasture nutrient levels, particularly pasture protein (CP) and soluble carbohydrate (SOLCHO) needed clarification. Pasture management factors are likely to have played a large part in the differences between farms for pasture CP and SOLCHO presented in Chapter 1 because environmental conditions were similar on all farms as they were located near each other. If milk production is depressed by high dietary CP and low SOLCHO, then factors influencing these nutrients needed identification. The influences of grazing and maturation on pasture nutrients do not appear to be especially well defined. The purpose of the study presented here was to identify nutrient changes in dairy pasture after grazing and maturation.

5.1 INTRODUCTION

Dairy cows require more than just dry matter or metabolisable energy (ME) to produce milk. Their requirements can be further broken down to protein, amino acids, acid

detergent fibre (ADF), neutral detergent fibre (NDF), readily fermentable carbohydrates, phosphorus, calcium, magnesium, sodium, potassium etc as outlined in nutrient requirement texts like NRC (1989). Pasture is a variable nutrient source, supplying different amounts of the above nutrients depending on season, environment and how it is managed. If pasture and stored pasture (hay and silage) are virtually the sole diet as in the dairy system practised here, then changes in pasture nutrients are obviously important and likely to impact on performance if the levels fall outside nutrient guidelines for their current level of productivity (see Chapter 2, Section 2.1).

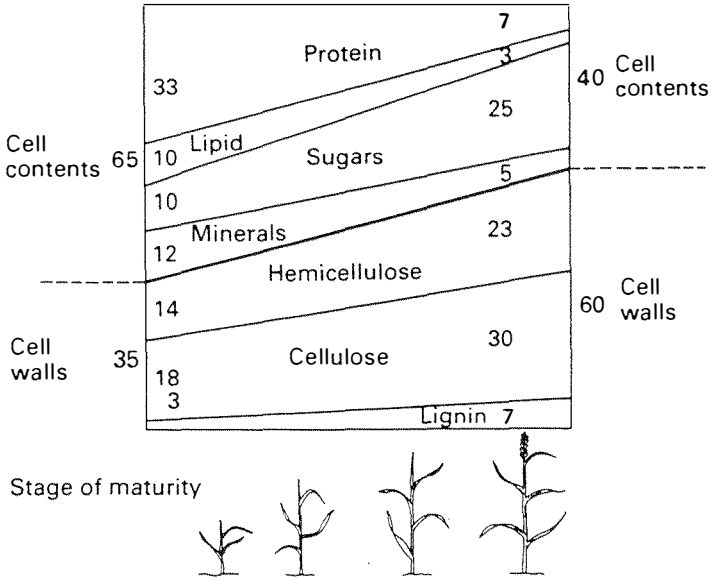
Digestibility is still the most frequently used measure of forage nutritive value (Reid, 1994). The composition and availability of the diet are major factors influencing voluntary intake. While the concentration of protein, the balance of amino acids and deficiency or excess of minerals can all affect intake, the major parameter of a food which determines the amount eaten is the concentration of available energy (Forbes, 1986). Lower digestibility forage fills the gut more and rate of passage of residues is slower. Pasture quality is a function of both forage intake and digestibility (Paterson *et al.*, 1994) and is affected by the environment for growth and by maturation (Nelson and Moser, 1994).

Solar radiation is the driving force for the upper limit to pasture productivity with temperature and rainfall the major modulators at each site (Nelson and Moser, 1994). Cool season grasses like ryegrass are induced to flower by lengthening days and the flowering stage is characterised by over 50% stem growth as a percentage of total growth. Temperature is the dominant environmental factor affecting pasture quality, with radiation less so (Henderson and Robinson, 1982; Buxton and Fales, 1994). According to Buxton and Fales (1994), plant maturity impacts the most on pasture quality but plant environment modifies the impact of plant maturity.

The progressive changes in nutrients present as pasture matures and after grazing have been described by Holmes (1989), Fulkerson (1994), Fulkerson *et al.* (1994), Fulkerson and Slack (1995), Fulkerson (1996) and Doyle *et al.* (undated). The changes are represented diagramatically in Figure 5.1 (after Holmes, 1989) and Figures 5.2 and 5.3 (after Fulkerson, 1996). Essentially cell contents decrease and cell wall increases as a

percentage of the plant. This means CP declines and neutral detergent fibre (NDF) increases as pasture matures. Grazing results in high CP and low SOLCHO initially and these levels then converge 12-20 days after grazing (see Figure5.2). Changes noted by Fulkerson in Australian studies of ryegrass (Figure 5.2) may not necessarily occur in local growing conditions and soil types.

Figure 5.1 Effects of stage of maturity (days since grazing) on pasture composition during spring (after Holmes, 1989).



Maturation effects on digestibility %, protein % and NDF % of ryegrass pasture varied with season in studies cited by Doyle *et al.* (undated) in northern Victoria with relatively constant digestibility and NDF until late spring for 6-8 weeks after grazing, but 5% declines in CP over the same 6-8 weeks. Grainger (1992) found a 0.6% digestibility decline per 1000 kg DM increase in pasture mass in Victorian pastures and Holmes (1987) found nearer 0.8% in New Zealand spring pastures.

Figure 5.2 Change in pasture nutrients after grazing (after Fulkerson, 1996).

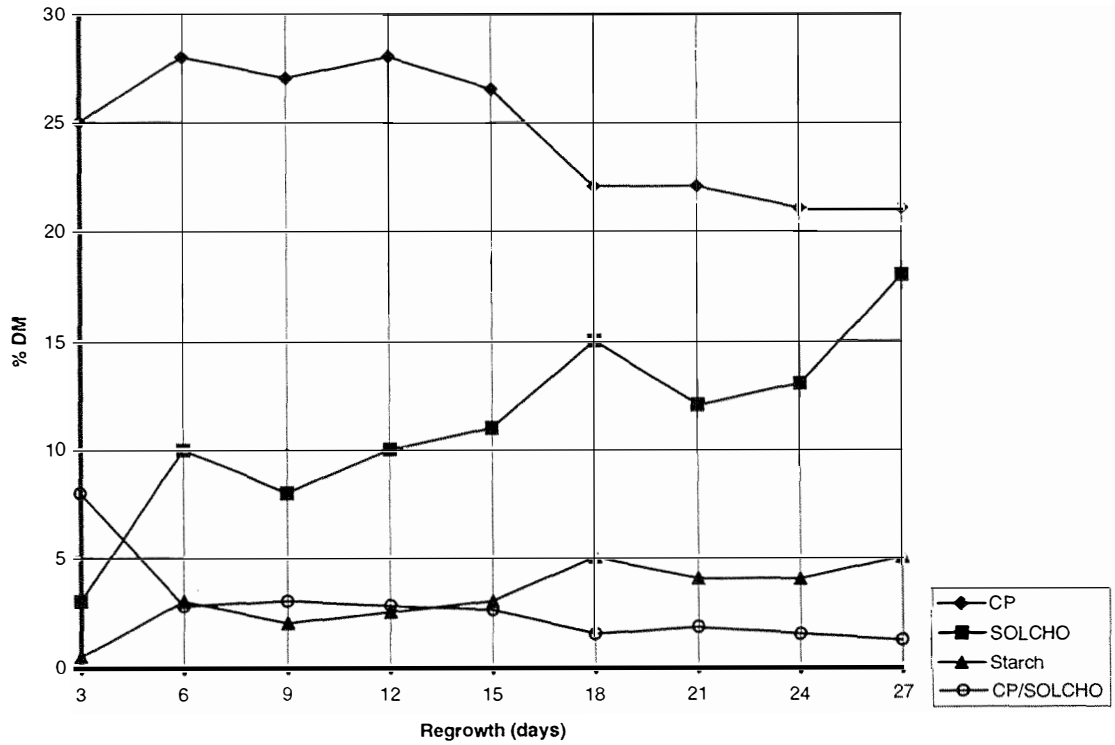
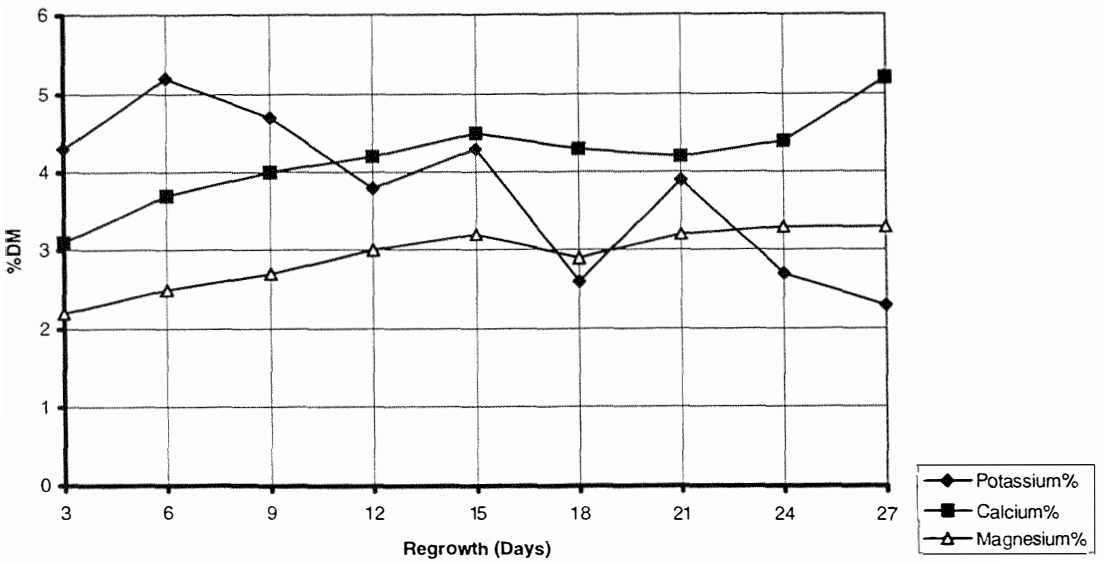


Figure 5.3 Mineral content of pasture after grazing (after Fulkerson, 1996).



Fulkerson and Slack (1994, 1995) have defined optimal grazing residual and maximal regrowth in terms of 1,2, 3 and 4 leaf stages of the ryegrass plant, with 3-3.5 leaf stage being the optimal stage to graze because the exponential growth phase is complete at the 3.5 leaf stage and water soluble carbohydrate (WSC) reserves in the plant have been replenished. Optimal residual grazing levels near 5 cm to maximise regrowth were also recommended by Fulkerson (1994). Higher residuals do not necessarily lead to a higher efficiency of converting solar energy to animal intake per hectare, however, because there may be less death and decay of dry matter with closer defoliation (Nelson and Moser, 1994; Fales *et al.*, 1995).

If grazing residuals were repeatedly nearer 2 cm and grazing occurred at the 1 leaf stage, then WSC reserves in stubble would be depleted and regrowth would be reduced to only 65% of that for residuals of 5 cm or 12 cm (Fulkerson, 1995). WSC accumulated preferentially in the stubble after the 1 leaf stage. At the 1 leaf stage the pasture plant was considered an inferior feed because the protein:WSC ratio is 4-5 times greater than at the 3 leaf stage. Magnesium and calcium increase if pasture is grazed at the 3 leaf stage rather than the 1 leaf stage. Potassium and phosphorus levels decrease at the 3 leaf stage (see Figure 5.3, Fulkerson, 1996). Phosphorus and potassium levels in pasture are known to decrease with maturity, but calcium does not alter (Fleming, 1973; Underwood, 1981). The progression of mineral concentrations in Australian conditions (K, Ca, Mg) after grazing as ryegrass pasture matures are represented in Figure5.3 (after Fulkerson, 1996).

The observational studies presented in Chapter 1 showed strong negative associations of pasture crude protein/soluble carbohydrate (CP/SOLCHO) ratio with milk productivity on 4 farms. Data from 1990 also suggested an association between higher phosphate in soil (Olsen P), and lower CP, and higher SOLCHO concentrations. This association between phosphate and lower CP/higher SOLCHO is not supported by Butler and Bailey (1973) where potassium was more likely to influence SOLCHO levels positively.

In order to provide more insight into the relationship between pasture CP, SOLCHO, pasture maturity after grazing and soil phosphate levels, an experiment was designed to follow pasture nutrient change as pasture matured and was either grazed or not grazed.

It was considered that this experiment would help provide recommendations on which way studies should continue on this subject, particularly in relation to factors influencing soluble carbohydrate and protein level in pasture.

5.2 METHODS

Three sites were chosen on one farm with high Olsen P levels (near Olsen P 35) and two sites were chosen on a lower Olsen P farm nearby (Olsen P 20). Both farms were on the same soil type (mixed Puniu silt loam and Otorohanga silt loam).

Two sites (one on the high P farm and one on the low P farm) were caged shortly after the previous grazing between 25 August and 5 September to allow sampling of ungrazed pasture for comparison. Samples were taken between 12 September and 3 November 1992. The cages consisted of wire mesh and a galvanised iron frame designed to minimise any change to the environment within the cage but prevented grazing by the cows.

Two other sites were grazed by cows as part of their normal rotation (one site each on the high P farm and low P farm). An additional site on the high P farm was introduced as a control that had had no recent N application when it was discovered that the high P grazed site had 35 kg N applied as urea shortly before the start of the experiment. This "control" site was grazed. No other fertiliser was applied to the sites during the trial.

Samples were collected every 5 days from each site in order to examine the changes in nutrient concentrations with time. Pasture was cut to the level of grazing (visually assessed) at each sampling. On the caged sites pasture was cut from regrowth from the original starting level and not from pasture regrowths (see Figure 5.4). Samples were taken between 12 September and 3 November 1992. Samples were placed in a cooled chillibin and then into a deep freeze within 30 minutes until further processing. Approximately 0.2 m^2 of pasture area was sampled from each caged site, but a larger area, nearer 1 m^2, was sampled from grazed sites.

Plate 5.1 Photo of cage and equipment used.

Pasture DM present was estimated using a rising plate meter calibrated with a standard formula (measure x 158 + 200), soil temperature using a 10 cm deep soil thermometer, and weather conditions (sunlight hours, rainfall /day) using recordings from the Ruakura weather station (located approximately 20 km from the sites).

Pasture samples were transported frozen on dry ice to Massey University, then freeze dried (lyophilised) in a Cuddon FD57 freeze drier and then ground through a 1 mm screen in a Wiley mill (A.H. Thomas, Philadelphia, PA, USA.).

Samples were then analysed for dry matter % (DM%), Digestibility (DOMD%), crude protein (CP%), neutral detergent fibre (NDF), soluble carbohydrate (SOLCHO) and pectin. Mineral analyses for P, K, Ca and Mg were measured using atomic absorption spectrophotometry. SOLCHO was analysed using serial extraction with hot water and Nelson's method for reducing sugars. Pectin was extracted with ammonium oxalate detected as D-galacturonic acid, NDF using sulphuric acid in a modified Van Soest method with neutral detergent at pH 7 (James and Theander, 1981), and CP using a

Kjeldahl method (Kjeltec auto system). More detail on methods can be found in Appendix 1.

5.3 RESULTS

Mean results with standard deviations on the data over time are presented in Table 5.1. No statistical analysis between treatments has been attempted on this data because of a lack of replicates, but the following observations can be made.

Higher SOLCHO, lower phosphorus, lower herbage accumulation rate (HAR) and higher DM% were observed on the low P sites (Figures 5.7, 5.11, 5.13, Table 5.1). Low P sites grew less dry matter (Figure 5.5, Table 5.1). Severe overgrazing on the low P grazed site was observed (see herbage mass and pasture accumulation rate, Figures 5.5-5.6, Table 5.1).

Highest CP was present on the high P site that received no nitrogen (Figure 5.9, Table 5.1). This site was visibly higher in clover %.

Higher NDF, and lower DOMD were present on the high P + N sites (Figures 5.10, Table 5.1).

Pasture P levels were lower in the two low soil P sites (Figure 5.13). Calcium and pectin levels were higher in the high P grazed site (Figure 5.12). The low P sites had higher DM%.

A correlation matrix was prepared to examine broad interactions between the parameters measured (Table 5.2) over time and across treatments.

Table 5.1 Mean values for pasture nutrients measured in spring dairy pasture (12 September - 3 November) from five sites (standard deviation (over time) in brackets).

	Site 1 - caged, high P + N n=12	Site 2 - caged, low P n=12	Site 3 - grazed, high P n=9[1]	Site 4 - grazed high P + N n=12	Site 5 - grazed, Low P n=12
Soluble carbohydrate %	9.8 (2.4)	13.1 (2.8)	10.6 (1.1)	10.1 (2.4)	11.2 (2.5)
Crude protein %	18.8 (3.5)	16.1 (2.0)	21.3 (2.0)	18.0 (3.2)	18.5 (2.3)
Neutral detergent fibre%	44.2 (3.9)	39.1 (3.9)	34.7 (3.9)	45.2 (5.4)	39.9 (4.3)
Pectin %	1.6 (0.4)	2.0 (0.9)	3.4 (3.4)	1.8 (0.6)	2.1(0.4)
Digestibility% (DOMD)	69.8 (3.16)	70.8 (2.51)	72.0 (2.42)	69.2 (4.27)	69.68 (3.68)
Phosphorus%	0.35 (0.03)	0.31 (0.03)	0.37 (0.02)	0.38 (0.04)	0.31 (0.03)
Potassium%	2.8 (0.49)	2.7 (0.57)	2.6 (0.24)	2.7 (0.30)	2.3 (0.49)
Calcium%	0.45 (0.07)	0.50 (0.1)	0.77 (0.17)	0.42 (0.1)	0.48 (0.11)
Magnesium%	0.15 (0.02)	0.16 (0.04)	0.18 (0.02)	0.16 (0.03)	0.16 (0.04)
Dry matter%	19.4 (2.8)	20.4 (3.2)	18.7 (3.4)	19.0 (2.6)	21.6 (3.4)
Herbage mass (kg DM/ha)	3300 (1333)	2763 (1245)	2188 (592)	2281(483)	1309 (250)
Herbage accumulation rate (kg DM/ha/d)	74 (43)	66 (40)	89 (52)	71 (71)	34 (20)

[1]Sampling from high P, grazed site with no N began 15 days later. This site had a higher clover content than the other sites but the exact % was not measured.

Table 5.2 Correlation matrix for the measured data[4].

	NDF	CP	DOMD	Pect	SOLCHO	Ca	Mg	K	P	HAR[1]	HA[2]	T°C[3]	DM%
NDF	1												
CP	-0.57	1											
DOMD	-0.7	0.4	1										
Pect	-0.58	0.29	0.34	1									
SOLCHO	-0.30	-0.36	0.10	0.10	1								
Ca	-0.65	0.32	0.51	0.81	0.23	1							
Mg	-0.40	0.26	0.35	0.42	0.03	0.63	1						
K	-0.16	0.15	0.39	0.14	-0.01	0.19	0.29	1					
P	0.06	0.31	-0.02	0.14	-0.45	0.16	0.23	0.38	1				
HAR	0.21	0.04	-0.14	-0.17	-0.08	-0.11	-0.24	0.00	0.00	1			
HA	0.18	-0.30	-0.21	0.02	0.27	0.17	0.08	0.23	-0.17	0.37	1		
T°C	0.02	-0.22	-0.08	0.42	0.21	0.49	0.49	0.35	0.09	0.00	0.46	1	
DM%	0.33	-0.49	-0.54	-0.15	0.10	-0.35	-0.33	-0.60	-0.33	-0.13	-0.13	-0.02	1

[1]HAR = pasture accumulation rate ("growth rate")
[2]HA = pasture accumulated at sampling
[3]T°C = soil temperature at 10cm deep, 8.30am
[4]Correlations significant at $P < 0.05$ when $r > 0.25$, at $P < 0.01$ when $r > 0.33$ and $P < 0.001$ when $r > 0.41$

Notable associations with correlations > 0.5 were :

- the negative association of higher NDF with CP, DOMD, pectin and calcium%.
- the positive association of pectin with calcium.
- the positive association of calcium and magnesium.

Dry matter % had positive associations with NDF but negative with protein, DOMD and the minerals. DOMD was associated with NDF and DM% but positively with CP, SOLCHO, K, Ca, Mg but not P. Temperature had positive associations with K, Ca and Mg but not P.

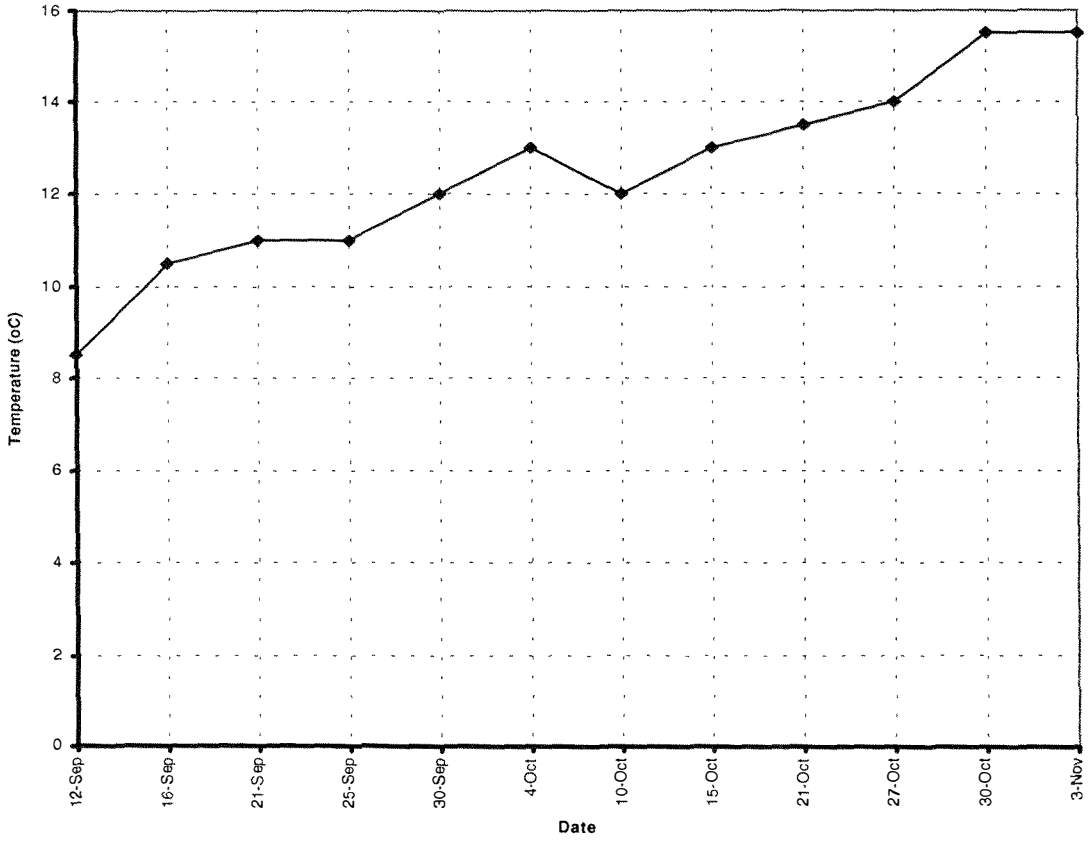
The changes in nutrient concentrations through time are represented graphically in Figures 5.5-5.16.

Soil temperature (10 cm deep) for the sampling period is presented in Figure 5.4.

The period from late September to mid October is of special interest. Ground temperature was increasing till near 4 October and reached 13°C, then fell to near 12°C for 1-5 days.

The data was rearranged to examine the effects of time from grazing vs non-grazing. Pasture accumulated as measured by a calibrated rising plate meter is presented in Figure 5.6. Both caged sites (ungrazed) accelerated markedly in herbage accumulation rate (HAR) near 1800-2200 kg DM/ha. The grazed Low P site was "overgrazed", and did not reach 1800 kg DM at any stage (Figure 5.5). Curves were smoothed using Microsoft Excel 5.0.

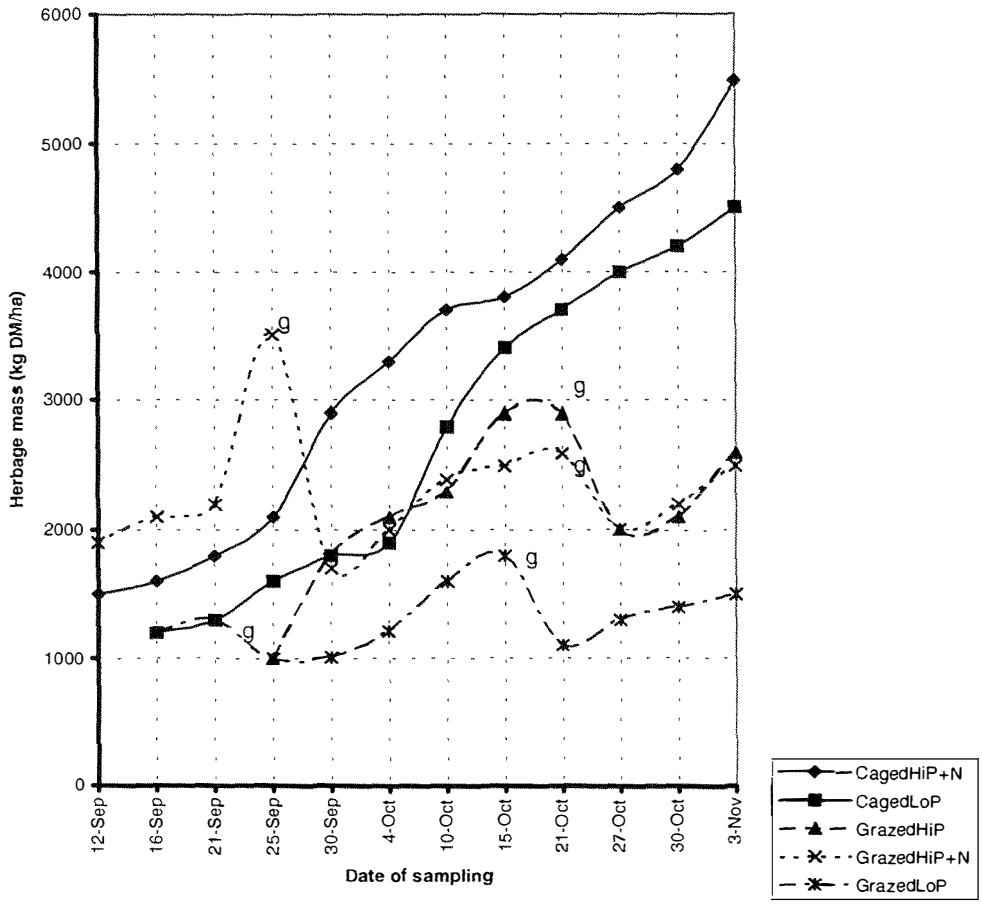
Figure 5.4 Soil Temperature °C at 10 cm depth vs date of sampling.



Herbage accumulation increased, DM% and SOLCHO decreased, and DOMD, CP, pectin, calcium, potassium, and magnesium all increased in early October (Figures 5.5-5.12; Figures 5.14-5.16). These changes were similar to those observed in mid-late October in Chapter 1.

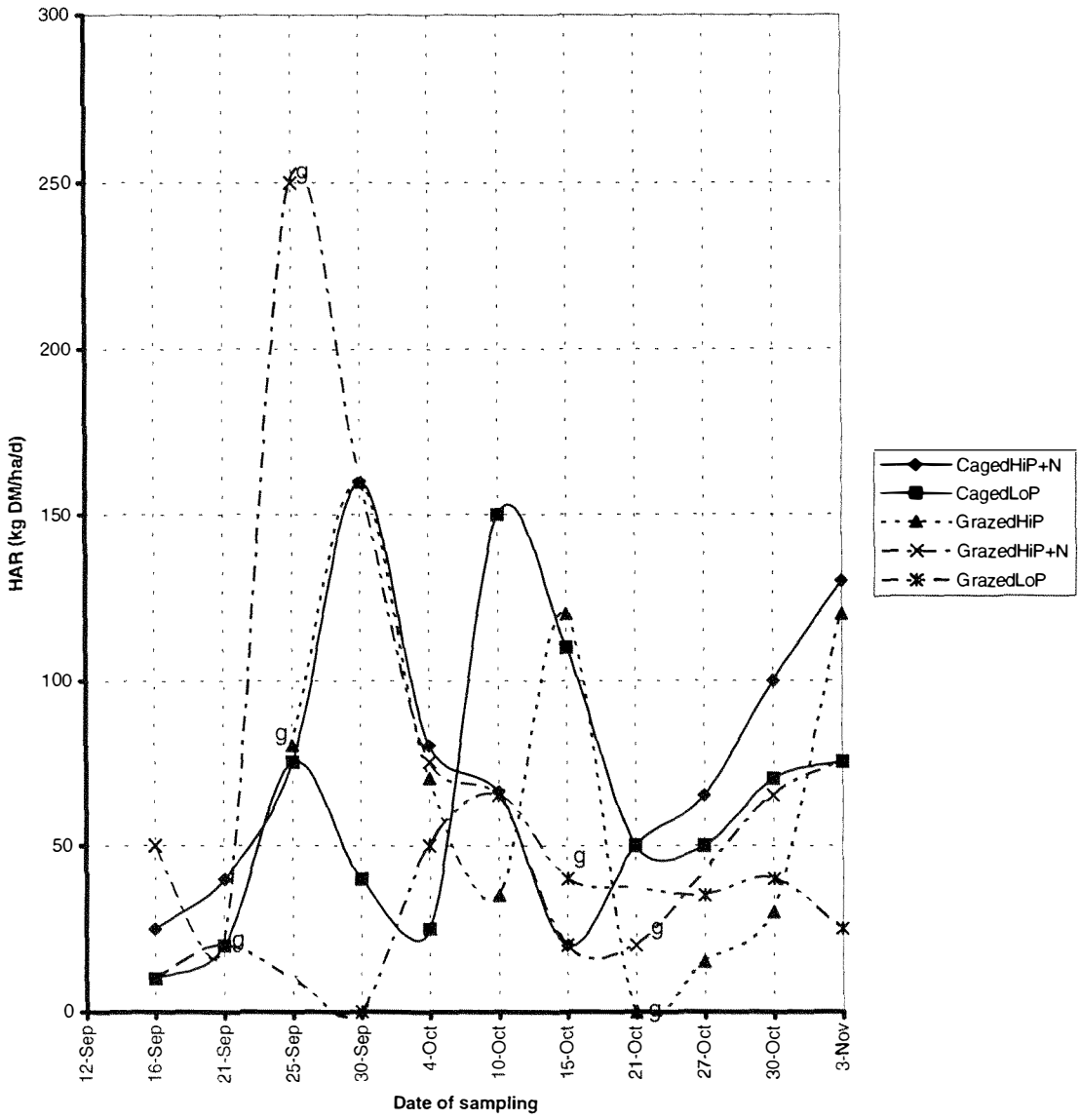
Figure 5.5 presents the herbage mass estimated at each site at each sampling date. The grazed LoP site did not exceed 1800 kg DM/ha at any stage. A faster phase of growth rate appears to start near 2000 kg DM/ha on the other 4 sites and continue to over 3500 kg DM/ha.

Figure 5.5 Herbage mass at each site vs date of sampling (g = grazed).



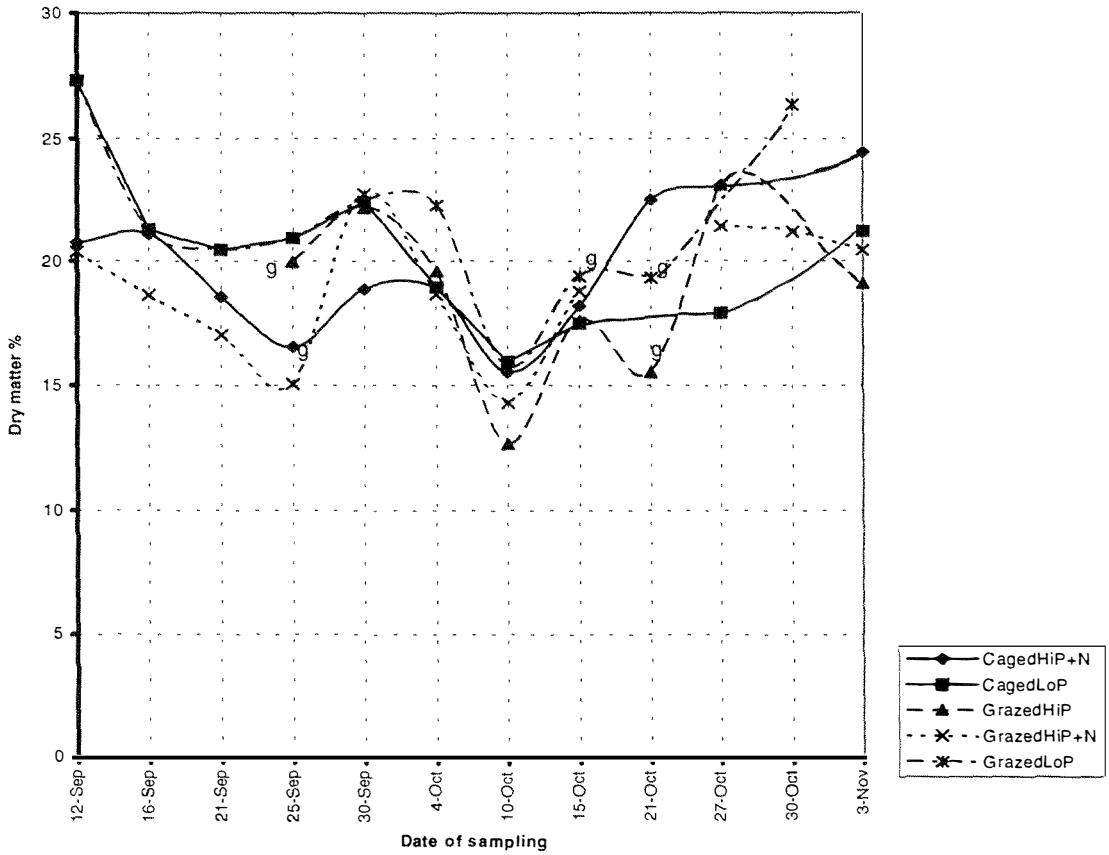
Herbage accumulation rate (HAR) results are presented in Figure 5.6. Fastest rates occurred for brief periods in pasture near 2000-2500 kg DM, and apparently reached 250 kg DM/ha/day on one site. These measurements were estimates with a rising plate meter by difference from the previous estimate using the formula (measure) x (158) + 200 = kg DM/ha. HAR decreased markedly after each grazing.

Figure 5.6 Herbage accumulation rate for the five sites vs date of sampling (g = grazed).



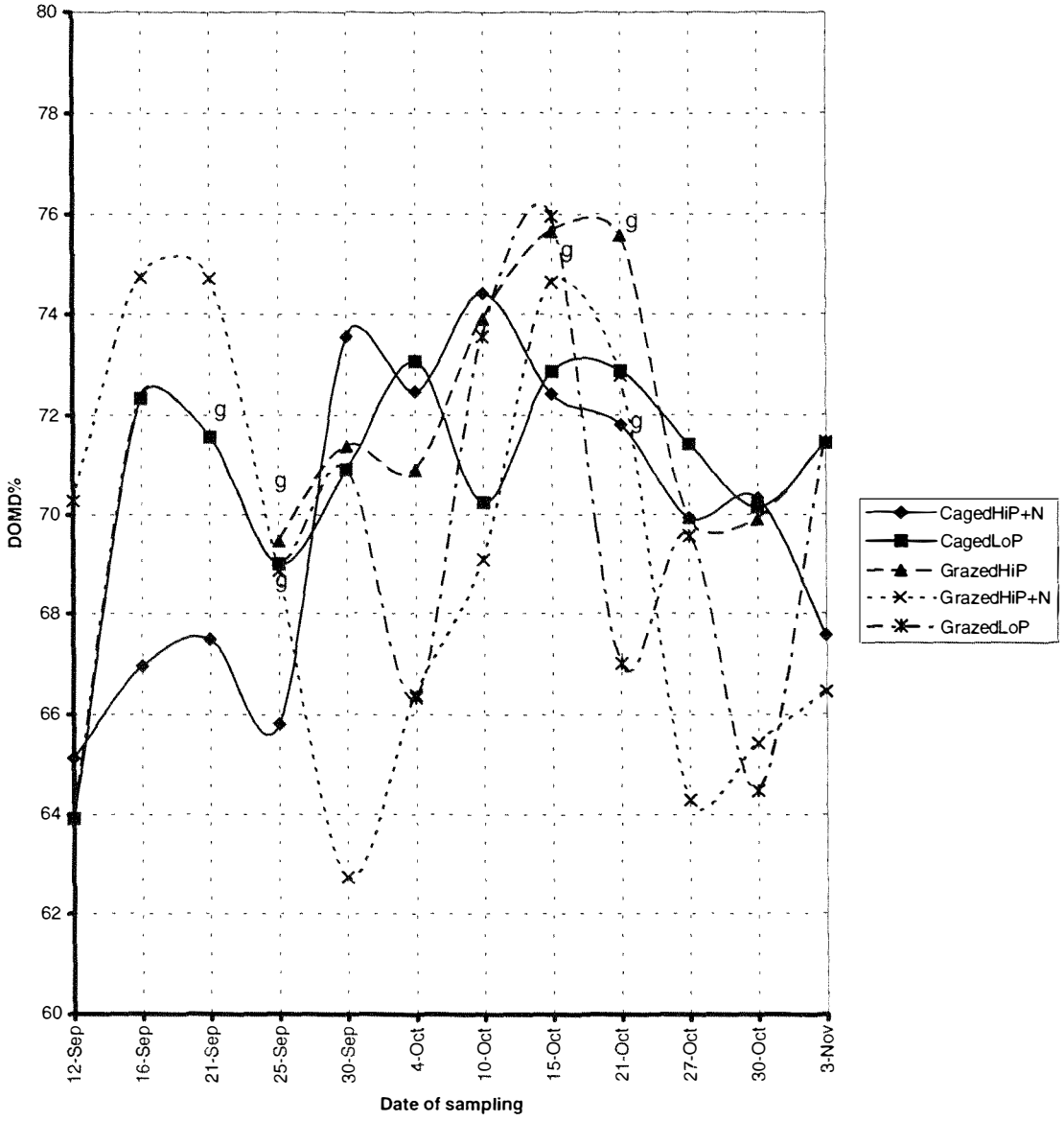
Dry matter % results are presented in Figure 5.7. A low point for all sites near 10 October preceded by a high point near 30 September are the most obvious features. Weather conditions (recorded at the Ruakura weather station) at this time were cool and sunny for the 3 days preceding 30 September (10 cm soil temp 9°C, 8.7 hrs sunlight/day) and warm and overcast for the 3 days preceding 10 October (10 cm soil temp 11.1°C, 1.7 hrs sunlight/day). Dry matter % results presented here are higher than those previously presented for other results, but still fluctuate with time. Samples were freeze dried for DM% after being deep frozen for 6 months.

Figure 5.7 Dry matter % for the five sites vs date of sampling (g = grazed).



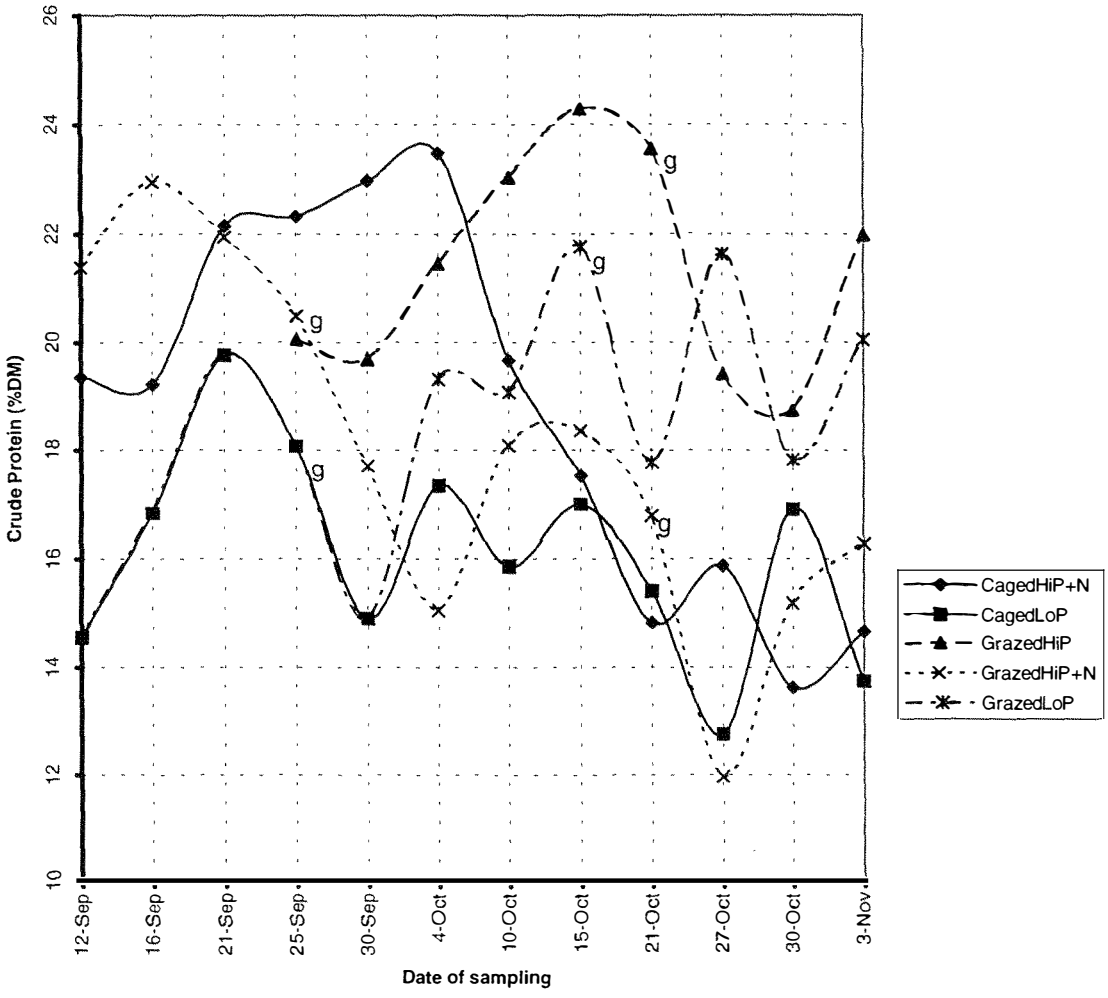
The *in vitro* digestibility results expressed in terms of DOMD (digestible organic matter in dry matter) are presented in Figure 5.8. There was a gradual decline in digestibility as the sampling period advanced into October of approximately 5%. Fluctuations of 3-4% occurred over 5-10 day periods on all sites and often at the same time. Ungrazed sites had similar DOMD to the grazed sites except the HiP plus N grazed site. DOMD declined immediately after each grazing.

Figure 5.8 Digestibility (DOMD) vs date of sampling (g = grazed).



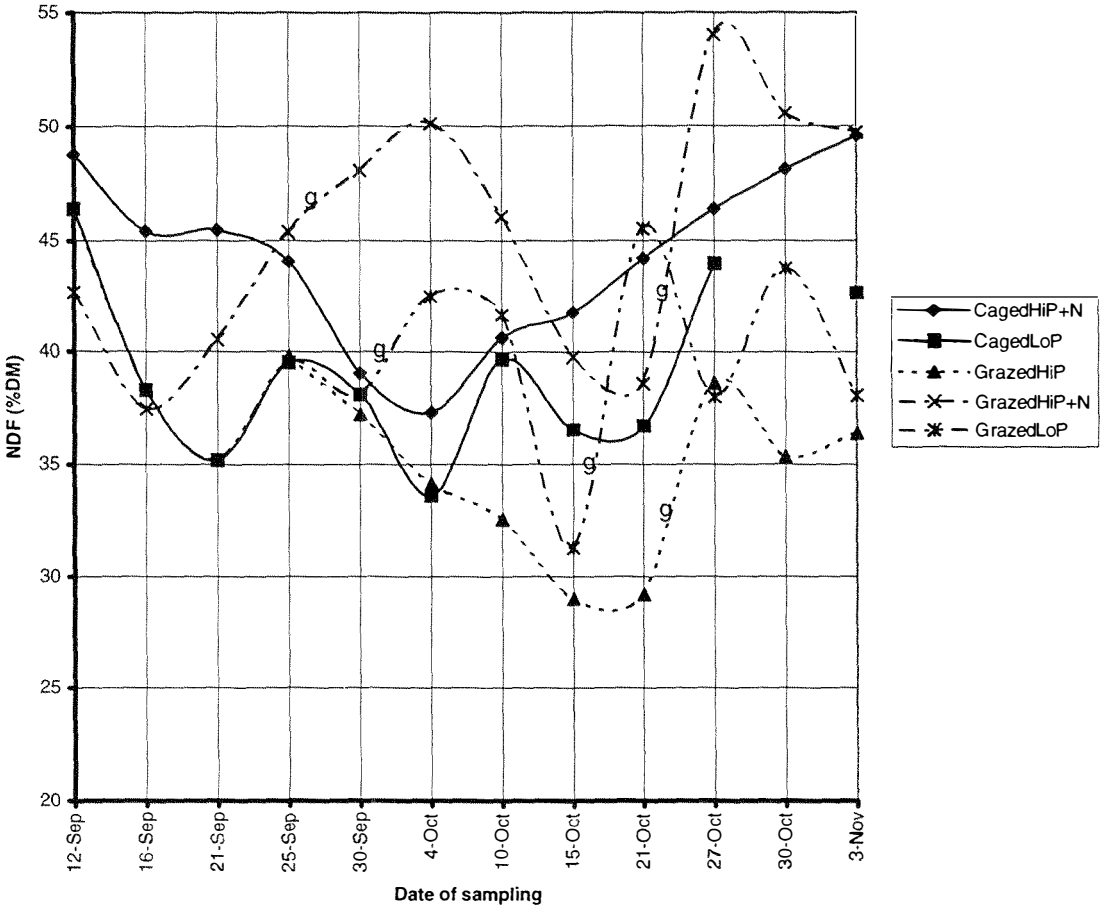
Crude protein % results are presented for the five sites in Figure 5.9. A gradual decline in CP levels occurs in the two ungrazed sites, particularly in October. The grazed HighP+N site also showed these changes. CP levels declined post-grazing (g) on every occasion for 5-10 days. Grazed sites increased in CP in early October.

Figure 5.9 Crude protein % for the five sites vs date of sampling (g = grazed).



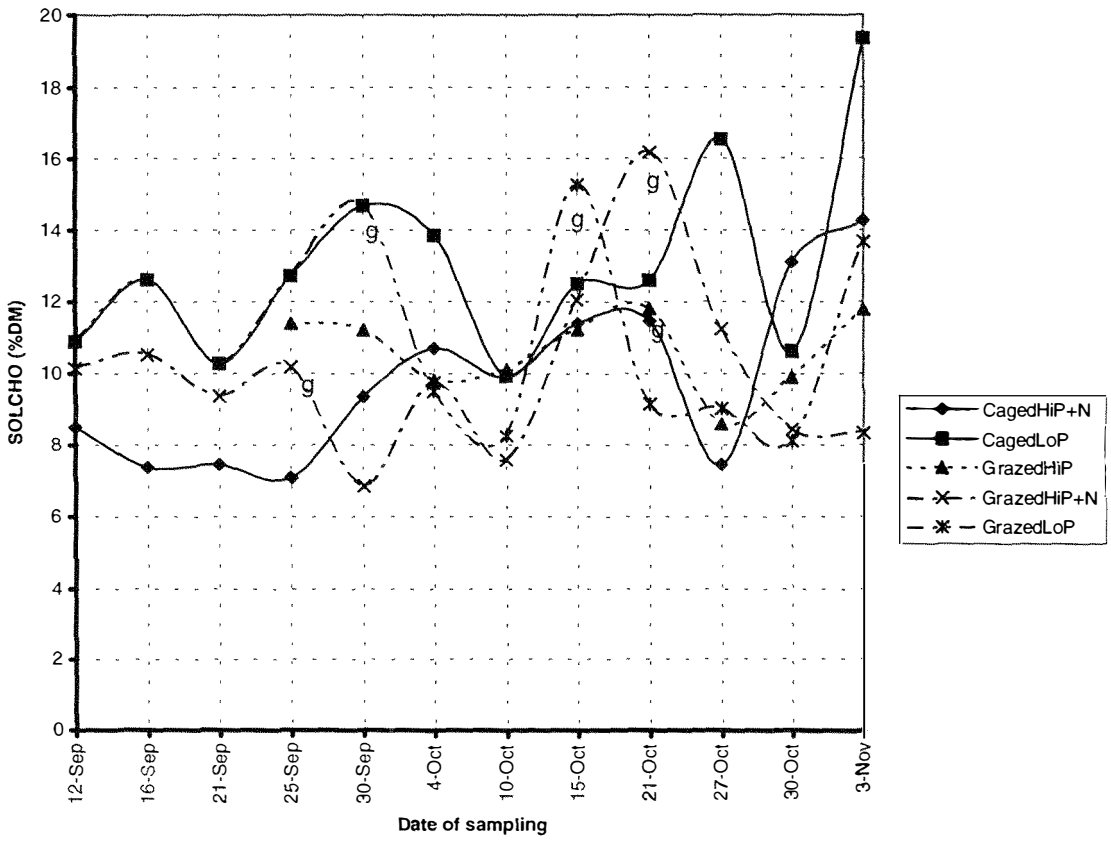
Neutral detergent fibre (NDF) results are presented in Figure 5.10. A gradual elevation in the ungrazed sites can be discerned from near 4 October onwards. Grazed sites showed reduced NDF in early October. An initial elevation in NDF after each grazing (g) is also present which subsequently declines. The grazed HiP site had the lowest NDF levels. This site also had more clover present, but the amounts were not quantified.

Figure 5.10 NDF% for the five sites vs date of sampling (g = grazed).



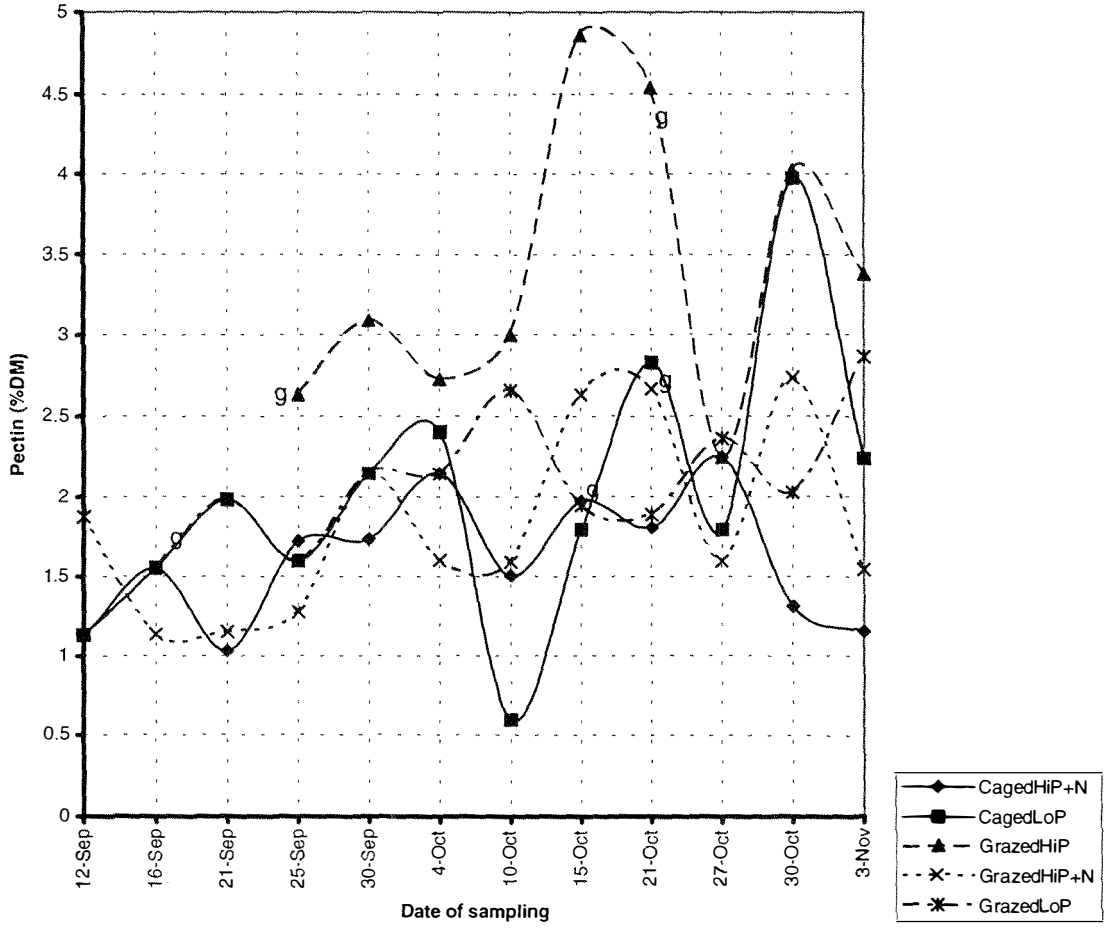
Soluble carbohydrate results are presented in Figure 5.11. Both the grazed LoP and ungrazed LoP sites had higher soluble carbohydrate (SOLCHO) levels. Grazed sites showed a post-grazing depression in SOLCHO for 5-15 days. A low point in SOLCHO occurred on all sites on 10 October (at the same time as the low point in DM%) that did not appear to relate to grazing. A gradual increase in SOLCHO in pasture as the experimental period advances is also present.

Figure 5.11 SOLCHO % for the five sites vs date of sampling (g = grazed).



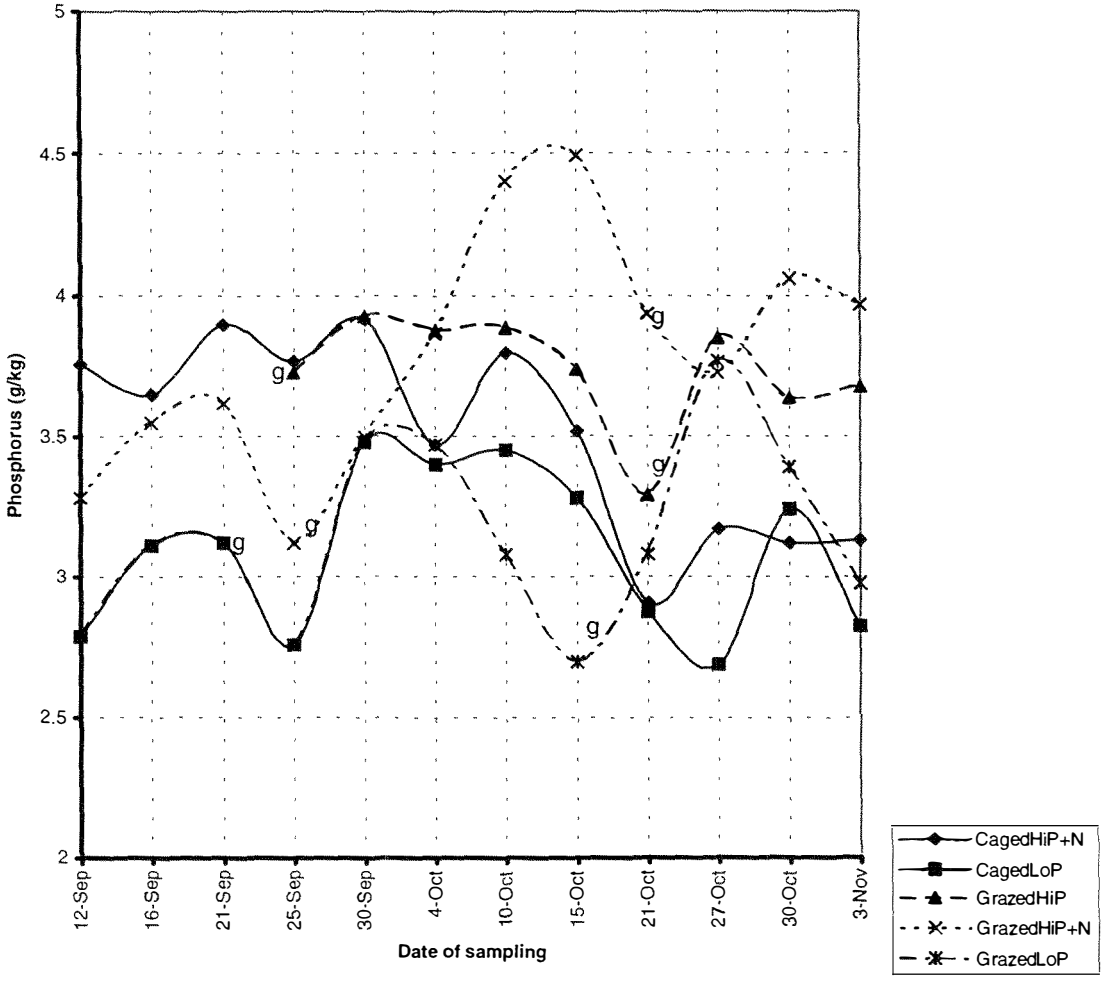
Pectin results are presented in Figure 5.12. The grazed high P site had distinctly higher pectin levels. This site visually contained higher clover levels. All sites gradually increase in pectin content (from 1.5 to 2.5%) through the experimental period.

Figure 5.12 Pectin % for the five sites vs date of sampling (g = grazed).



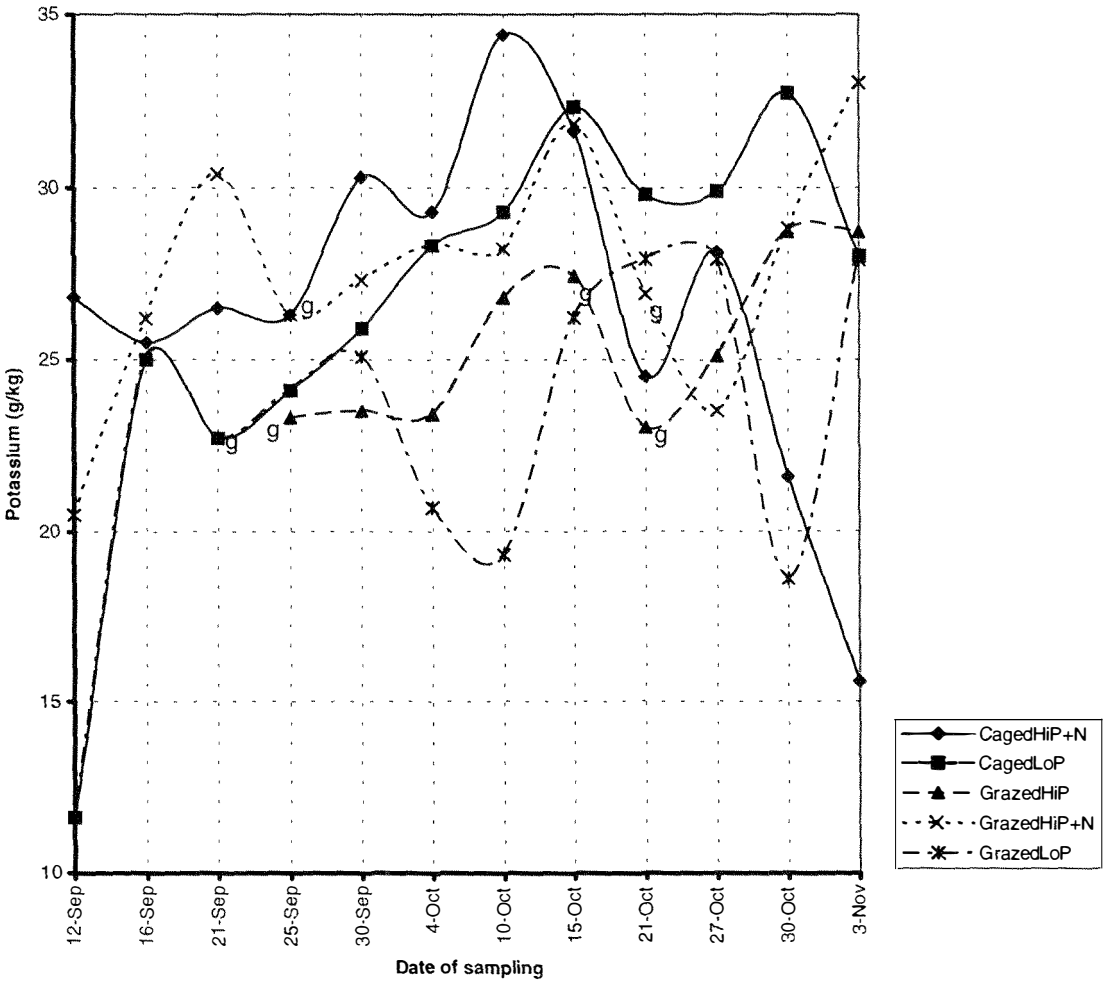
Phosphorus results are presented in Figure 5.13. High P sites (both grazed and ungrazed) had higher pasture phosphorus (P) levels. The high P sites had a soil Olsen P level of 35 and the low P sites an Olsen P level of 20. Phosphorus levels in pasture gradually declined in the ungrazed sites during October.

Figure 5.13 Phosphorus in pasture (g/kg) for the five sites vs date of sampling.



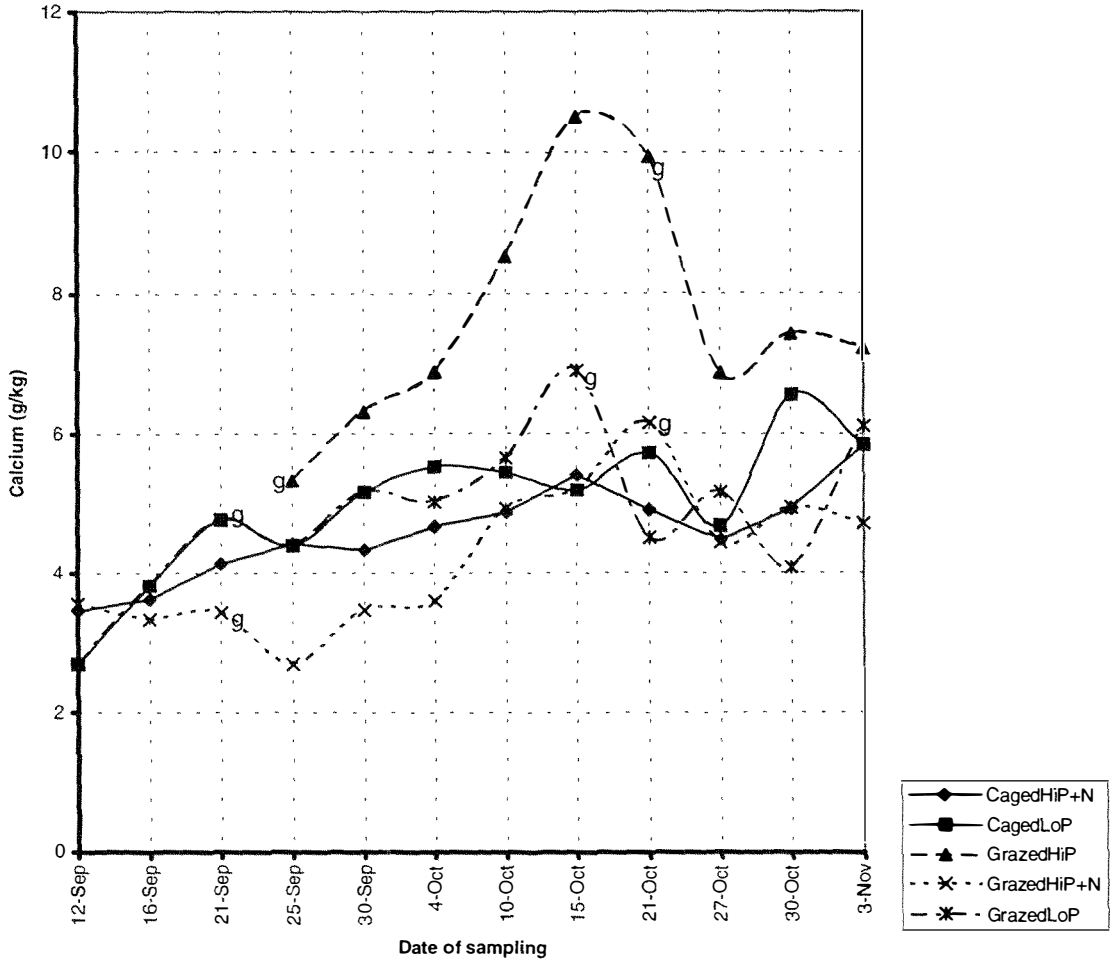
Potassium results are presented in Figure 5.14. Potassium levels on the ungrazed sites gradually increased till early October, then appeared to decline.

Figure 5.14 Potassium in pasture (g/kg) for the five sites vs date of sampling.



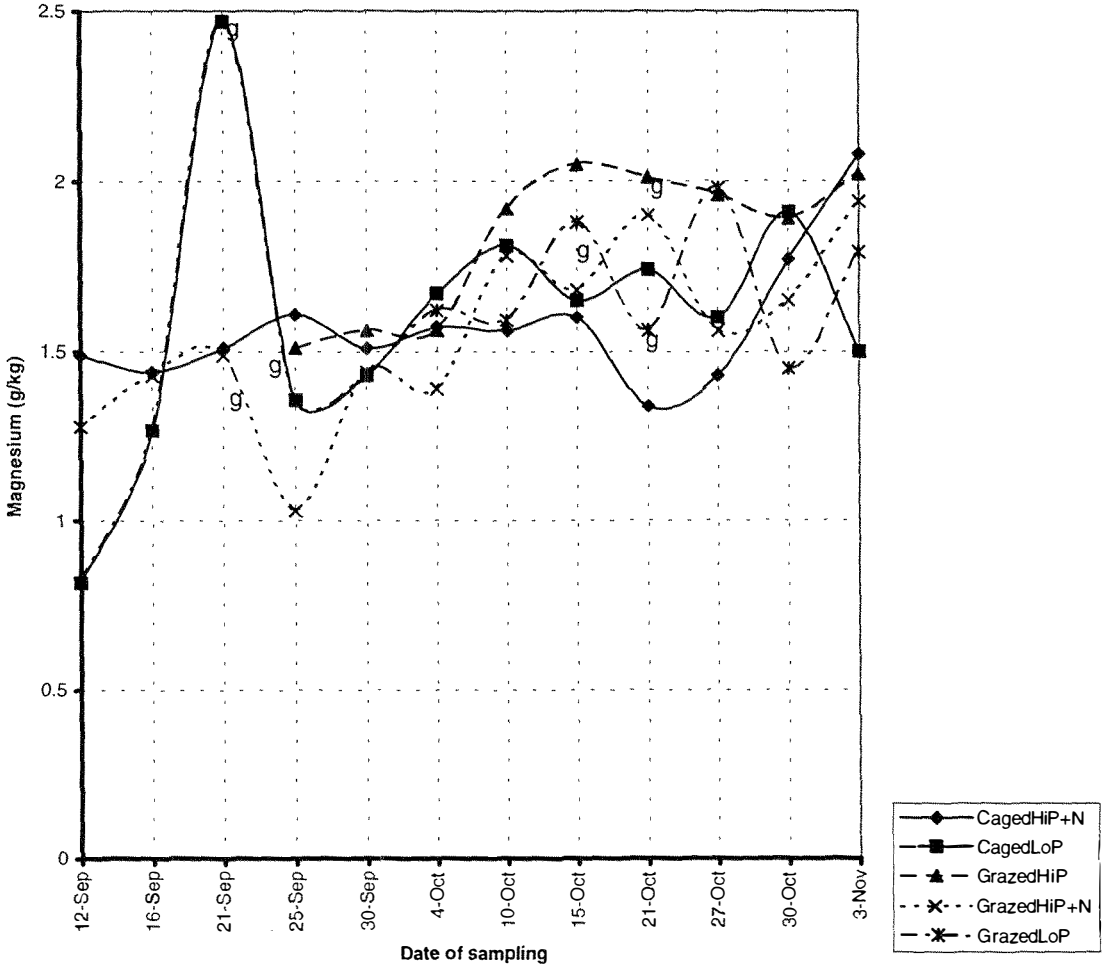
Calcium results are presented in Figure 5.15. There was a gradual elevation in pasture calcium levels as the sampling period advanced. After most grazings pasture calcium declines for 5-10 days. The high P grazed site had the highest calcium levels.

Figure 5.15 Calcium in pasture (g/kg) for the five sites vs date of sampling.



Pasture magnesium results are presented in Figure 5.16. There was a gradual elevation in levels as the sampling period advanced. Magnesium levels often declined post grazing.

Figure 5.16 Magnesium in pasture (g/kg) for the five sites vs time.



5.4 DISCUSSION

As noted in the preface, no statistical significance exists for the data presented in this chapter. The data were included as background information to other parts of the thesis.

5.4.1 Grazed vs Non-grazed Sites:

Grazing appeared to result in an immediate decline in SOLCHO, protein and digestibility and increase in NDF levels. This is similar to the results observed by Fulkerson (1996) for SOLCHO and Davies (1965). The initial increase in less digestible nutrients (increased NDF) after grazing may represent the physical elevation of this material to a higher level in the sward as part of the new shoot development or may be an artefact of the sampling procedure, where at lower levels of pasture the samples were inadvertently cut at lower levels than at the previous occasion. As the proportion of intracellular material vs cell wall in the plant increases again after grazing the NDF% decreases and CP% increases. The SOLCHO is replenished as photosynthesis occurs and storage of excess SOLCHO in the stem occurs again after the 1-leaf stage (Fulkerson, 1994 and 1995).

DOMD for ungrazed sites was similar to the grazed sites, with the exception of the high P plus N grazed site, except that there was a decline in DOMD immediately after grazing. SOLCHO and NDF gradually increased and CP gradually decreased on the ungrazed sites. This would be expected as pasture matures and the proportion of cell wall increases (Holmes, 1989). The reproductive phase of ryegrass will also have affected results in October where there appears to have been a marked decline in pasture quality as defined by DOMD. These changes in pasture nutrients (increased NDF, decreased CP, decreased DOMD) tended to accelerate in pasture near 3200-3500 kg DM/ha. The effects of temperature, sunlight, maturity, clover content and the reproductive phase of ryegrass cannot be separated here.

5.4.2 High P vs Low P sites

Lower soil phosphate sites had higher soluble carbohydrate, higher DM%, lower HAR, higher pectin %, lower P levels and usually lower protein %. Whilst most of these nutritive changes might seem desirable from the cow's point of view when compared to recommended dietary levels (NRC, 1989; Muller, 1993), they are offset by the reduced DM produced. To a large extent the less dry matter grown on the low P sites was because the pasture was being overgrazed or started growing from a lower point (eg ungrazed low P site vs ungrazed high P site). This is indicated by the DM estimates (HA) which did not exceed 1800 kg DM/ha at any stage on the low P grazed site.

5.4.3 Herbage accumulation rate

Ungrazed sites grew at similar rates once they reached a certain level of pasture present (near 1800-2200 kg DM/ha) suggesting that P levels in soil and pasture were not limiting growth in this experiment. Ungrazed sites declined in HAR when they reached 3200-3500 kg DM/ha. Grazed sites exceeded 100 kg DM/ha/d for short periods, with the exception of the low P site which appeared to be overgrazed throughout.

Increases in HAR occur as more pasture accumulates. This is consistent with the slow recovery phase, the rapid growth phase and the period of net decline as described by Brougham (1956, 1958) and higher leaf area index producing more DM growth (Hodgson, 1990). The low P grazed site did not grow well probably because sufficient leaf area index did not develop. The low P ungrazed site grew at almost the same rate as the high P sites once it gained more leaf area index (=HA). Very high growth rates (HAR) occurred for short periods of 5 days approximately. Up to 250 kg DM/ha/d was estimated using the rising plate meter (this may not have been an actual growth rate as plate meter estimates are subject to errors). These high growth rates occurred at different times on three sites and seem to relate more to conditions at that site rather than external weather conditions, ie. the stage of growth of the pasture seemed more important in determining growth rate, although temperature, and lengthening days must have played a large part also.

From a pasture production point of view, leaving residuals nearer 5 cm would be expected to accumulate more pasture (Fulkerson, 1995). Higher residuals also place pasture closer to the 1800 kg DM level in this experiment where pasture appears to accelerate in growth rate (pasture accumulation rate), which must then grow more total pasture. On the other hand efficiency of conversion of solar radiation to animal intake per unit land area can halve through senescence and decay if close defoliation is not practised (Nelson and Moser, 1994).

5.4.4 Mineral levels

Elevations in magnesium and calcium as the sampling period advanced probably reflect an increase in clover % in the pasture, although this was not detected visually or measured. The higher clover site (high P grazed) did have higher calcium levels. Pectin also increased on all sites as the period of observation advanced, suggesting clover % was increasing. Alternately the increased concentrations may reflect increased mineralisation of soil nutrients as the ground temperature increased. Fulkerson noted gradual increases in magnesium and calcium as time from grazing advanced toward the 4 leaf stage. This could also have been a contributing factor. The pattern for potassium is less distinct except that increasing pasture maturity (with the consequent increase in % of cell wall components and decrease in cell contents) tends to reduce K levels. Grazing produced a temporary decline in K levels. The pattern for phosphorus is not distinct, except that maturity of pasture appears to result in P level decline, and grazing produces a temporary decline in P levels. These effects are consistent with those of Fleming (1973), Underwood (1981) and Fulkerson (1996), who found that phosphorus and potassium decline with maturity.

Phosphorus % and Calcium % were adequate for moderate levels of milk production but may not be quite enough for higher levels (NRC, 1989). Magnesium was below recommendations throughout the sampling period. Potassium was always in excess of requirements.

The excessive potassium levels present in pasture have major implications for anion/cation balance which have been shown to cause metabolic disease at calving. Reduction in the DCAB (difference between cations and anions) before calving has reduced milk fever, improved periparturient health and increased milk protein production post-calving (Aseltine, 1990; Delaquis and Block, 1994; Wilson, 1996).

5.4.5 Correlations

The correlation matrix (Table 5.2) is mainly of speculative interest, but confirms expected relationships between NDF, CP, DOMD, pectin, and SOLCHO. Negative correlations of calcium and magnesium with NDF, yet positive with CP suggest increases in cellular content increase these mineral levels. A positive correlation between calcium and magnesium, and between pectin, calcium, magnesium and temperature also exists. Lower DM% correlates with increased mineral and protein levels. Rising ground temperature, seasonal change and maturation will all have affected values found. Higher calcium and pectin levels would be expected in clover, and rising temperatures should increase mineralisation in soils and subsequent mineral uptake.

5.4.6 General comments

Elevated pectin levels were present on the high P grazed site. These are likely to relate to the increased clover content at this site, as clover usually contains 7% pectin and ryegrass only 2% (Ulyatt and Waghorn, 1993).

DOMD declined during the sampling period by 5 units approximately, whether the site was grazed or not, and occurred whether maturity increased or not. This occurred as protein in pasture declined and NDF increased, and thus reflects a change from cell content dominance to increased cell wall content. Temperature elevation is likely to have played a large part in this as digestibility of ryegrass pasture is known to decline 0.66 units per °C rise in temperature (Minson, 1990). Temperature influences the

relative proportions of leaves and stems (Nelson and Moser, 1994). Sunlight has a lesser effect on digestibility than temperature (Deinum *et al.*, 1968). The high P plus N grazed site appears to have been at the opposite end of the grazing spectrum (compared to the low P grazed site) with quality (DOMD) only just kept under control by the grazing management.

The gradual decline of DOMD through the experimental period is particularly marked from early October onwards, and may be explained as the arrival of the reproductive phase of ryegrass pasture and higher temperatures. This has particular significance for the maintenance of peak lactation in dairy cows at this time of the year in the seasonal system practised in New Zealand because most cows are reaching peak at this time and will encounter pasture with reducing DOMD. This will lead to lower dry matter intakes (Hodgson, 1977) and reduced productivity (higher NDF leads to lower intakes). Reduction of peak milk per month can be maintained at 3-5% per month in controlled diets overseas. A 10% decline in peak milk per month is common in October/November in New Zealand (LIC). It is likely that the reduced DOMD at this time contributes to this through reduced intakes. In a review by Holmes (1987) limited effects of *in vitro* OMD (digestibility of organic matter) on DMI were found in a number of local studies except at high allowances in summer. Another study found increases in DMI of 0.55 kg DM per 1% increase in OMD within a range of 64-80% (Stehr and Kirchgessner, 1976). Grainger (1992) found that OMD declined by 0.6% per tonne increase in pregrazing mass. The effects noted in this experiment, especially over 3500 kg DM/ha, were larger but probably confounded by temperature and the physiological state of the ryegrass.

Dry matter % of pasture declined uniformly on 10 October and this coincided with reduced SOLCHO and NDF levels as well. Ground temperature reached 13°C on 4 October, then declined to 12°C on 10 October before increasing again. Magnesium, calcium and potassium increased at this time but phosphorus declined. These changes are similar to those seen in the 1990 data presented in Chapter 1 that had associations with detrimental effects on cow production.

High P and High P plus N sites had lower soluble carbohydrate levels than Low P sites. For the high P grazed site this may be explained by the increased clover content, which

is known to contain less SOLCHO. The main explanation probably relates to increased HAR at these sites, which would then require more photosynthate (SOLCHO) for growth (Figure 5.6, Table 5.1).

5.4.7 Consequences for dairy cow nutrition

From the nutritional point of view for the cow grazing these pastures, more desirable ratios of CP:SOL CHO in pasture occur as pasture protein declines near the 2500 kg DM to 3000 kg DM stage. Muller (1993) states that NFC:DIP ratios (Non fibre carbohydrate: degradable intake protein ratios) of 3:1 are sought by nutritionists for high productivity. Extrapolation of the results in this experiment suggest that fresh pasture is frequently at less than 2:1 and sometimes 1:1.

NFC = 100 - (CP + NDF + Lipid + Ash)
DIP = 75-80% of CP in pasture (65% if mature)

Imbalances in protein and SOLCHO supply to the rumen are likely to lead to a "protein penalty", where extra metabolic energy is required in the liver to metabolise excess protein above the cow's requirements (Reid and Jung, 1974; NRC, 1989; see Chapter 1 and 2) and wastage of pasture protein (Beever *et al.*, 1986; Ulyatt *et al.*, 1988).

Increased water soluble carbohydrate in pasture should lead to increased protein production in the rumen and bacterial supply to the small intestine (Thomson and Beever, 1980). At higher temperatures respiration and transpiration rates are high in pasture, and soluble carbohydrates do not accumulate. The opposite applies in cooler conditions where growth of pasture is limited by temperature but photosynthesis is still active. In these conditions soluble carbohydrate can accumulate up to 30% of total DM (Chatteron *et al.*, 1989). Pasture grazed too frequently may not have sufficient time to replenish leaf reserves of soluble carbohydrates which are required for pasture regrowth after grazing. Root supply of soluble carbohydrate may also suffer in frequent grazing with consequent poorer root development. In the context of this experiment, grazing every 10 days would have been too frequent because SOLCHO replenishment would

not have time to occur (Figure 5.11). Diurnal fluctuations in SOLCHO are well known with SOLCHO increasing in the afternoon by up to 50% (Holt and Hilst, 1969). This can increase digestibility by 1.6% in the afternoons, and effectively dilute the protein content of pasture by 5-15%. Samples were collected at 8.00 am in this experiment, at the expected low point in SOLCHO, and these changes will not have been detected. Doyle *et al.* (undated) state that soluble and storage carbohydrates have a major influence on intake through influencing rumen function and through effects on palatability.

The fibre content of pasture in terms of NDF appears adequate for milk production but at levels over 40% may begin to interfere with intake potential at higher production levels on pasture. To achieve high intakes and high production, milking cow diets are formulated nearer to 30% NDF in the US (Muller, 1993; Hutjens, 1995). NDF levels are known to negatively affect intake (Mertens, 1987). This does not take account of the "effective fibre" component of pasture or the "scratch factor" required for good rumen function, which may be inadequate in fresh pasture because of it's elasticity (Muller *et al.*, 1995; Kolver, 1996).

Voluntary intake has been linked to digestibility (Blaxter, 1962; Hodgson, 1977; Wilson *et al.*, 1995). Maintenance of high digestibility of pasture is the most important pasture management priority after ensuring adequate DM intake of dairy cows for dairy farmers. An additional 1 MJ of ME per kg of DM of pasture in the form of better quality or digestibility in a lactating cow consuming 15 kg DM of pasture should lead to 3 litres extra milk (4% fat corrected) produced daily from this cow (1 litre of 4% fat milk requires 5 MJ of ME). In addition reduced quality will mean higher NDF levels which may limit DM intake to less than 15 kg anyway.

The variable composition of pasture presented to cows at consecutive grazings as well as the quantity presented must play a considerable part in the rapid post-peak decline in milk production seen on most farms in New Zealand. Once the cows propensity to produce milk begins to decline after peak milk production is reached then any suboptimal nutrition or production limiting nutrient is likely to have an irretrievable effect on milk production because the cow is unable to completely recover milk

production to its former level. In ME terms, cows often have limited body reserves at peak and thus do not have a great ability to respond from reserves when underfed.

The major factor determining what nutrients a cow will receive from pasture appears to be the pasture maturity at grazing, with seasonal influences superimposed over this.

5.5 CONCLUSIONS

The experiment provided an indication of the size of changes in pasture composition due to grazing or maturation and also differences between high P and low P sites. It was designed to study the differences between grazed and ungrazed pasture on a range of sites in order to improve understanding of changes that occur and point to areas that deserved further study. Any suggestion that higher P in soil was leading to increased SOLCHO (as suggested by results in Chapter 1) does not appear to be worth pursuing further - in fact the opposite appears to be indicated here.

Controlled environment studies would be required to clearly separate effects of temperature, sunlight, rainfall, shading, daylength, etc and were well beyond the resources and scope of this experiment. Interpretation and statistical analysis were limited by lack of adequate replication and control of variables. Further statistical analysis was thus not considered nor recommended for these reasons.

CHAPTER 6

Effects of nitrogen fertiliser (urea) on pasture nutrient composition - results of a replicated split plot trial

PREFACE

Nitrogen use has been increasing rapidly on New Zealand dairy farms in recent years and is now averaging near 60-80 kg N/ha, with some farms using over 200 kg N/ha. Some dairy farmers resist using N because they have experienced cow health problems, poor production and weight loss after N application. The intention in the experiment reported in this chapter was therefore to examine in more detail the impact of nitrogen use on the nutritive value of spring pasture.

This chapter examines the effect of nitrogen fertiliser on the major pasture nutrients (crude protein, soluble carbohydrate, neutral detergent fibre, acid detergent fibre) and other pasture variables (herbage accumulation, dry matter % and brix measurement). Nitrogen use on pasture was identified as being likely to have a strong influence on pasture crude protein (CP) and soluble carbohydrate (SOLCHO) levels. Excess CP and low SOLCHO have been identified in earlier chapters as nutritional weaknesses of fresh pasture for dairy cows (Chapter 1, Sections 1.1.2, 1.4 and Chapter 2, Sections 2.1, 2.3). The literature on the effects of nitrogen application on pasture nutrients suggest that it is likely to exacerbate these nutritional weaknesses.

6.1 INTRODUCTION

Observations and experimental work in this thesis suggest that pasture nutrients tend to be unbalanced (in comparison to recommended nutrient levels) in spring and autumn with excessive crude protein levels and insufficient levels of readily fermentable carbohydrate (soluble carbohydrate and pectin) compared to nutritional recommendations for dairy cattle (NRC, 1989; Moller *et al.*, 1993; Muller, 1993; Ulyatt and Waghorn, 1993; Moller, 1996).

Nitrogen fertiliser is often used in spring and autumn to enhance dry matter production in the seasonal pasture based dairy system practised in New Zealand (O'connor *et al.*, 1989; Roberts and Thomson, 1989; Roberts *et al.*, 1992), although not always successfully (Roberts *et al.*, 1992). Responses in yield depend especially on soil temperature and moisture levels. There is some evidence in the scientific literature that use of nitrogen will further exacerbate the imbalance in nutrients noted above. Elevation of crude protein is mentioned in several references (Beheaghe and Carlier, 1973; Whitney, 1974; Ross *et al.*, 1978; Saibro *et al.*, 1978; Whitehead *et al.*, 1986; Korte *et al.*, 1987; Van Vuuren *et al.*, 1992; Beever, 1993; Delaby *et al.*, 1995; Huguet and Gillet, undated) with varying rates of N and applied to various pasture species. Increased NPN (non protein nitrogen) (Korte *et al.*, 1987; Beever, 1993), increased nitrates (Saibro *et al.*,1978; Whitehead *et al.*, 1986), increased rumen degradability (Van Vuuren *et al.*, 1992), increased digestibility (Wilson and Mannetje, 1978; Korte *et al.*, 1987; Delaby *et al.*, 1995), reduced dry matter % (Gomide *et al.*, 1969; Beheaghe and Carlier, 1973; Korte *et al.*, 1987; Huguet and Gillet, undated) reduced soluble carbohydrates (Beheaghe and Carlier, 1973; Saibro *et al.*, 1978; Wilson and Mannetje, 1978; Ross *et al.*, 1978; Westhafer *et al.*, 1982; Korte *et al.*, 1987; Delaby *et al.*, 1995; Huguet and Gillet, undated), reduced fibre levels (Delaby *et al.*, 1995), no change in fibre levels (Korte *et al.*, 1987; O'connor, undated) have also been recorded. Reduced dry matter intake of nitrogen fertilised pasture has also been noted (Dittrich and Winklemann, 1994; Mackle *et al.*, 1995). Some dairy farmers would prefer not to use N fertiliser because they see variable responses and sometimes apparent weight loss and diarrhoea in cows consuming nitrogen fertilised pasture. The palatability of N fertilised grass is sometimes also questioned.

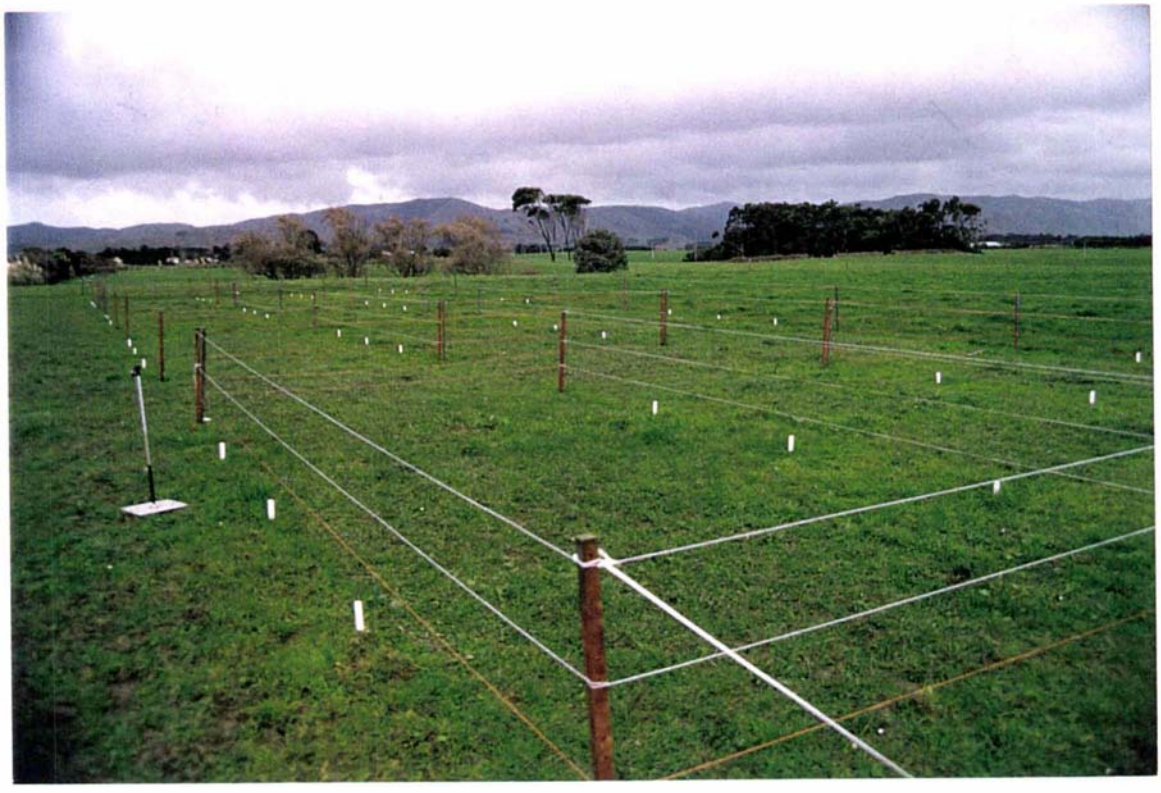
This trial was designed to examine the effects of nitrogen fertiliser (urea) application at different rates on the major nutrient components of typical dairy pasture in spring. An attempt was made to mimic the range of "normal" management decisions in relation to nitrogen fertiliser on dairy farms with 3 separate start dates for the trial, four rates of N applied and regular sampling over 8 weeks after N application.

6.2 METHOD

The experiment was laid out on an area of approximately 15 m x 30 m fenced off from one paddock at the Massey University Dairy Research Unit, Palmerston North in the winter of 1993. There were four replicates of a plot trial involving four rates of N (0, 20, 40, and 80 kg N per hectare) and three start dates for sampling. The trial was designed to mimic the range of likely management strategies employed on dairy farms as far as N application was concerned. The three start dates were included in anticipation of increasing ground temperatures as spring arrived. For each start date pasture samples were collected on four occasions 2, 4, 6 and 8 weeks after application of N. The trial could be described as 4 replicates of a split plot design with starting dates randomised on the main plot and nitrogen levels randomised on the subplots (for ease of collection the sampling dates were not randomised on each individual N plot) (see Figure 6.1).

The trial area was prepared by hard grazing with dairy cows 5 weeks before each start date to approximately 1200 kg DM (or 2 cm stubble). On each of the start dates N was applied at the specified rates after the pasture was lightly rolled to remove uneven soil levels and mown to 1000 kg DM/ha (assessed by using a rising plate meter with formula = (measurement x 158) + 200 = kg DM/ha). Faecal pats were removed after the last cow grazing as it was felt these may interfere with results. Soil temperature (10 cm deep) was recorded daily at 8.00 am throughout the trial period. Nitrogen was applied by dissolving urea in water then sprinkling it on the required area at the specified rate.

Plate 6.1 Site of replicated plots at Massey University.



Start Date 1, when N was applied, was on August 15, Start Date 2 was on August 31 and Start Date 3 was on September 14. Sampling began 2 weeks after N application for Start Date 1, on 31 August and then every 2 weeks after this on 14 September, 26 September and 12 October. The same pattern was followed for the following two start dates. This resulted in the following pattern for sampling (Table 6.1). On each date four replicates were sampled for each N rate:

Table 6.1 Sampling pattern

Start Date 1 Aug 15	Sample 1 Start Date 1 Aug 31	Sample 2 Start Date 1 Sept 14	Sample 3 Start Date 1 Sept 26	Sample 4 Start Date 1 Oct 12		
	Start Date 2 Aug 31	Sample 1 Start Date 2 Sept 14	Sample 2 Start Date 2 Sept 26	Sample 3 Start Date 2 Oct 12	Sample 4 Start Date 2 Oct 26	
		Start Date 3 Sept 14	Sample 1 Start Date 3 Sept 26	Sample 2 Start Date 3 Oct 12	Sample 3 Start Date 3 Oct 26	Sample 4 Start Date 4 Nov 9

Each sampled area consisted of 2 square meters (2 m x 1 m). Pasture was sampled using a mower and pasture was cut back to the same residual level as at the start of the trial (1000 kg DM , or 1-1.5 cm stubble). Samples were placed in a chillibin containing crushed ice and transferred to a freezer after weighing. A subsample was dried in a force fan oven and reweighed. This allowed calculation of DM% and DM grown/ha/day. Another subsample was freeze dried and then ground through a Wiley mill grinder (1 mm sieve) and sent to Penn State University for NIRS analysis for pasture nutrients. Previous calibrations at Penn State were used for the CP, ADF and NDF analysis based on methods outlined by Shenk and Westerhaus (1994) and SOLCHO levels were analysed separately at AgResearch, Palmerston North using their NIRS calibrated to the soluble carbohydrate test as described by Southgate (1976).

Hemicellulose was estimated from the difference between NDF and ADF and also subjected to anova to detect differences between N rates and start dates. Fermentable carbohydrate (NDF + SOLCHO) was also analysed in a similar manner.

Brix values were assessed for their value in predicting SOLCHO in pasture. This was done by applying modified vise grips to a sample of pasture to extract intracellular fluid, which was then placed on a brix refractometer for a reading (Campbell and Hume, 1970).

Pasture accumulation effects following N application were examined both for the current accumulation rate and the cumulative accumulation rate.

6.3 RESULTS

Analysis of variance using Genstat (1993) was performed on the nutrient data.

Three start dates were used where N was applied to new areas prepared as specified in the methods. These dates were 15 August, 31 August, and 14 September. Sampling began 2 weeks after each start date and continued fortnightly on 3 more occasions from

pasture left since N application on the start date. This allowed examination of the progression of effects on pasture of N application over 8 weeks as well as with 3 different application times (start dates). Four replicates of each sample were collected. Start dates were randomised within the replicated areas and N rates were randomised within each start date, but sampled area within each N rate was not randomised for ease of sampling.

6.3.1 Mean results

Tabulated results are presented in Table 6.2-6.4 for mean herbage accumulated and herbage accumulation rate for the four replicates. Table 6.5 presents means and SED for the net herbage accumulated for all start dates combined.

Soil temperature data (10 cm deep) are presented below:

Soil temperature (weekly mean at 8.00 am, 10 cm depth)

15 August	7.8
31 August	9
14 September	10
26 September	10
12 October	12.5
26 October	13
9 November	13

Table 6.2 **Mean herbage accumulated and herbage accumulation rates (kg DM/ha daily) of four replicates - Start date 1.**

Herbage accumulated (kg DM/ha)	Kg N applied	Kg DM/ha 1st sample	Kg DM/ha 2nd sample	Kg DM/ha 3rd sample	Kg DM/ha 4th sample
	0	1100	1300	1550	2700
	20	1150	1420	1950	3400
	40	1140	1440	2100	2800
	80	1160	1500	2500	3500
HAR (kg/ha/d) (current)		**Kg DM/ha/d**	**Kg DM/ha/d**	**Kg DM/ha/d**	**Kg DM/ha/d**
	0	6	12.5	18	82
	20	9	17	38	104
	40	9	19	47	50
	80	10	21	71	71
HAR(kg/ha/d) (cumulative)		**Kg DM/ha/d**	**Kg DM/ha/d**	**Kg DM/ha/d**	**Kg DM/ha/d**
	0	6	10	13	30
	20	9	14	22	41
	40	9	15	26	32
	80	10	17	36	43

Table 6.3 **Mean herbage accumulated and herbage accumulation rates (kg DM/ha daily) of four replicates - Start date 2.**

Herbage accumulated (kg DM/ha)	Kg N applied	Kg DM/ha 1st sample	Kg DM/ha 2nd sample	Kg DM/ha 3rd sample	Kg DM/ha 4th sample
	0	1250	1275	2300	4100
	20	1220	1450	2760	4600
	40	1330	1640	3100	4200
	80	1310	1900	3500	5000
HAR(kg/ha/d) (current)		**Kg DM/ha/d**	**Kg DM/ha/d**	**Kg DM/ha/d**	**Kg DM/ha/d**
	0	17	2	73	128
	20	16	16	94	131
	40	24	19	104	79
	80	22	37	114	107
HAR(kg/ha/d) (cumulative)		**Kg DM/ha/d**	**Kg DM/ha/d**	**Kg DM/ha/d**	**Kg DM/ha/d**
	0	17	10	31	56
	20	16	17	42	64
	40	24	24	50	57
	80	22	34	60	71

Table 6.4 Mean herbage accumulated and herbage accumulation rates (kg DM/ha daily) for four replicates - Start date 3.

Herbage accumulated (kg DM/ha)	Kg N applied	Kg DM/ha 1st sample	Kg DM/ha 2nd sample	Kg DM/ha 3rd sample	Kg DM/ha 4th sample
	0	1150	1920	3200	3600
	20	1200	2050	3600	4600
	40	1320	2400	4300	5000
	80	1300	2600	4300	5300
HAR (Kg/h/d) (current)		**Kg DM/ha/d**	**Kg DM/ha/d**	**Kg DM/ha/d**	**Kg DM/ha/d**
	0	13	48	80	29
	20	17	53	110	71
	40	27	67	135	50
	80	25	81	121	71
HAR (Kg/h/d) (cumulative)		**Kg DM/ha/d**	**Kg DM/ha/d**	**Kg DM/ha/d**	**Kg DM/ha/d**
	0	13	33	52	47
	20	17	37	62	64
	40	27	50	79	72
	80	25	58	52	76

Herbage accumulated represents the actual DM weighed from each plot from the start of the trial. Current HAR represents the growth during each 2 week period as opposed to the cumulative HAR which represents overall growth over the trial period from N application.

Table 6.5 Net herbage accumulated (kg DM/ha) means and SED for the combined start dates[1].

N rate	0	20	40	80	SED	SED
					Start date	N rate
Sampling date					4 replicates	3 replicates
1st sample	170	190	263	258	25.8**	29.8*
2nd sample	498	640	821	1010	91.0***	105.0**
3rd sample	1344	1766	2169	2447	106.2***	122***
4th sample	2485	3172	3036	3576	234.6***	270.9*

[1]Footnote: Data were lost from individual start dates but combined rates were still available.

6.3.2 Correlation matrix for all the trial data

Table 6.6 Correlation data across treatments and through time[1].

	N rate applied	Sampling date	KgDM accum	HAR Instant	HAR Start	Soil Temp	%NDF	%CP	%ADF	%SolCHO	NDF - ADF	NDF+ CHO
N Rate applied	1.00											
Sampling Date	0.00	1.00										
KgDM accumulated	0.19	0.90	1.00									
HAR Inst	0.16	0.73	0.81	1.00								
HAR from start	0.27	0.90	0.94	0.84	1.00							
Soil Temp 10 cm	0.00	0.93	0.87	0.83	0.88	1.00						
%NDF	-0.07	0.09	0.30	-0.10	0.12	0.01	1.00					
%CP	0.22	-0.87	-0.87	-0.59	-0.74	-0.78	-0.43	1.00				
%ADF	-0.26	0.08	0.24	-0.08	0.06	-0.01	0.91	-0.45	1.00			
%SolCHO	-0.23	0.34	0.16	0.10	0.16	0.19	-0.38	-0.30	-0.43	1.00		
NDF-ADF	0.31	0.07	0.26	-0.08	0.18	0.05	0.68	-0.18	0.31	-0.11	1.00	
NDF+CHO	-0.18	0.26	0.40	-0.06	0.21	0.11	0.90	-0.60	0.77	0.07	0.68	1.00

[1]DM grown=pasture level sampled, CP%=crude protein % in pasture, SOLCHO=Soluble carbohydrate level, NDF=Neutral detergent level, Temp=10 cm soil temperature, HAR-instant=fortnightly growth rate, Nrate=0,20,40,80 kg N applied, Day=day of trial between 1/9 and 10/11, HAR-start=growth rate from time of N application, NDF-ADF="hemicellulose", NDF+CHO="fermentable carbohydrates". Correlations significant at $P < 0.05$ when r exceeds 0.19; $P < 0.01$ when r exceeds 0.25 and at $P < 0.001$ when r exceeds 0.32. Some relationships should be expected as one is a function of the other, but have been retained in the matrix for completeness.

This correlation matrix includes variation from all sources, including seasonal change.

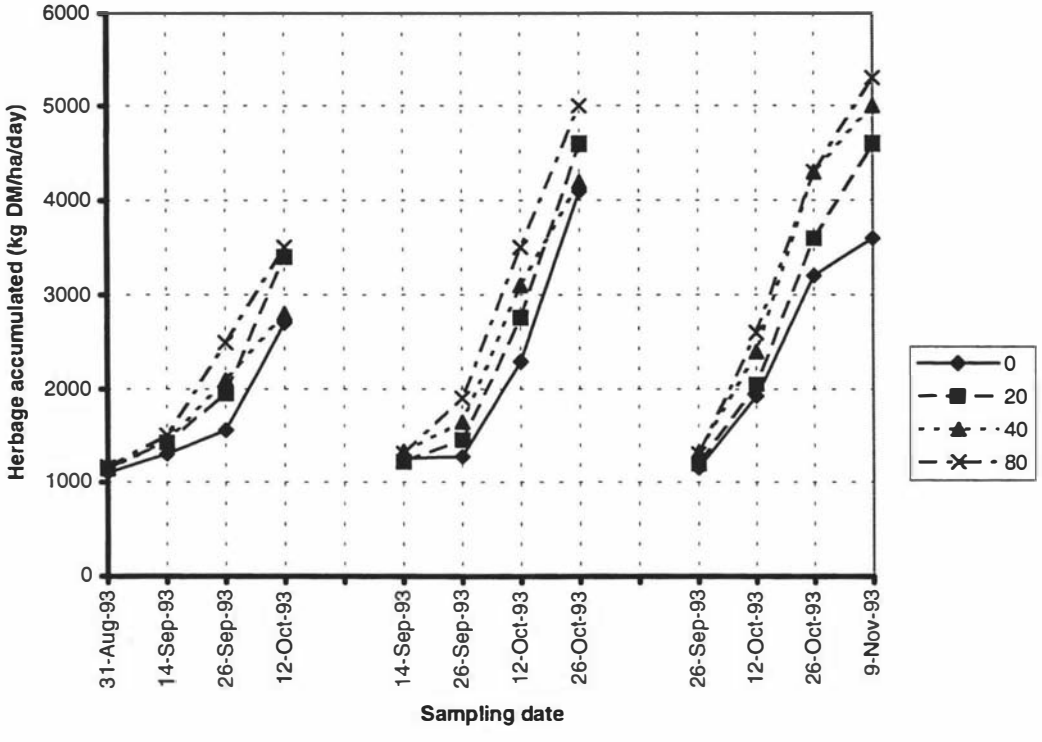
There were strong negative correlations between DM accumulated and CP present, and strong positive correlation of temperature with HAR, negative correlation of fibre (NDF) with soluble carbohydrate and strong correlation of growth rates with DM present (see also Figures 6.1-6.11). Hemicellulose expressed as the difference between NDF and ADF correlated weakly with increased HA (Figure 6.13).

Results are presented as time trends for each parameter for all 3 start dates and the 4 N-rates. Each point represents the mean of 4 replicates. The anova results are tabulated for each pasture nutrient measured and presented according to each sampling date.

6.3.3 Herbage accumulation

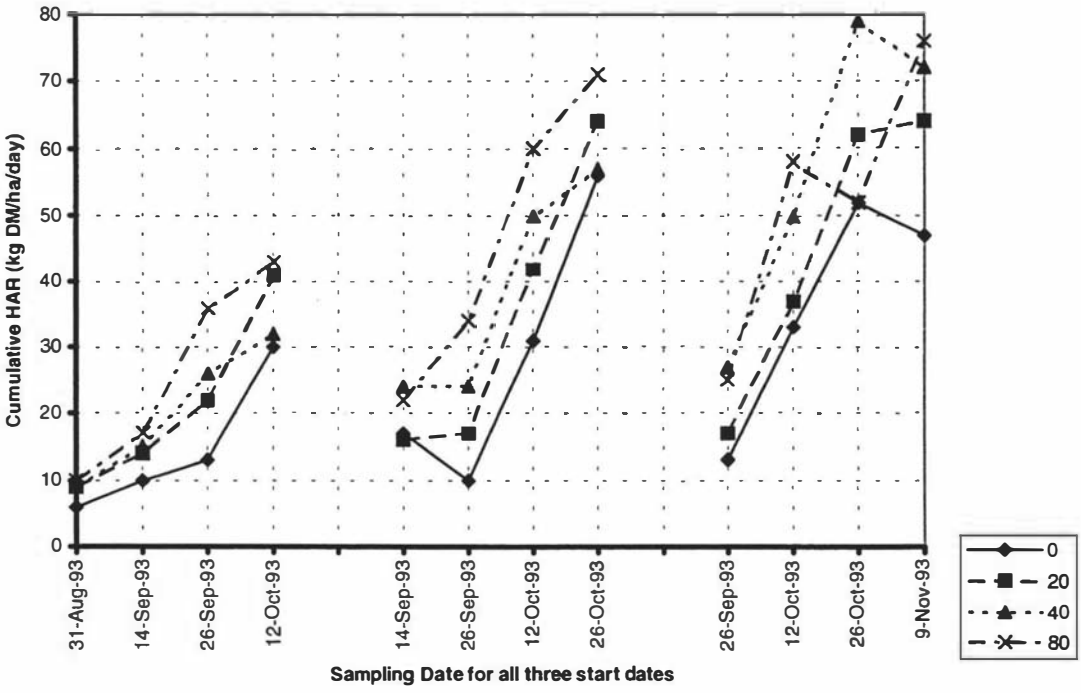
The following Figures present information about herbage accumulation during this trial (see also Tables 6.2-6.4). Unless stated otherwise the herbage accumulation rate (HAR) measure is the fortnightly or "current" HAR as opposed to the cumulative growth rate from when N was applied. In general, the growth achieved with N application was considerably greater than without N. Different rates of N did not necessarily achieve greater pasture growth with increased N applications but generally this was the case (Tables 6.2-6.4, Figures 6.2-6.4). Pasture growth slowed as greater maturity occurred (more HA). This occurred earlier with no N application (see Start Date 3, Figure 6.1 and 6.2 and Figures 6.4-6.6).

Figure 6.1 Pasture accumulated (HA) from time of N application versus date of sampling.



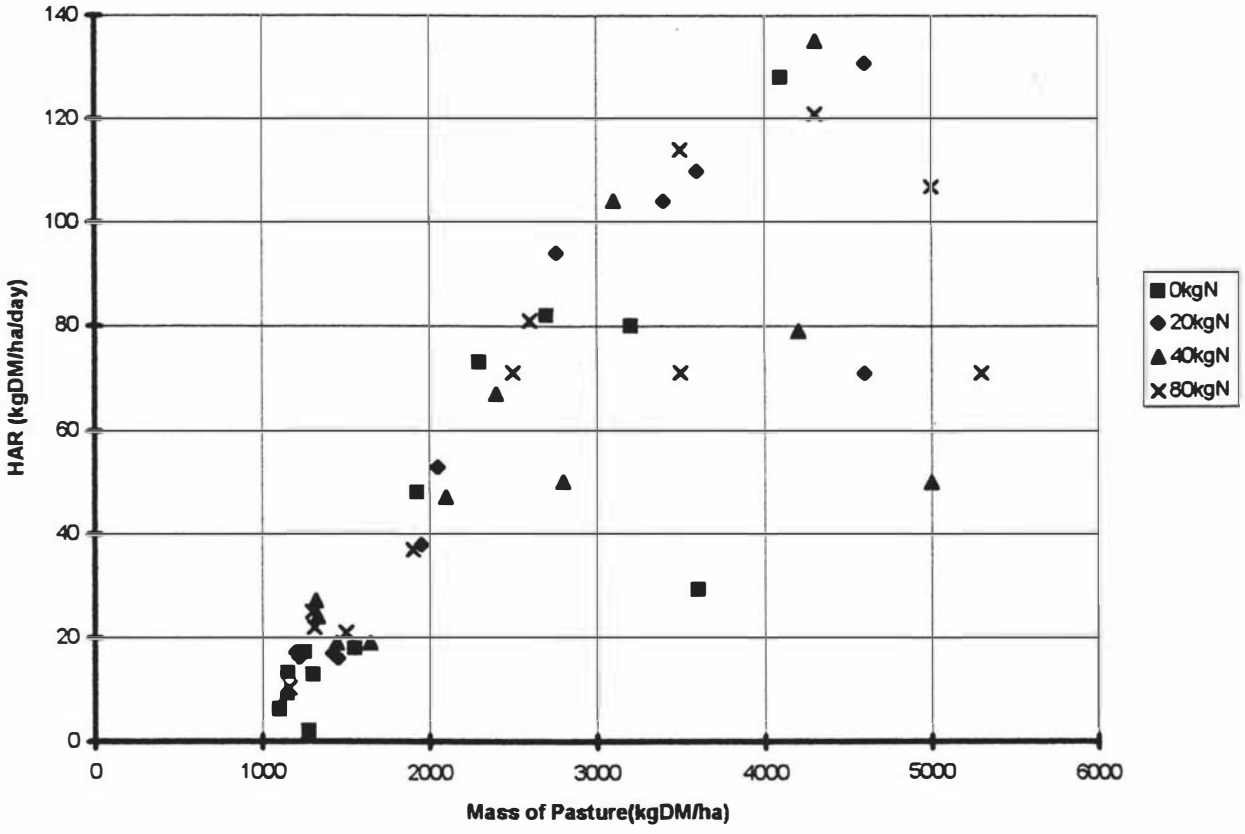
All growth rates accelerated in late September whether N was applied or not. Differences in response between N rates were most pronounced 4 weeks after N application (2nd sample) and with N application near September 1 (soil temperature 9°C at September 1). If N was applied in mid August then differences were not obvious between N rates for 6 weeks (soil temperature 7.8 at August 15). Statistical significance of differences are presented in Table 6.5

Figure 6.2 Pasture accumulation rate (HAR) from time of N application versus date of sampling.



HAR increased with increased herbage mass present from 1000 to 3000 kg DM/ha, but there was no clear relationships at greater levels of herbage mass whatever the N rate applied. Data from each start date showed a similar pattern (Figure 6.3; Tables 6.2-6.4, 6.6). See Table 6.6 for correlations.

Figure 6.3 Current herbage accumulation rate (kg DM/ha/day) versus herbage mass at sampling.



6.3.4 Efficiency of N response

Extra dry matter accumulated per kg N applied above the control (0 kg N) dry matter accumulated was highest for 20 kg N and especially at 6 and 8 weeks after N application. Responses to 20 kg N/ha were 20-30 kg DM/kg N at 6 weeks after N application, and 20-48 kg DM/kg N after 8 weeks. Application of 40 kg N and 80 kg N were intermediate in response per kg N, ranging from 12-30 kg DM/kg N at 6 weeks and 3-35 kg DM/kg N at 8 weeks. The response to 20 kg N/ha was slower to take effect, however, with differences only becoming apparent at 4 weeks after N application.

6.3.5 Acceleration of HAR

When HAR was plotted against HA, the point at which growth rates accelerated can be approximately identified for each start date (Figs 6.4-6.6). Figures for Start date 1 and 2 indicate marked HAR acceleration at 1300-1600 kg DM/ha. This was not apparent at Start date 3 however, where a steady increase in HAR occurred with higher DM present (HA). At some point between 3000 and 4500 kg DM/ha HAR fell at all sites. This occurred at lower HA for lower N application rates.

Figure 6.4 Current herbage accumulation rate versus herbage accumulated for Start date 1.

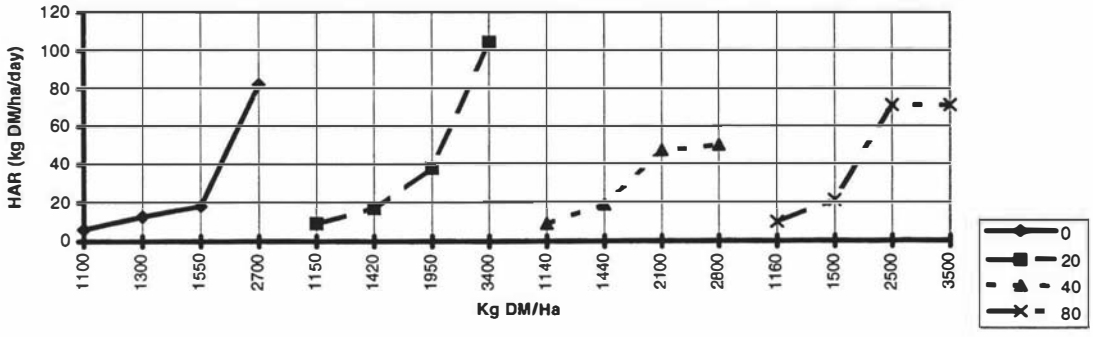


Figure 6.5 Current herbage accumulation rate versus herbage accumulated for Start date 2.

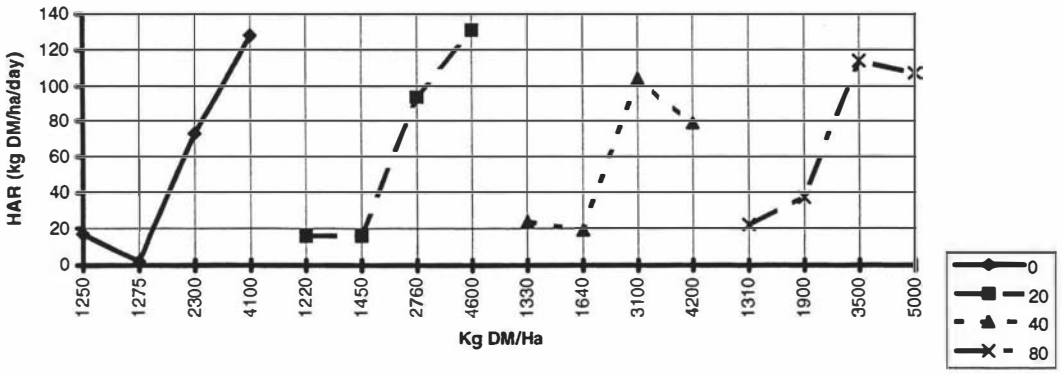
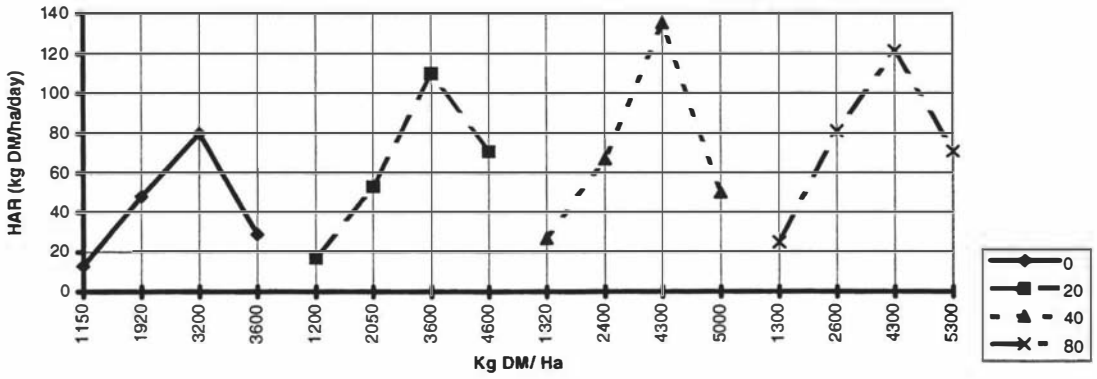


Figure 6.6 Current herbage accumulation rate versus herbage accumulated for Start date 3.



6.3.6 Dry matter %

Significant reductions in dry matter % (DM%) occurred with N use at the third sampling dates for all start dates combined ($P < 0.05$), but not at the fist, second and fourth sampling dates (data not tabulated). SED for DM% were 1.3, 1.8, 0.7 and 0.9% for first, second, third and fourth samplings. This effect had disappeared by 8 weeks from N application (Figure 6.7). Dry matter % declined at the 2nd and 3rd sampling for all four N rates including the control (0 kg N) indicating this effect was not only related to N use.

6.3.7 Crude protein

The crude protein values are presented in Figure 6.8. Crude Protein levels were positively influenced by N application rate. This effect was most marked at the first and second samplings (Figure 6.8 and Table 6.7) and lasted for approximately 4-6 weeks after N application. The effect of N waned more rapidly at the later start dates. Start date had a negative influence on crude protein, particularly on the third and fourth samplings. These effects of N rate were significant at the first, second and third sampling dates ($P < 0.001$, $P < 0.001$, $P < 0.01$) (Table 6.7). Although not significant the N effect on CP appeared to be reversed at the 4th sampling (Figure 6.8, Table 6.7). Protein in pasture declined at each sampling after N application and this was more

pronounced at consecutive start dates. Pasture CP increased by approximately 0.075% per kg N applied in the first 4-6 weeks after N application.

Figure 6.7 Dry matter % for the four N rates versus sampling date.

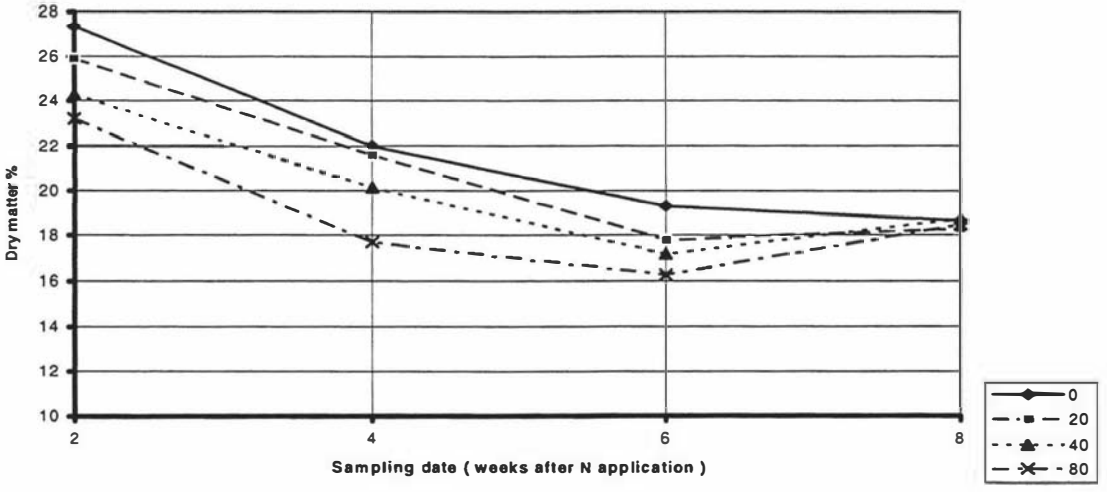


Figure 6.8 Crude protein % in pasture with the four N rates versus sampling date.

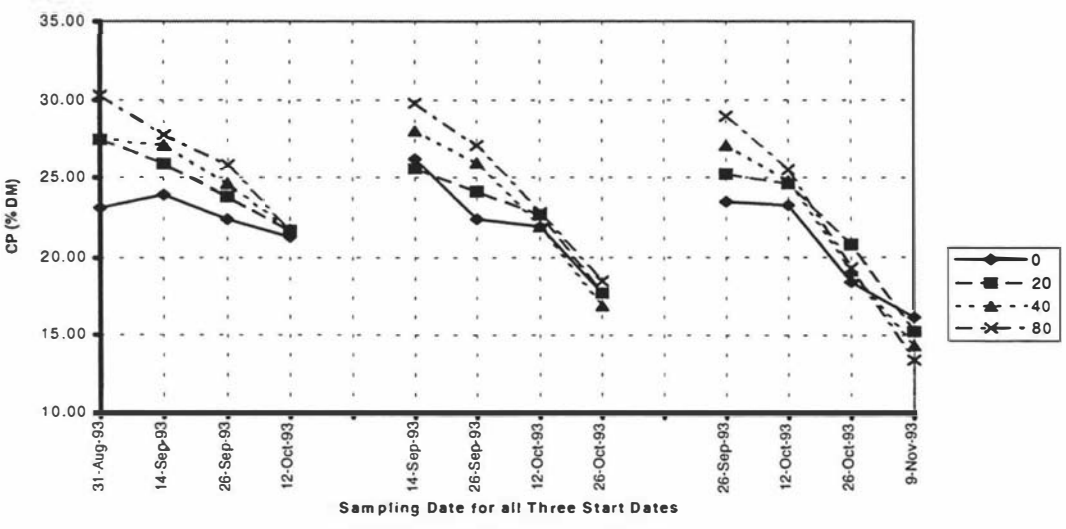


Table 6.7 SED and means for crude protein (%DM)

CP(%DM)	Start Date	N Rate	Start Date & N Rate
Replicates	16	12	4
1st sample	0.82	0.64***	1.26
2nd sample	1.18	0.49***	1.39
3rd sample	0.59***	0.46**	0.91+
4th sample	0.68***	0.43	0.93

CP	0 kg N	20 kg N	40 kg N	80 kg N	Mean
1st sample					
Start Date 1	23.1	27.5	27.5	30.3	**27.1**
Start Date 2	26.2	25.6	28.0	29.8	**27.4**
Start Date 3	23.5	25.2	27.1	29.0	**26.2**
Mean	**24.3**	**26.1**	**27.5**	**29.7**	
2nd sample					
Start Date 1	23.9	25.9	27.1	27.8	**26.2**
Start Date 2	22.4	24.1	26.0	27.1	**24.9**
Start Date 3	23.2	24.7	24.8	25.5	**24.6**
Mean	**23.2**	**24.9**	**26.0**	**26.8**	
3rd sample					
Start Date 1	22.4	23.8	24.6	25.8	**24.2**
Start Date 2	21.9	22.7	21.9	22.8	**22.3**
Start Date 3	18.5	20.8	19.1	19.3	**19.4**
Mean	**20.9**	**22.4**	**21.9**	**22.7**	
4th sample					
Start Date 1	21.3	21.7	21.7	21.6	**21.6**
Start Date 2	17.7	17.7	16.9	18.5	**17.7**
Start Date 3	16.2	15.2	14.3	13.4	**14.8**
Mean	**18.4**	**18.2**	**17.7**	**17.8**	

6.3.8 Soluble carbohydrates

Soluble carbohydrate levels are presented in Figure 6.9. These results were analysed by the AgResearch NIR at Grasslands, Palmerston North on the same dried samples used at Penn State University.

Soluble carbohydrates were negatively affected by N rates in the order of 1-1.5% of DM. These effects were significant ($P < 0.05$) on two of the sampling dates only (Table 6.8). Patterns were difficult to discern in soluble carbohydrate levels and the day of sampling seems to have been important. Although not significant, there is some indication that the N effect on soluble carbohydrate lasted longer than 8 weeks (Figure 6.9).

Figure 6.9 Soluble carbohydrate % in pasture with the four N rates vs sampling date.

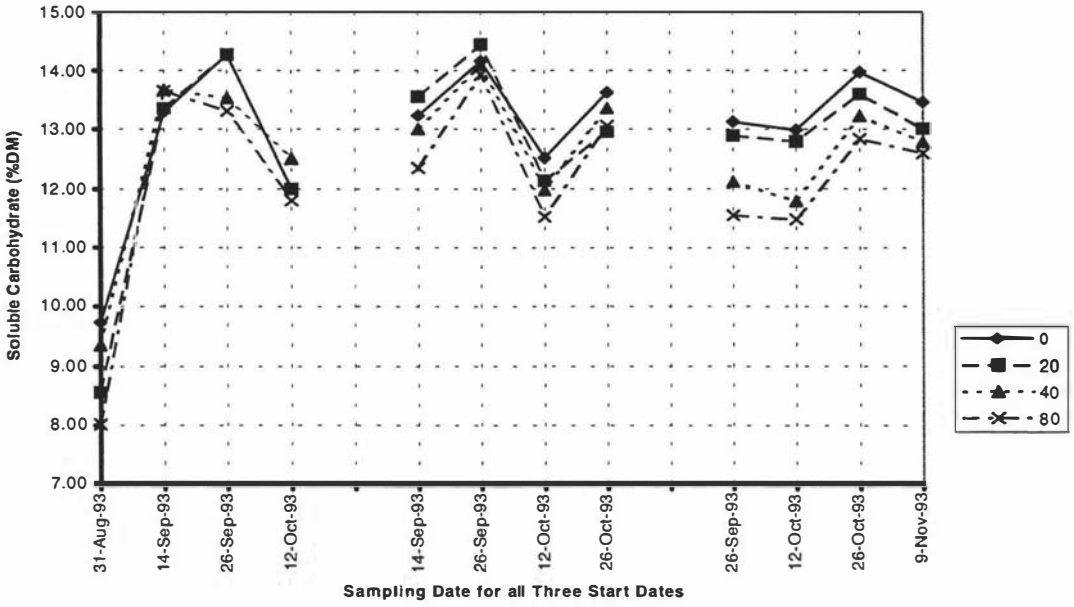


Table 6.8 SED and means for soluble carbohydrate (%DM)

Sol CHO (%DM)	Start Date	N Rate	Start Date & N Rate
Replicates	16	12	4
1st sample	0.52***	0.41*	0.80
2nd sample	0.23***	0.28	0.48
3rd sample	0.62+	0.36*	0.82
4th sample	0.35*	0.29	0.49

Sol CHO	0 kg N	20 kg N	40 kg N	80 kg N	Mean
1st sample					
Start Date 1	9.7	8.6	9.4	8.0	**8.9**
Start Date 2	13.2	13.6	13.0	12.4	**13.1**
Start Date 3	12.9	13.2	12.1	11.6	**12.5**
Mean	**11.9**	**11.8**	**11.5**	**10.6**	
2nd sample					
Start Date 1	13.3	13.4	13.7	13.7	**13.5**
Start Date 2	14.2	14.4	14.1	13.9	**14.2**
Start Date 3	12.9	12.9	11.8	11.5	**12.3**
Mean	**13.4**	**13.6**	**13.2**	**13.0**	
3rd sample					
Start Date 1	14.3	14.3	13.5	13.3	**13.9**
Start Date 2	12.5	12.1	12.0	11.5	**12.0**
Start Date 3	14.2	13.6	13.2	12.8	**13.5**
Mean	**13.7**	**13.3**	**12.9**	**12.6**	
4th sample					
Start Date 1	12.0	10.9	12.5	11.8	**11.8**
Start Date 2	13.6	13.0	13.4	13.1	**13.3**
Start Date 3	13.3	13.0	12.8	12.6	**12.9**
Mean	**13.0**	**12.3**	**12.9**	**12.5**	

6.3.9 Neutral detergent fibre

Neutral detergent fibre results are presented in Figure 6.10.

Neutral Detergent Fibre levels in pasture were mainly reduced by N application, especially at the first start date. The higher the N rate the lower the value. The negative effect of nitrogen on NDF was still noticeable at 6 weeks after N application for Start Date 1, but only noticeable at 4 weeks for Start Date 2 and 2 weeks for Start Date 3. The effect of N on NDF levels was initially negative, but as the time span from N application lengthened this effect was reversed into a positive effect. Both effects were statistically significant (see Table 6.9).

The reduction in NDF at 2 weeks after N application was of the order of 0.07% per kg N applied, and at 4 weeks after N application was 0.03% per kg N applied.

Figure 6.10 Neutral detergent fibre % in pasture with the four N rates versus sampling date.

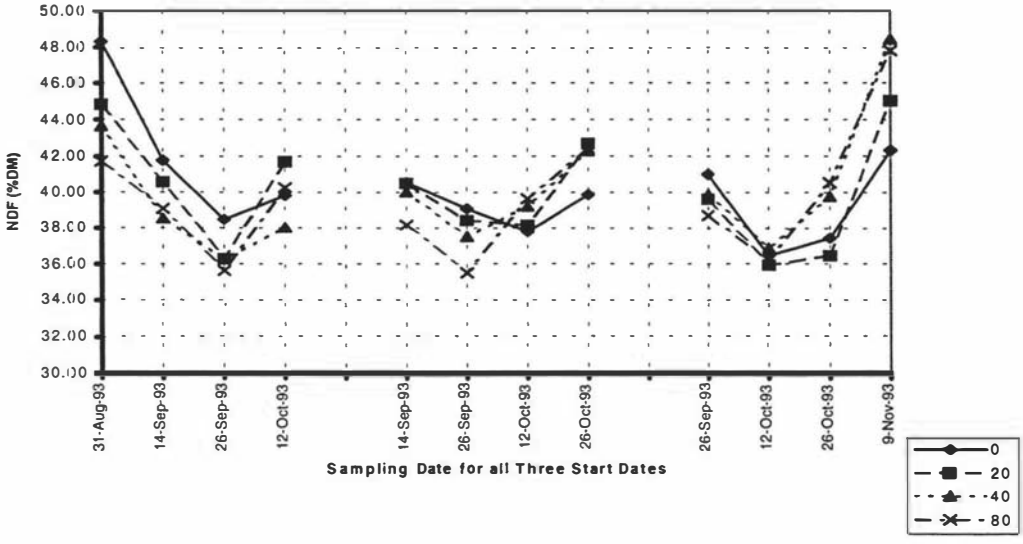


Table 6.9 SED and means for NDF (% of DM).

NDF(%DM)	Start Date	N Rate	Start Date & N Rate
Replicates	16	12	4
1st sample	0.96**	0.84***	1.58
2nd sample	1.10*	0.65*	1.46
3rd sample	0.60*	0.63+	1.12**
4th sample	0.86***	1.02*	1.76+

*P< 0.05 **P< 0.01 ***P< 0.001 +P< 0.1

NDF	0 kg N	20 kg N	40 kg N	80 kg N	Mean
1st sample					
Start Date 1	48.3	44.8	43.7	41.7	**44.6**
Start Date 2	40.6	40.5	40.1	38.2	**39.9**
Start Date 3	41.0	39.6	39.9	38.7	**39.8**
Mean	**43.3**	**41.7**	**41.2**	**39.6**	
2nd sample					
Start Date 1	31.8	40.6	38.6	39.1	**37.6**
Start Date 2	39.1	38.4	37.5	35.5	**37.6**
Start Date 3	36.5	36.2	36.9	36.2	**36.5**
Mean	**39.1**	**38.4**	**37.7**	**36.9**	
3rd sample					
Start Date 1	38.5	36.3	36.3	35.6	**36.7**
Start Date 2	37.8	38.2	39.2	39.7	**38.7**
Start Date 3	37.5	36.5	39.8	40.5	**38.6**
Mean	**37.9**	**37.0**	**38.4**	**38.6**	
4th sample					
Start Date 1	39.8	41.7	38.0	40.3	**40.0**
Start Date 2	39.9	42.7	42.3	42.5	**41.9**
Start Date 3	42.3	45.0	48.4	47.8	**45.9**
Mean	**40.7**	**43.1**	**42.9**	**43.5**	

6.3.10 Acid detergent fibre

Results for ADF analysis are presented in Figure 6.11 and Table 6.10.

Nitrogen influenced ADF levels in a similar manner to that for NDF. There was an initial negative influence of higher N use on ADF which then became positive. The negative influence on ADF appeared to last longer at the earlier Start Dates and was more distinct than that of NDF. The effect of N rate on ADF was significant at the first, second and third sample dates ($P < 0.001$, $P < 0.001$, $P < 0.05$). The reduced ADF at 2 and 4 weeks after N application was approximately 0.06% per kg N.

Figure 6.11 Acid detergent fibre % in pasture with the four N rates versus sampling date.

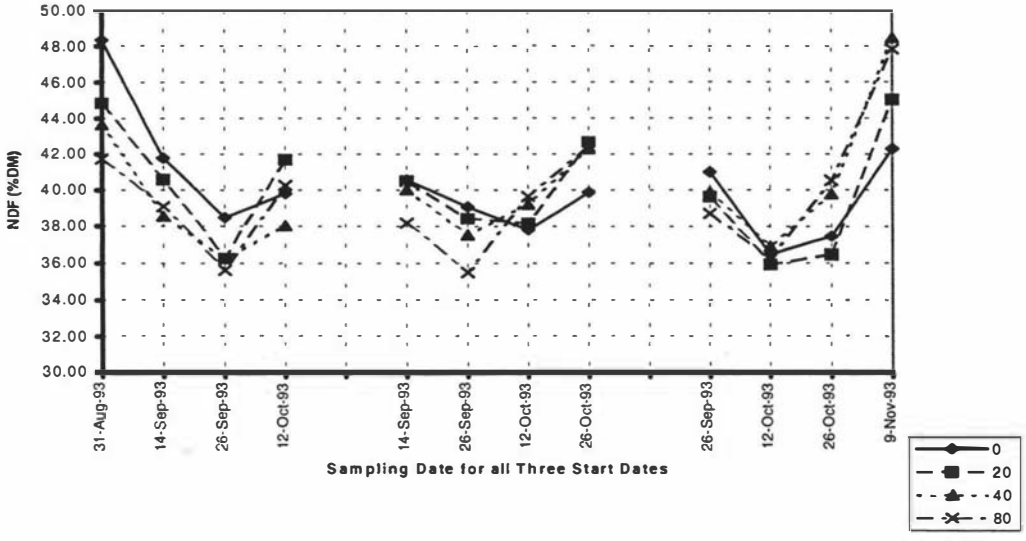


Table 6.10 SED and means for ADF.

ADF(%DM)	Start Date	N Rate	Start Date & N Rate
Replicates	16	12	4
1st sample	1.19***	0.66*	1.55
2nd sample	1.28	0.60***	1.56
3rd sample	0.27**	0.40*	1.56
4th sample	0.41***	0.45	0.78**

ADF	0 kg N	20 kg N	40 kg N	80 kg N	Mean
1st sample					
Start Date 1	33.4	30.2	29.4	27.9	**30.2**
Start Date 2	24.7	25.1	23.8	22.7	**24.1**
Start Date 3	27.5	26.0	25.9	24.2	**25.9**
Mean	**28.5**	**27.1**	**26.4**	**25.0**	
2nd sample					
Start Date 1	26.6	24.7	23.4	22.5	**24.3**
Start Date 2	26.3	24.5	23.0	22.2	**24.0**
Start Date 3	24.1	22.7	22.9	22.3	**23.0**
Mean	**25.7**	**24.0**	**23.1**	**22.3**	
3rd sample					
Start Date 1	25.3	23.4	24.0	23.0	**23.9**
Start Date 2	24.6	24.4	24.7	24.2	**24.5**
Start Date 3	25.7	24.5	25.5	25.6	**25.3**
Mean	**25.2**	**24.1**	**24.8**	**24.3**	
4th sample					
Start Date 1	25.6	25.9	24.5	25.0	**25.3**
Start Date 2	26.7	27.7	27.5	26.4	**27.1**
Start Date 3	27.4	29.1	29.9	30.6	**29.3**
Mean	**26.6**	**29.1**	**29.9**	**30.6**	

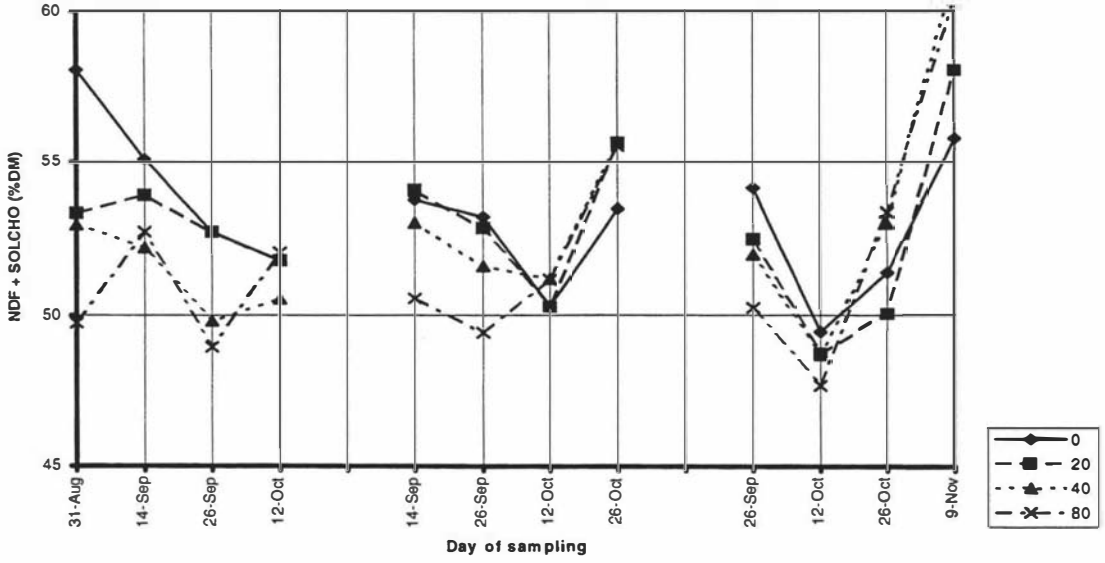
6.3.11 "Total Carbohydrate"

"Total carbohydrate" is a term that has been used to represent carbohydrate sources in general whether they be fibre or soluble carbohydrate. The addition of NDF (which in pasture will represent most of the fibre) and soluble carbohydrates (which will represent most of the other forms of carbohydrate in pasture) should give a measure of "total carbohydrate". A few minor carbohydrates in pasture like pectin may not be fully represented, however. NDF also contains some indigestible lignin.

Results are presented in Figure 6.12 (no tabulated data presented).

The total carbohydrate levels were significantly lower with higher N rates on the first and second sampling dates ($P < 0.001$) then changed to a significantly higher level on the third and fourth sampling ($P < 0.05$).

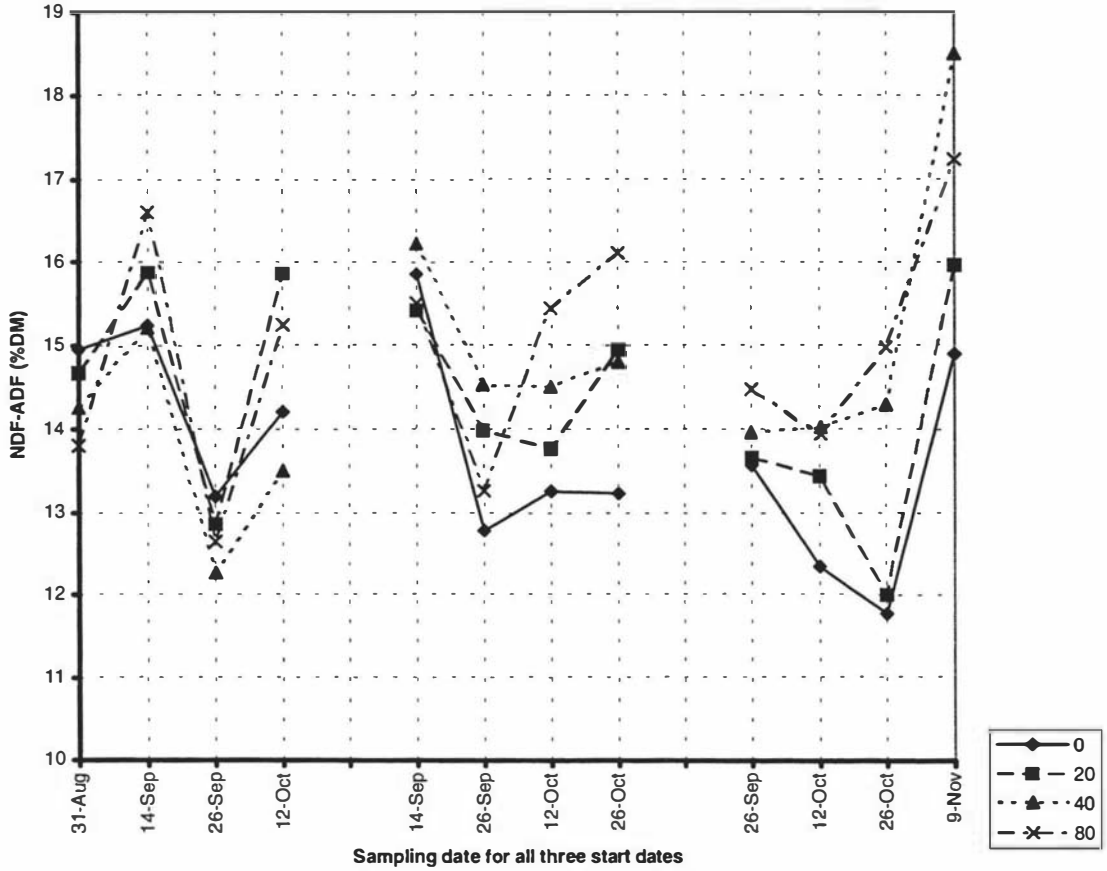
Figure 6.12 "Total" carbohydrate (NDF + SOLCHO) % in pasture with the four N rates versus sampling date.



6.3.12 Hemicellulose

Hemicellulose can be crudely estimated from the difference between NDF and ADF. This assumes that lignin percentage is the same in both ADF and NDF. Figure 6.13 represents hemicellulose levels in the different treatments (no tabulated data presented). Increased N use has increased hemicellulose at the 3rd and 4th sampling (except for Start date 1). Only small differences can be seen at the 1st and 2nd sampling with different N rates (Figure 6.13).

Figure 6.13 "Hemicellulose" (NDF-ADF) % in pasture with the four N rates versus sampling date.



6.3.13 Brix

Mean brix values for different levels of nitrogen are presented in Figure 6.14. Treatment effects were not statistically significant, but at 6 weeks from N application were close to $P < 0.1$.

Figure 6.14 Brix values for all start dates combined versus sampling date.

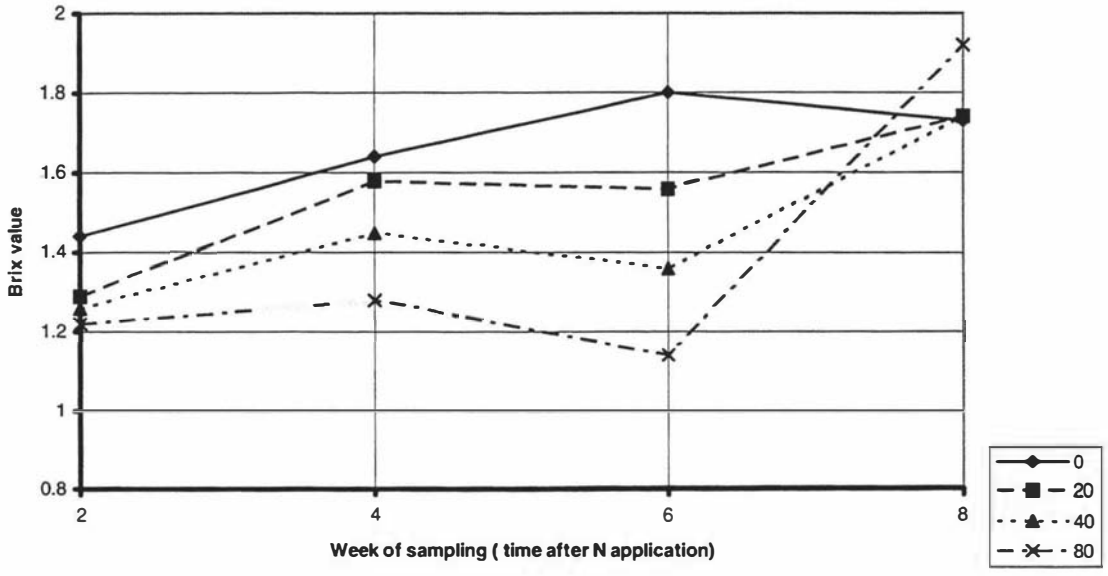


Table 6.11 Brix means and SED for combined start dates.

N rate	0	20	40	80	SED	SED
					Start date	N rate
Sampling date					4replicates	3 replicates
1st sample	1.44	1.29	1.26	1.22	0.15	0.17
2nd sample	1.64	1.58	1.45	1.28	0.15	0.17
3rd sample	1.80	1.56	1.36	1.14	0.20	0.23
4th sample	1.73	1.74	1.74	1.92	0.18	0.21

6.4 DISCUSSION

It is likely that several variables have influenced the experimental results. In particular, rising ground temperature, increasing day length, increasing maturity of the pasture, and the reproductive phase of ryegrass plant growth were or would all have been occurring during the trial period. These variables are all likely to have impacted later in the experimental period and at the 3rd and 4th sampling. These factors do not mask clear effects of N on CP, NDF, ADF, SOLCHO, DM%, and DM accumulated (HA).

6.4.1 N responses

The trial was performed in late winter/spring when ground temperature was increasing and day length increasing. Ryegrasses reached their reproductive stage at some time during the trial and this is likely to have affected the growth rates measured (Brougham, 1959; Whitney, 1974; Korte *et al.*, 1987). Increasing pasture maturity over approximately 3000 kg DM/ha appears to have affected levels of pasture nutrients measured and this has confounded some of the results near the last sampling dates for each start date as far as the N effects are concerned (Gomide *et al.*, 1969).

In general, increasing levels of N application in this trial have provided extra growth of pasture with a few exceptions. This is consistent with literature on this subject (Cowling and Lockyer, 1965; Field and Ball, 1978; Holmes, 1982) and some trials have linked this response to extra milk production (Bryant *et al.*, 1982; Roberts and Thomson, 1989; Thomson *et al.*, 1991; Penno *et al.*, 1996). The higher rates of N provided pasture of "grazeable" height (> 2000 kg DM) approximately 2 weeks earlier in this trial than no or smaller rates of N. However, growth rates of pasture receiving no N achieved high growth rates eventually, but at a later date. That is, nitrogen application advanced the timing of supply of increased dry matter. Application of 20 kg N was the most economic in terms of kg DM grown per kg N applied but did not provide the extra DM as quickly as higher N rates. This is in agreement with conventional recommendations for N use in New Zealand (Roberts and Thomson, 1989).

6.4.2 Herbage accumulation rates

In general, HAR (herbage accumulation rate) increased substantially with increasing N application. HAR increased with time from an initially low level following all dates of N application. HAR declined in the final sampling period of the 3rd Start date. This reduction in HAR with later sampling occurred at a lower herbage mass (HA) with 0 kg N applied (Figures 6.1-6.6).

Growth rates followed the pattern described by Brougham (1956, 1959) and Hodgson (1977, 1990) and Parsons (1988) with a sigmoid curve showing rapid increase in growth rate as pasture approached 1500 kg DM-1800 kg DM. Growth rate (fortnightly) slowed again somewhere in the 3000 kg DM-4500 kg DM region (see Figure 6.2 and Figure 6.3). The sigmoid curve was not visible in data from Start Date 3, presumably because the period of slow growth passed between the first and second samplings. There is currently some discussion about the validity of the sigmoid curve in dairy pasture as described by Brougham (1956, 1959). These results suggest that at least at the time of the year that this trial was performed the sigmoid growth curve (especially the "acceleration phase" at the start of the sigmoid curve) is a feature of dairy pasture growth, especially in cooler weather and shorter daylength conditions in late winter.

The data appear to suggest that growth is strongly influenced by DM present (ie. "That grass grows grass"). The literature on this topic does not appear to be consistent, with lax defoliation sometimes apparently growing more pasture (Kerrisk and Thomson, 1990) and in other studies less pasture (Fulkerson and Michell, 1987; Parsons, 1988; Roberts *et al.*, 1992). Increased leaf area index (LAI) is known to result in increased HAR at about these levels of HA but the relationship between LAI and HA does not appear to have been calculated for local conditions. The confounding variables mentioned above make conclusions on acceleration in HAR difficult in this data. HAR is known to accelerate in the reproductive phase of ryegrass growth. A more rigidly controlled experiment may be necessary to disentangle these factors if it was considered worthwhile . This is an important point in pasture management, particularly in winter, as any positive influence on growth at this time would be very useful (see Figures 6.2-6.6). The accelerated growth rate around 1500 kg DM to 1800 kg DM is less distinct on

Start date 3 but this is probably because the pasture passed this stage between fortnightly measurements.

Improved pasture growth responses occurred to higher N rates especially soon after N application - the effect was less marked at 8 weeks when 20 kg N reached similar growth rates to 80 kg N. This would tend to agree with conventional use of N fertiliser on pasture, where lower N rates are recommended unless the need for increased DM is great to replace a feed deficit (Roberts and Thomson, 1989). The efficiency of response (kg DM grown per kg N applied) was greater for lower rates of N applied and this would be expected from the literature also (Roberts and Thomson, 1989). Nitrogen use appeared to have kept pasture in a growing state for longer (see Fig 6.4). This effect was not consistent, but indicates another potential use for N in grazed pastures.

Soil temperature has had a strong influence on HAR as would be expected (Korte *et al.*, 1987). This is indicated in the correlation matrix (Table 6.6).

The results also suggest that grazing pasture at the 2500-3000 kg DM/ha HA level and leaving residual pasture HA near 1500 kg DM/ha would produce more dry matter on a given area because the acceleration phase of the sigmoid growth curve is taken full advantage of. This takes no account of other changes in the sward that may occur if this practice was followed, however.

6.4.3 Effect of N on nutrients in pasture

Higher N rates significantly reduced NDF levels 2, 4 and 6 weeks after N application (probably by increased leaf formation). This effect was reversed at 8 weeks to higher NDF levels with higher N rates. It is postulated that this latter effect was a maturation effect (an increased stem:leaf ratio) and not related to the N application per se (see tabulated mean data, Table 6.2) (Gomide *et al.*, 1969). Data in previous chapters supports the suggestion that this effect was from maturation (Chapter 5).

The effect of N on NDF can be quantified as approximately 0.07% per kg N reduction in NDF 2 weeks after application and approximately 0.03% per kg N reduction at 4 weeks. Higher N rates similarly reduced ADF levels highly significantly at 2, 4 and 6 weeks after N application. The effect of N on ADF lasted longer at Start date 1 compared with the later start dates. Again, a maturation effect on ADF appears to confound results at later samplings. Literature on the N influence on fibre is equivocal with increased cellulose in one study (Huguet and Gillet, undated), reduced cell wall polysaccharides in another study (Ford and Williams, 1973) and "no effect" in another (O'Connor, undated) .

Higher N rates had a highly significant effect on crude protein levels in pasture that lasted for 4-6 weeks after N application. Pasture maturity appeared to have a more dominant influence on crude protein level as length of pasture and time from N application increased. This result is consistent with results cited in the introduction. The earlier start date showed effects on pasture crude protein lasting longer than at later start dates. This is consistent with literature showing N responses are faster in warmer weather (Ledgard, 1989).

The increase in CP can be quantified as approximately 0.075% increase in CP per kg N applied for the first 4-6 weeks after application.

Higher N rates significantly reduced soluble carbohydrates on two of the sampling dates. Factors other than those measured in this trial appeared to have greater influences on soluble carbohydrates (see Fig. 6.9 for fluctuations between samplings), like weather conditions. Earlier work in this thesis also indicated the labile nature of soluble carbohydrate levels in pasture in comparison with other plant nutrients even when sampled in a consistent manner. The lower soluble carbohydrate levels noted with increased N use were not large in this study and this is consistent with the literature cited earlier. This will contribute to a lowered fermentable carbohydrate energy supply in the rumen, but lowered soluble carbohydrate does not appear to be a major effect of N fertiliser on it's own. The effect on soluble carbohydrate was greater at the third start date, when longer day length and warmer temperatures were present.

Dry matter % was reduced by N application also, especially at 2,4 and 6 weeks after N application. The reduced DM % can be quantified as approximately 0.075% per kg N for this period. When estimating dry matter present (HA) in pasture adjustment should be made for this change in dry matter % unless the method of estimation takes account of this (eg rising plate meter vs capacitance meter).

6.4.4 Correlations

Pasture DM present would appear to have some predictive value for crude protein % according to the correlation values (R = -0.85). It should be remembered, however, that this trial occurred in spring with pasture in a growing state and little sign of senescence. The same relationship may not occur in summer, for instance. As might be expected (Korte *et al.*, 1989) DM grown related strongly to temperature, growth rate, day of trial but perhaps surprisingly not strongly to N rate applied. This latter observation may relate more to the design of the trial rather than be a reflection of the degree of importance of the variables influence on growth rate, however.

Crude protein correlated negatively to soluble carbohydrate, NDF, temperature, growth rate and day of trial and was positively related to N rate. Some of these correlations can be explained by the timing of the trial in advancing daylength, increased maturity (length) of pasture and rising ground temperature. The reproductive stage of ryegrass occurs in these conditions and this is likely to have confounded the growth rates observed and fibre and protein levels (Korte *et al.*, 1989).

Soluble carbohydrate was negatively correlated to NDF but positively to growth rate, temperature and day of trial. Fermentable carbohydrate (NDF+SOLCHO) were negatively correlated to Crude protein.

N rate did not feature strongly in this data set as an influence on growth. This presumably indicates much stronger influences came from temperature, HAR etc. It may relate to the trial design as mentioned above, however.

6.4.5 Significance of the findings for dairy nutrition

Pasture CP can be further subdivided into non-protein nitrogen (NPN), peptides, nitrates, rumen degraded protein (RDP), rumen undegraded protein (RUDP or UIP), soluble protein, acid detergent insoluble nitrogen (ADIN) and other fractions. The nature of the increased crude protein with extra N applied has not been established in this experiment. Beever (1993) states that in fresh forages after N fertilisation the NPN component of crude protein may reach 40-50%. Other studies have reported smaller increases in NPN after N use (Dittrich and Winkelmann, 1994; Mackle *et al.*, 1995). Only a certain level of NPN is useful in the rumen and the rest is likely to be absorbed mainly as ammonia, which is then converted to urea in the liver and excreted (Kelly *et al.*, 1993). Toxic effects from circulating ammonia and urea on fertility have been recorded and the detoxification of ammonia in the liver requires considerable energy which is then not available for production. The increased crude protein levels after N application lasted for at least 4 weeks and sometimes 6 weeks in this trial (with the longer effect in the colder weather at Start date 1). At the times of the year that N was applied in this trial dairy farmers are often reducing their rotation length to shorter intervals than 4-6 weeks through necessity, or in anticipation of spring growth and to control pasture and keep it in a digestible state. Longer grazing intervals are difficult for the farmer to achieve at this time. This means that dairy farmers would be unable to avoid exposing their cows to at least some extra crude protein as a consequence of the N application. This is of importance if pasture is the sole diet, because it seems likely that high crude protein pasture will involve some milk production penalty (Danfaer *et al.*, 1980; Gordon, 1980; Fox *et al.*, 1990). Recommended crude protein levels in the cows diet at peak production are near 18% (NRC, 1989). Repeated N applications every 4-6 weeks or less are also likely to ensure that the cows will encounter pasture unbalanced by the N application.

On the other hand, the N effects noted in this trial could be seen as useful later in spring when farmers are attempting to maintain pasture quality when ryegrass is stalky and in its reproductive stage and protein levels are falling to suboptimal levels (see below), ie. the N is keeping the pasture less fibrous and consequently likely to have higher digestibility. Pasture control by aggressive management would also be necessary at this

stage because growth rates are high during the reproductive phase and N will accelerate this further. Stocking rate may not be sufficient to control the pasture on its own.

This effect could also be useful in summer when fibre levels increase and protein levels drop in pasture. The difficulty in summer may be to get an N response because of low moisture levels, however .

Another effect of the N application in the trial has been on ADF, NDF and soluble carbohydrate (the "total carbohydrates") levels which were reduced for 4-6 weeks by N, especially at the higher N rates. Only one reference to reduced fibre levels was located (Delaby *et al.*, 1995) and two references stating no effect of N on fibre (O'Connor, undated; Van Vuuren, 1992). The data show that cows consuming N fertilised pasture will consume a diet lower in potentially fermentable carbohydrates (NDF plus SOLCHO) as well as excessive in crude protein.

Protein metabolism is inextricably linked with energy metabolism (Horn *et al.*, 1979), especially in the rumen. The potential effect of this is to reduce capture of pasture protein by rumen microbes because they require fermentable energy to capture the pasture protein and convert it into microbial tissue. A cow consuming fresh pasture may already be consuming a diet considered marginal for fibre in some circumstances with lush pasture (NRC, 1989; Muller, 1993) and excessive for crude protein. Nitrogen fertilisation appears likely to exacerbate these imbalances. Muller (1993) states that NFC:RDP ratios of 3:1 are recommended for high productivity in dairy cows. Making assumptions for ash and fat content of pasture (10% and 5% based on published values, the NFC value can be calculated from 100-(NDF%+CP%+Fat%+Ash%). The RDP value can be estimated from published values at 80% of CP (Muller, 1993). This means that, for example the NFC:RDP ratio for the third sampling on the first start date was 1.1:1 for 80 kg N and 1.5:1 for 0 kg N. This indicates the degree of imbalance between fermentable energy in the rumen and protein and also the degree to which N use may exacerbate this.

At a slightly later date in the seasonal dairying calendar, N use may be useful in retaining digestibility as ryegrass is in the reproductive stage and is inclined to become

stalky. This would be likely to lead to higher DM intakes (Hodgson, 1977; Horn *et al.*, 1979) and hence productivity from N use resulting from an increase in digestibility.

An ideal supplement to balance a dairy cows diet closer to that recommended in early lactation when N is applied to pasture should then have extra carbohydrate (both fibre and soluble carbohydrate) and a reduced protein level (NRC, 1989). At the same time it should not have a markedly lower metabolisable energy value, which would also reduce productivity. Maize silage is such a supplement now commonly in use in dairy herds. Grass silage is not so suitable because much of the soluble carbohydrate has been converted to volatile fatty acids in the ensiling process and so is not available to bacteria as an energy source to assist with plant protein capture in the rumen (Beever, 1993; Thomas, 1996).

Weight loss and thin cows are common features of New Zealand dairy cows grazing pasture as well as relatively low productivity per cow. It would appear that the increased use of N in recent years on New Zealand dairy farms, particularly in winter and spring, could exacerbate some of these features, despite the nitrogen growing more dry matter. The potential penalties of the increased crude protein and reduced fibre would be offset by the increased dry matter grown. This trial was not designed to identify problems in cows consuming N fertilised pasture but does indicate the possible nature of adverse effects sometimes observed by the use of N. The effects on fibre, soluble carbohydrate and protein appear to be of practical significance as normal dairy farm grazing management at this time of the year is likely to result in cows grazing pasture with an imbalance of fermentable carbohydrates and crude protein. Theoretically this could lead to a reduced supply of NAN (non ammonia nitrogen) to the duodenum through poor rumen fermentation and hence poorer milk protein production. This effect was observed by Van Vuuren *et al.* (1992, 1991) where less NAN reached the small intestine on N fertilised grass. Reduced milk protein production from nitrogen fertilised pasture has been observed by Hermansen *et al.* (1994). Increased N application has led to less efficient plant protein use in other studies (Deenen and Lantinga, 1993; Mackle *et al.*, 1995).

Reduced dietary fibre levels in cows consuming N fertilised pasture would theoretically lead to lower milk fat production if total dietary levels became suboptimal and the excess protein penalty (caused mainly by energy used to detoxify ammonia in the liver into urea) would lead to reduced milk production and/or weight loss through limited ME intake. A high propensity to milk soon after calving as occurs in seasonal dairying in New Zealand would perhaps make it more likely that a cow would lose weight as well as produce less milk. There is some debate about the degree of protein penalty on lush pasture which still needs to be resolved in the New Zealand context. This is especially so because of our reliance on grazed pasture, and the potential losses that may be occurring.

Digestibility and ME values are sometimes calculated indirectly from analysed ADF and crude protein levels using a formula (Holland and Kezar, 1990). If this was used, digestibility and ME of pasture would be increased by N use. The true feed value of N fertilised pasture might be best measured by *in vivo* digestibility rather than a laboratory formula. This is usually impractical because of the cost of performing in vivo digestibility tests. Some feeding systems for dairy cattle now take account of fermentable carbohydrate and it's effect on protein uptake. This would seem to be a more desirable way to calculate the feed value of pasture. Literature would suggest that increased digestibility will lead to increased DM intakes (Hodgson, 1977; Wilson and Mannetje, 1978; Horn *et al.*, 1979; Wilson *et al.*, 1995). Visually this is not always the case on farms (eg. Mackle *et al.*, 1995), where cows will sometimes seek pasture in the paddock that has not received N fertiliser in preference to that which has. This effect (lack of palatability) may have been responsible for the lower DM intake with higher N use observed in other work (Dittrich and Winkelmann, 1994; Mackle *et al.*, 1995).

The effects on pasture nutrients noted in this trial are only of practical significance if the dairy cow is fed pasture of the appropriate length and within a specific time after N application. Longer rotation lengths and lower rates of N use are likely to reduce the chances of problems occurring with excess crude protein and lower fermentable carbohydrate levels. Applications of N in close to winter conditions (cold and wet) are likely to lengthen the time that N affects the nutrient balance.

There is some indication in the soluble carbohydrate results that depression of soluble carbohydrate may last longer than 8 weeks in some conditions although the results were not significant at 8 weeks. This may merit further investigation.

The scientific literature would suggest that the rumen degradable fraction (RDP) of crude protein is likely to be elevated by N use. Combined with the reduced "fermentable" carbohydrate found with N use in this trial, it is highly likely that cows grazing this pasture will have higher circulating blood urea levels and milk urea levels. High urea levels in comparison to overseas already exist in New Zealand cows grazing only pasture (Moller, 1991; Moller *et al.*, 1993; Moller *et al.*, 1995). These have been linked to reduced conception rates in dairy cows (Ferguson *et al.*, 1988; Moller, 1991; Moller, 1993), although the results to date are mainly circumstantial in New Zealand. Nitrogen use on pasture near the concentrated mating time in October/November could possibly affect conception rates.

An awareness of the effects of N on nutrients within pasture would assist the mainly pasture based dairy farmer in New Zealand avoid the potential detrimental effects on production that could occur. Awareness of the need to balance the increased protein in nitrogen fertilised pasture with additional fermentable carbohydrates would assist dairy cow performance from the pasture diet.

6.5 CONCLUSIONS

This trial indicates that crude protein is elevated and NDF, ADF and soluble carbohydrate are initially depressed by N use in a linear fashion with increasing N rate in some conditions. N use may exacerbate a "protein penalty" on milk production and may also create additional imbalances of fibre and soluble carbohydrate for dairy cows grazing lush pastures (NRC, 1989). These imbalances in pasture could also be considered an advantage if pasture is already too fibrous and too low in protein for optimal milk production as in the reproductive stage of ryegrass.

These effects on pasture nutrients are temporary and eventually overridden by pasture maturation. The practical significance for farmers of these effects will depend on management (rotation length, application rate, time of N application) decisions employed at this time.

The effects of N on pasture nutrients were more marked in cooler weather at the first start date. It would appear that N should be used with more caution in cooler weather if potential effects on cattle grazing the pasture are to be avoided .

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CHAPTER 7

Final Discussion

PREFACE

This final chapter combines the findings of the seven studies into concluding comments on each of the studies, explores themes common to all of the studies and then provides conclusions.

The thesis has attempted to concentrate on practical issues concerning commercial dairy farming in New Zealand. In particular, factors affecting variation in pasture nutrients (and especially CP) have been studied. The studies have crossed traditional disciplines of veterinary science, agronomy, and animal nutrition and attempted to link these as they apply to dairy cow performance. Commercial herds and dairy pastures were used in most of the studies with the aim of making the results of practical value to dairy farmers.

7.1 INITIAL HYPOTHESES

The initial hypothesis was that changes in nutrients within pasture were in part responsible for differences in herd reproductive performance, and that these changes would reflect in blood parameters within these herds.

There were clear and strong associations between blood and milk urea levels, pasture crude protein, pasture crude protein/soluble carbohydrate ratio, milk production and herd reproductive performance in the data presented in Chapter 1.

The hypothesis formed after obtaining the data presented in Chapter 1 was that dietary protein excess as indicated by elevated milk or blood urea could be responsible for the marked reductions in milk production and reproductive performance in dairy cows. High milk or blood urea levels reflect excess rumen degraded protein (RDP), the urea being produced in the liver following the absorption of rumen ammonia not used by rumen bacteria (Moore and Varga, 1996). Of total pasture protein (CP), the RDP component of CP is estimated at 60-85% (NRC, 1989; Muller, 1993). Subsequent work in this thesis (see late September and October in Chapters 1, 3, 4) and elsewhere (Trevaskis, 1996) indicates that the explanations for differences in herd or cow performance in relation to urea levels are more complex than this. Blood (and milk) urea levels will be altered by dietary protein content and degradability and also dry matter intake - that is, dietary RDP excess will elevate urea, and high DMI will multiply this effect. High urea levels indicate dietary RDP excess (Moore and Varga, 1996), but cows with high milk urea levels can still be milking well through high intakes. It is likely that the protein excess creates an energy penalty for detoxification in the cow's liver. This can be met by high ME intake, but at some cost to milk production compared to cows receiving recommended levels of dietary protein (Danfaer *et al.*, 1980).

An increased intake of readily fermentable carbohydrate (RFC) has the ability to reduce urea levels (in blood or milk, Chapter 3), probably through enhanced bacterial capture of the excess protein in the rumen, which then has the potential to be absorbed and to enhance milk production, particularly milk protein.

Little conclusive evidence of negative effects of high urea in blood or milk on most parameters of herd reproductive performance has been gathered in this thesis, although significant improvements in end of season empty cow percentages occurred following RFC supplementation (Chapter 3), in which milk urea was reduced by supplementation. This response may have been due to factors other than the reduction in urea levels however, eg improved ME intake at mating time.

Precise measurement of dietary intake, or indeed individual pasture nutrients, is difficult to achieve with grazed pasture. More controlled experiments where diets are hand fed, and responses measured in the rumen, blood, milk, bodyweight and DMI to pasture

protein manipulation by nitrogen application levels would help to clarify the extent of the protein penalty as it applies to pasture. More controlled diets may also help define the effects of high protein intake upon reproduction but high numbers of cows would again be needed.

7.2 PROTEIN PENALTY

Central to much of the work in this thesis was the issue of dietary protein excess in pasture diets. Specific studies were concerned with the measurement of protein excess (Chapter 1), assessment of seasonal variation in protein level in pasture (Chapter 2), addition of carbohydrate supplement to reduce dietary protein and assist with rumen capture of dietary protein (Chapter 3), pasture management factors affecting dietary protein (Chapter 5), and the specific effects of nitrogen application to pasture on nutrient composition (Chapter 6). Chapter 1 highlighted negative associations of high blood and milk urea with milk production and herd reproductive performance. Chapter 2 indicated that protein excess was more likely to occur in spring and autumn, although the lack of readily fermentable carbohydrate in pasture in summer may affect rumen capture of protein then also. Chapter 3 showed that urea levels in milk could be reduced by addition of small quantities of readily fermentable carbohydrate to the diet, and positive effects on productivity and herd fertility were measured that may have been due to reduced dietary protein excess. Chapter 5 indicated the effects of maturity and grazing on pasture protein levels, CP content declining steadily with increasing pasture maturity. Chapter 6 showed that nitrogen application raised pasture protein levels for varying periods from 4-8 weeks after application depending on environmental conditions and stage of growth at the time.

It is likely that the energy required to detoxify excess ammonia absorbed from the rumen and convert it to urea is considerable (Blaxter, 1962; Moore and Varga., 1996), as several authors have shown with diets based on grass silage or high CP concentrates or totally mixed rations (TMR) (Danfaer *et al.*, 1980; Gordon, 1980). Using the prediction of Moore and Varga (1996) for an ME penalty of 1.3 MJ/100 g protein excess, a cow consuming 15 kg DM of 25% CP pasture and requiring 17% CP for its

production level (NRC, 1989) will have an excess of 1.2 kg protein in the diet to dispose of, requiring an extra 15.6 MJ of ME. This is equivalent to nearly 1.5 kg DM extra pasture required. More experiments with pasture diets are still needed, however. There may be differences with pasture because it has lower RFC levels than most dairy cow diets, and has rumen fermentation patterns with different pH and higher acetate levels compared to recommended TMR diets (A. Hogden, pers. comm., 1996). There is evidence that urea formation in the liver may require an amino acid source for one of the ammonia radicles in urea, as well as the one absorbed ammonia radicle from the rumen (Lobley *et al.*, 1995). If this is so, a depletion of absorbed amino acid or tissue protein may be more likely with a pasture diet; weight loss, and/or reduced protein production in milk could result. Observation of pasture fed dairy cows suggests that both effects may occur, as difficulty in holding condition is a feature of pasture fed cows (Muller, 1993) and protein/fat ratios in milk are not high on pasture diets in New Zealand (typically 0.65-0.7 in Jersey cows and 0.7-0.75 in Friesian cows).

It is possible that most of the damaging effects of high urea in the body are in fact associated with spillage of ammonia into the blood stream when high amounts of ammonia are presented to the liver (Symonds *et al.*, 1981; Visek, 1984). Ammonia is known to be very toxic to tissues. The liver is known to be able to enhance the detoxifying process with constant exposure to ammonia (Blood and Henderson, 1968; Danfaer *et al.*, 1980), and this may help explain the lack of detrimental toxic effect on fresh pasture diets which are usually excessive in CP. Sudden exposure may be much more detrimental, particularly at the start of mating in October. Differences in ammonia absorption may help to explain variation in the effects of high urea levels in non-structural carbohydrate dominant (NSC) diets like TMR's and herbage based diets (A. Hogden, pers. comm., 1996). Another possible explanation for differences in the response of New Zealand cows to high protein grass is that indirect selection for cows more able to detoxify ammonia has occurred through culling of non-pregnant cows. Lower rumen pH is known to reduce ammonia absorption from the rumen (Payne, 1977) and this is likely to be a feature of high NSC diets. In addition, higher NSC levels in the rumen will provide carbon skeletons for bacterial assimilation of excess dietary protein, thus reducing ammonia absorption and reducing urea levels in blood or milk. Despite recording higher urea levels in this thesis than those shown to cause reduced conception in overseas studies (Ferguson *et al.*, 1988), little proven effect on

non return rate but a marked effect on end of season "empty" rate was seen (Chapter 1 and Chapter 3).

Further experimentation to establish the extent of the "protein penalty" on pasture diets is needed. High RDP pasture could be produced by N application and NIR analysis could be used to monitor the diet daily. N application may alter other plant nutrients like DM%, ADF%, NDF%, and SOLCHO however, so some form of supplement may also be required to stabilise the diet for nutrients other than RDP.

There are many aspects of rumen fermentation of pasture diets that are poorly understood, and better understanding of these is likely to lead to new ways of improving dairy cow performance. Use of "artificial rumens" or detailed monitoring throughout the year of individual cows could provide a wealth of information.

7.3 PASTURE CRUDE PROTEIN (CP) AND READILY FERMENTED CARBOHYDRATE (RFC)

Pasture CP exceeded 25% and occasionally reached 30% in studies in this thesis (Chapters 1, 2 and 3), which is well in excess of dietary requirements for high producing dairy cows (NRC, 1989; Muller, 1993). It was assumed that sampled pasture reflected dietary protein "as grazed", which may not necessarily be the case. Cows can select a diet containing 5-10% more protein than that offered in the total pasture, which complicates this assumption (Moran *et al.*, 1993). In addition, the laboratory method used for protein analysis in these studies was Kjeldahl N x 6.25. This method may not detect all non-protein nitrogen present in pasture, and total CP as measured by combustion methods could be even higher than measured here (C. Moller, pers comm., 1996).

Supplements containing higher RFC clearly reduced milk urea levels (Chapter 3). There is an upper limit to bacterial protein production in the rumen (Orskov,1981), so presumably addition of RFC to a pasture diet will only assist in reducing ammonia absorption and urea production in the liver up to the point where maximal bacterial

production occurs. Dietary CP must also be reduced (as well as RFC increased) to even approach recommended dietary levels for high production. It is possible that the production responses obtained to concentrate or molasses supplement to the pasture diet in this thesis (Chapter 3) occurred because the "protein penalty" on production was reduced by enhanced bacterial capture of ammonia and not by increased DMI or ME intake.

Managing pasture to maximise RFC content would appear to have some potential to address the issue of protein excess in pasture. Both soluble carbohydrate (SOLCHO) and pectin are important in temperate pastures, starch more so in warm-season pastures, and possibly other fermentable ingredients like organic acids play a part as well (Ulyatt and Waghorn, 1993). Pasture will increase in soluble carbohydrate content by as much as 50% as photosynthesis occurs during the day. SOLCHO accumulates in the plant more in cooler temperatures when photosynthesis occurs but growth is limited by the lower temperature. Hence levels of 30% SOLCHO have been recorded in ryegrasses in winter (Vartha and Bailey, 1973). Warmer temperatures result in more plant respiration and growth that will utilise SOLCHO immediately rather than allowing it to be stored. Hence the lower SOLCHO levels in summer noted in Chapter 2. Increased dead pasture in summer may also have contributed to this, as SOLCHO is intracellular, and dead pasture would contain less intracellular material.

Opportunities to feed pasture later in the day exist in winter, when cows are break fed on restricted DMI, and possibly in the rest of the season when 24-hour paddock grazing is practised on many farms. Soluble carbohydrate levels will gradually reduce in pasture in overcast conditions, as often exist in Waikato springs for 1-2 months (W.J. Fulkerson, pers. comm.). Awareness of this may assist farmers decide on appropriate diets to reduce potential damaging effects when these conditions occur. Pasture takes some time to increase soluble carbohydrate levels after grazing, with full plant replenishment of leaves and roots not occurring till the 3-leaf appearance stage (Fulkerson and Slack, 1994; Fulkerson, 1994). The 3-leaf appearance interval varies with season. The depression in SOLCHO after grazing occurred for 5-15 days in data presented in this thesis, and suggests that rotation lengths of shorter than 15 days in these circumstances may not be ideal from a dietary viewpoint. As pasture matures dietary protein also starts to reduce. The net effect of this on pasture CP/SOLCHO ratio

can be 5:1 at 5 days post grazing versus 1.3:1 at 20-25 days post grazing. The lower P sites in Chapter 5 had higher SOLCHO, suggesting that faster growth reduced SOLCHO. This needs to be confirmed, but seems logical in that growth requires photosynthate which is mainly SOLCHO. Thus more rapid growth would be detrimental to improving the pasture diet for SOLCHO. In several datasets presented in this thesis, a period of reduced SOLCHO in October occurs when ground temperature warms and/or weather is overcast and this appears to be detrimental to milk production (Chapter 1, Chapter 3, Chapter 5). This effect may be related to rapid pasture growth and/or reduced photosynthesis.

Clover enhancement in pasture is likely to be beneficial to cow performance, because higher quantities of dry matter are usually consumed and the ME value of clover is higher than that of grass (Ulyatt and Waghorn, 1993). The higher pectin present in clover compared to ryegrass is probably beneficial to rumen fermentation and rumen bacterial populations because it has a slower rate of release and less rumen pH effects (Ulyatt and Waghorn, 1993). Some of the benefit seen with increased clover consumption may also be due to increased levels of bypass protein (UDP) (Doyle *et al.*, undated) and minerals. Higher protein levels in clover could offset some of these advantages.

Pasture CP% is higher in shorter pasture, and gradually decreases as herbage mass increases from approximately 3000 kg DM/ha upwards. The reproductive state in ryegrass and warming temperatures have probably confounded maturation effects presented here (Chapter 5), with reduced cell contents occurring in the reproductive phase and in warmer temperatures. Protein levels generally decreased in October/November.

There is a need to more clearly define readily fermentable energy sources in pasture, as SOLCHO, starch, pectin, organic acids and possibly others all provide energy for bacterial growth. Ease of breakdown of cell wall components (NDF) must also influence the supply of carbohydrate energy in the rumen.

7.4 SEASONAL TRENDS IN PASTURE NUTRIENTS

Chapter 2 outlines the likely changes in major pasture nutrients through the seasons on dairy farms. The protein excess and lack of RFC have been discussed above. At times, however, pasture CP was close to being deficient for high productivity in October/November and in summer.

Considerable variation from sample to sample occurred on the same farm, suggesting that daily variation in pasture offered to cows must be considerable. This would seem likely to limit peak milk production and speed the decline in milk production after peak since cows would need to draw on body reserves or produce less on some days when the diet is less than ideal.

A change in the nature of carbohydrates from spring and autumn (higher SOLCHO) to summer when higher neutral detergent fibre is present, seems likely to affect DMI and rumen fermentation in several ways. Higher NDF is likely to limit DMI if cows are fully fed (Mertens, 1987), and hence reduce productivity and possibly cause weight loss if the cow has a high propensity to milk. Slower gut passage of digesta (the fill effect of high fibre), and increased milk fat % (the end product of high NDF) would be likely. However, increased SOLCHO should favour increased milk protein production.

An awareness of seasonal trends in pasture nutrients becomes important when supplements are added to the pasture diet. Nutrient deficiencies or excesses may be exacerbated by supplement addition. For example, addition of grass silage to a summer diet will usually add more fibre to a diet already high in fibre from pasture; soluble carbohydrate in silage will also be lower than pasture, and non protein nitrogen (NPN) in silage will be higher than in pasture (Spartan dairy ration balancer, undated; Thomas, 1996). These factors could be detrimental to productivity, although the objective of supplementation in some circumstances may be simply to avoid a major dry matter shortage rather than enhance or hold productivity.

The decline in digestibility (DOMD) and CP, and increased NDF and ADF that occur in October/November are of special significance for seasonal calving dairy cows as they

reach peak milk production and are mated at about this time on most farms. Reduced DMI will occur just as cows reach peak and in these circumstances rapid falls in production from peak seem likely. Rapid falls from peak milk are a feature of New Zealand cow lactation curves (Edwards and Parker, 1994). Weight loss may also occur if the cows are of high genetic quality and are strongly inclined to produce milk despite consuming less ME due to the lower DOMD pasture. Weight loss at mating is likely to be detrimental to conception. An increase in clover production as summer approaches will ameliorate the ryegrass quality decline, as will the return of ryegrass to the vegetative phase, but this may not occur till December. Production responses to supplementation through the spring period in this thesis were most marked in October/November when pasture quality was poorer, and indicate that the timing of concentrate supplementation at pasture for increased production should be reexamined.

Once pasture growth exceeds demand in late September/October most concentrate supplementation ceases in New Zealand at present. The fall in pasture quality in October/November coincides with the reproductive phase of ryegrass growth, rising ground temperature and increasing daylength, and as long as ryegrass remains the base species of dairy pasture there would appear to be limited opportunity to manipulate this with grazing management. Topping, short rotation lengths and "shutting up" of surpluses for silage are the standard methods used but have only limited effect. Choosing later flowering ryegrass varieties may help slow the decline in quality. Choice of other pasture species for this time of the year may also help eg. a legume that is active in cooler conditions than white clover and can be grazed without damage (eg cultivars of red clover, haifa or persian clover, subterranean clover).

Summer milk production will be affected by a lack of available ME as herbage accumulation rates (HAR) decline below requirements in many districts, and also by a decline in pasture quality as ryegrass plants senesce or decay leaving increased NDF, reduced CP and reduced SOLCHO. Increased NDF over 35-40% has been shown to reduce DMI through increasing gut fill (Mertens, 1987). Reduced CP may also limit production if it falls below optimal levels (see earlier discussion) and reduced SOLCHO will be likely to reduce rumen fermentation. Increased dietary fibre is likely to increase rumen pH through increased salivation and hence favour fibre digestion and fat

production rather than protein production. Appropriate supplements for summer dry periods should probably not increase the fibre content of the diet.

Pasture mineral levels identified in Chapter 2 indicated potential effects on production from low magnesium levels and high potassium levels. Potassium excess has large effects on anion/cation balance (DCAB) in pasture and this is known to affect the incidence of metabolic disease, cow health and milk production (Wilson, 1996). Calcium and phosphorus levels found would seem unlikely to lead to reduced productivity (with the possible exception of September calcium levels presented in Chapter 5) unless supplementation lowered dietary levels further, or very high productivity was occurring which increases the requirement for calcium and phosphorus.

There is ample evidence in this thesis of weather and environment effects on nutrients within pasture, both in the short term and with seasons. More precise identification of climatic effects on pasture nutrients could be achieved in controlled climate studies by mimicking typical weather conditions.

7.5 INDIRECT MEASUREMENT OF DIETARY NUTRIENTS

Urea concentrations in milk from farm vats is a useful and easy parameter to measure and will give an indication of dietary protein intake and dietary protein excess. While other factors can affect milk urea, like stage of lactation, parity, mastitis, etc RFC will lower milk urea and will help the rumen bacteria harvest more of the protein (Chapter 3). Some indication of DMI would help to interpret milk urea, because high intakes of diets containing over 18% CP will increase milk urea as well as high CP per se. Production level and information about weight loss or gain will improve the prediction of intakes determined by pasture assessments. If milk urea analysis became available with regular production information from the dairy company, it would be useful information to some farmers. Provision of milk urea levels with regular monthly herd testing information would probably not be a useful option however, as this sampling is not frequent enough.

Pasture mass had strong negative correlations with CP% in some of the data (Chapter 5 and 6), with higher CP at the lower pasture mass (more immature pasture). Within seasons this may provide a good indication of likely levels of CP (eg. in spring or autumn). This relationship may not hold in summer however, and it is likely to be affected by nitrogen (N) application.

The effect of N application on dietary CP appeared to be predictable in the conditions of the N experiment in Chapter 6, and so could be used to predict likely CP% if timing and level of N application are recorded. This could be useful in designing experiments to study protein excess in pasture, because pasture CP could to some extent be controlled. However, weather effects could not be controlled.

Dry matter % and SOLCHO% declined and rose together in many of the spring experiments, and one could possibly be used to predict the other. Neutral detergent fibre was a better predictor of digestibility (DOMD) than acid detergent fibre, which is often used to determine DOMD of feeds.

Although the brix measurements did not successfully predict SOLCHO in the N experiment, there were indications that brix varied with N rate applied, and further work may help to clarify what the brix technique is measuring in pasture. High clover % in pasture resulted in very green juice that was difficult to read in the refractometer.

The NIRS technology proved very useful in these studies and appears to have a considerable future both as a research tool and for rapid, low cost feed analysis to assist farmers making decisions about diets. Formerly cost prohibitive research may now be undertaken by first calibrating the NIRS with a limited number of analysed samples then placing the rest through NIRS. The NIRS frequently was able to detect poor reference chemistry analysis.

Further calibration of pasture nutrients on NIRS could become a valuable research tool. Carbohydrates could be broken down to cellulose, hemicellulose, lignin, pectin, individual plant sugars and starch. Pasture CP could be further broken down into NPN, nitrate, peptides, soluble protein, RDP, UDP and ADIN. Lipids could also be included.

7.6 RESPONSES TO SUPPLEMENTATION

Supplement trials were only undertaken in spring (Chapters 3 and 4) and should be extrapolated with caution to different conditions and seasons.

Responses in milk production to supplementation of pasture diets with RFC sources like molasses and high energy concentrates were of a similar order to those reported previously and were up to 1 litre of milk per kg DM of supplement over the period of supplementation (Chapter 3). Higher responses occurred in higher producing herds, and those in better condition. This would not be expected from some literature on responses to supplements. Another feature of the results was a better response to supplementation in October/November than in September. The reason for this is unclear, but may relate to a fall in quality of pasture in October/November which occurred on every farm measured in these studies. The higher quality supplement may then have raised intake of ME at this time, which is approximately when cows in early lactation reach peak intake and production. An additional effect of supplementation was a halving of end of season empty cow rates. This was a surprising result, and the reason it happened was not obvious from other data collected. The explanation may relate again to pasture quality and ME intake in October/November when mating occurs. The cows receiving supplement may have gained more weight during mating which is likely to favour improved conception rates or prevent early abortion.

The "balanced" diet supplementation trial reported in Chapter 4 suggested that better responses to concentrate supplementation will occur if the concentrate is formulated to address all known specific dietary weaknesses. The suggestion that cows may have actually eaten more pasture when fed the supplement in place of some maize silage is a novel one, but is supported by other literature showing reduced substitution when supplements contain more bypass protein (UDP) (Kellaway and Porta, 1993). In this trial, there was a genuine supplementary effect. The large carryover effect noted will not occur if cows are subsequently underfed. Nutritional advisors refer to the "1 in 200 rule" where cows achieving 1 litre more milk at peak then have the opportunity to produce 200 litres more milk over the rest of the lactation. This trial appears to indicate

a response of this magnitude. The increased fibre content of pasture in October/November tends to negate any potential for the "1 in 200" rule in New Zealand.

If the reproductive effect of supplementation is combined with the milk production response, and current market values used for "empty" cows, milk payout, supplement costs, treatment costs for herd reproduction problems, then responses were economic on more than half of the farms in the molasses/concentrate supplementation trial (see Chapter 1 for costings). However, this does not account for the extra work involved in feeding the supplement. The extent to which these results can be extrapolated to other situations is difficult to gauge.

The balanced diet trial showed larger responses than reported in most previous supplementation trials. Maize silage has become a common supplement to pasture diets in New Zealand in recent years, and this trial indicates potential areas for improvement in response from pasture/maize silage diets. Although the results presented in Chapter 4 were only one trial, the results suggest that "balancing" was effective in improving the response to supplement as opposed to addition of only molasses or concentrate to the diet as in Chapter 3.

7.7 USE OF APPROPRIATE SUPPLEMENTS

Armed with a knowledge of the nutrients in the supplements, and the ability to measure pasture nutrient composition, knowledge of likely seasonal changes in pasture composition or other indirect detection methods as outlined above, it is possible to decide on the most appropriate supplements for different situations. If the only information available about the diet is DM intake, or ME intake, then improvements are difficult to achieve.

In situations where the priority is to address diet quality rather than a deficiency of dry matter, there seems little point in attempting to enhance or even hold milk production in a dry summer with high NDF, low CP and low SOLCHO pasture by adding grass silage with even higher NDF, low CP and lower SOLCHO. Reduced milk production would

be almost certain to result. If grass silage is the only economic feed available, then it needs to be very high quality and for preference should have additional SOLCHO or RFC added. It is possible occasionally to have silage available that is of higher quality than the pasture on offer in summer. A more appropriate supplement for summer would be a high ME grain, byproduct or crop which does not reduce dietary CP unduly, for example barkant turnip, maize gluten meal, brewers grain, earlage or squash (NRC, 1989).

Similar comments apply to the October/November period when ryegrass is in the reproductive stage and has lower DOMD, but the clover content of pasture is still low. Appropriate supplements should be energy dense and designed to enhance rumen fermentation and fibre digestion.

Autumn, spring and winter supplements to pasture might concentrate more on reducing dietary CP closer to recommended levels, providing more RFC and possibly small quantities of "effective" fibre. A combination of grain and small quantities of hay or straw often is appropriate, or high grain maize silage, or small grain silage. Concentrates can provide specific requirements for UDP and minerals if desired or appropriate. Attempts should be made in early lactation to increase the energy density of the diet, to reduce weight loss post calving when DMI does not match requirements. Addition of protected fats at this stage may be advantageous but this would require further experimentation (Hutjens, 1996).

Supplementation of pasture diets with concentrates is a topic that raises strong opinions amongst farmers and their advisors in New Zealand, with concern often expressed about the economics of embarking on such a policy when pasture is so much cheaper and easier to feed (Penno *et al.*, 1996). Substitution creates additional complications in that pasture can be left to senesce or decay if concentrates are inappropriately used. The economic response to concentrate feeding then needs to be even higher to make up for lost pasture. Several misconceptions need clarifying in this debate. When capital costs of land are included the cost of pasture can exceed 20c/kg DM at present (Beca and Moller, 1997). Several supplements like maize silage and byproducts can cost less than this per kg DM or per MJ of ME (Maize silage presently costs 12c/kg DM (home grown) or 20c/kg DM purchased in stack; squash cost 15-20c/kg DM delivered).

Certainly if the land has been purchased, then efficient use should aim for high utilisation of pasture grown to reduce the cost per kg DM. In most circumstances, addition of extra supplement per ha of pasture should be accompanied by increases in stocking rate per ha to ensure that pasture utilisation remains high. If per animal productivity was low because stocking rate was already too high, then this may not require increases in stocking rate when extra supplements are purchased.

Substitution can be used to advantage sometimes in that pasture residuals can be increased to allow faster regrowth. The winter/spring period, when grazing to less than 1000 kg DM/ha is common in mainly pasture systems, is an example where supplements can assist pasture growth, adding to the supplement response.

Except for short term use or more specialised use, more expensive supplements (> 50c/kg DM) would appear to have a very limited place in the New Zealand dairy system with current payouts well below those received in other countries for milk. Economic responses to supplements require low cost, high energy density supplements designed to enhance production from pasture alone by addressing dietary deficiencies or excesses in pasture. In many cases the most appropriate supplement may be home grown, or a byproduct suitable to feed cows like maize silage, earlage, apple pomace, carrot pulp, or barkant turnips.

7.8 NITROGEN EFFECTS ON PASTURE NUTRIENTS AND DRY MATTER ACCUMULATION

Nitrogen application to winter/spring pasture affected dry matter %, CP%, SOLCHO% and NDF% and ADF%. The effects on the fibre components were unexpected, with reduced NDF and ADF for 4-6 weeks after N application, particularly in cooler conditions and shorter days. The effects were dependant on rate of application and timing of N application. The reduction in fermentable carbohydrate and elevation of CP further imbalanced pasture which already is nutritionally skewed in this direction. In an all grass system as practised in New Zealand these effects point to possible detrimental effects on animals grazing N fertilised pasture in some circumstances. Appropriate

grazing management or supplementation could be used to alleviate the problem if it occurs. Longer rotations will reduce the effects, and addition of fibre and SOLCHO should assist.

Responses in herbage accumulation increased with increasing N application rate, were greater at later application dates and ranged from 10-40 kg DM per kg N applied above the control at 6-8 weeks after application. Application of 20 kgN/ha was the most efficient response per kg N applied, but responses to 20 kg N/ha were slower than 40 kg N/ha or 80 kg N/ha.

The effects of nitrogen application on the NPN component of CP could provide useful information because NPN is solely in the RDP fraction, and thus is likely to impact greatly on the "protein penalty". There is likely to be large variation in NPN content of pasture (Beever, 1993), with particularly high concentrations in rapidly growing pasture.

7.9 GRAZING MANAGEMENT FOR OPTIMISING PASTURE NUTRIENTS

As discussed above, longer rotations will provide pasture of lower CP/SOLCHO ratio because protein levels begin to fall and SOLCHO should increase with increasing pasture maturity. Just which rotation length is appropriate will depend on accumulation rates and season. In periods of rapid growth, shorter rotation will avoid loss in digestibility, but in winter/early spring longer periods of regrowth are needed. In terms of mass of pasture or maturity for grazing, dairy pasture in the range of 2500-3000 kg DM/ha would appear to be the most appropriate in spring for balance of CP and fermentable carbohydrate, but this takes no account of what is best for pasture management or utilisation as opposed to the animal's requirements.

Additional growth can be achieved in winter/early spring by leaving higher residuals (approximately 4-5 cm residuals; Chapter 5 and 6, Brougham, 1956, 1959). Accelerated growth occured at approximately 1500-1800 kg DM/ha and residuals left

closer to these levels provided additional DM earlier. This will provide extra ME for grazing.

7.10 MILK PRODUCTION FROM MAINLY PASTURE BASED DIETS

If dry matter shortage or ME shortage has been addressed in a pasture based dairy system, which nutrients are then limiting performance? If maximum intakes of ME are required then it is clear pasture must remain highly digestible. This is particularly difficult to achieve in the reproductive phase of ryegrass growth in October/November and in dry summers. Stocking rates set too low for the farm make pasture digestibility harder to maintain, and mechanical topping and conservation of surpluses become more necessary. Senescence and decay can be important sources of lost ME per hectare. These losses are more likely to occur with low stocking rates, and can easily reach 40% of DM (Nelson and Moser, 1994).

Excess CP in fresh pasture could potentially account for losses of over 10% in milk production per ha if the predicted energy losses in pasture diets can be extrapolated from other studies (Danfaer *et al.*, 1980; Moore and Varga, 1996). So on the one hand we should aim for high DOMD pasture to maximise DMI, but this effect of extra DMI on productivity will be modified by the energy penalty created by the excess CP. The predicted scale of this effect (based on studies using other diets like grass silage; Danfaer *et al.*, 1980) would justify further research effort to clarify the degree of the protein penalty as it applies to pasture.

Managing pastures to minimise CP excess and maximise SOLCHO will help but not fully address the protein excess and RFC deficiency in pasture. Species of pasture plant other than ryegrass may prove in time to be more appropriate if they are more suitable in the above respects.

The issue of UDP adequacy in pasture is still not sufficiently clear, but recent experiments suggest there will be little response by average cows in average condition

to addition of protected methionine to the diet (Salam *et al.*, 1996). High productivity will require higher UDP content in the diet. It is possible clover will prove to have higher UDP content than ryegrass. Responses to UDP may occur with pasture diets, but can also result in condition loss, and UDP addition should always be accompanied by RFC addition to the diet.

Suggestions that "effective" fibre is inadequate in fresh pasture (Muller *et al.*, 1995; Kolver *et al.*, 1996) and that there will be responses to addition of small quantities of stalky fibre to a pasture diet are borne out by observation, but were not studied in this thesis.

7.11 BLOOD PARAMETERS MEASURED

Supporting information, especially relating to the "mechanisms" involved following dietary change, was gathered in several of the trials reported in this thesis from blood parameters in the cows (Chapter 1, Chapter 3, Chapter 4). Apart from blood urea which has already been discussed, these were Albumin (Alb), Betahydroxybutyrate (BOH), non esterified fatty acids (NEFA) and Magnesium (Mg). Both BOH and NEFA appeared to reflect fat mobilisation and respond quickly to dietary change (Chapter 1, Chapter 3). Albumin appeared to respond more slowly to changes in body protein status (Payne, 1977) and may be an effective indirect measure of body condition. It also has been related to conception rate (Payne, 1977; Wilson, 1989; Wilson *et al.*, 1985). Further work would be needed to confirm the correlation between condition and Alb. Magnesium was of little assistance in assessing nutritional status. Blood glucose was used in the original study in 1990, but could not be interpreted in any useful way.

7.12 HERD FERTILITY

Herd reproductive performance was monitored in the studies presented in Chapter 1, and in the supplementation trials reported in Chapter 3 and 4. Cows calving in good condition and fed well just before and just after calving could be expected to have low

anoestrus levels at mating 8 weeks later (McGowan, 1981; McDougall, 1994). The influence of feeding at mating on conception is less well established on pasture diets. Production levels in the higher performing herds in Chapter 1 would suggest they had higher DMI in early lactation and at mating and this was one reason for improved herd reproductive performance in this data. The influence of high blood or milk urea levels on both anoestrus and conception is suggestive only in data presented in this thesis. However, "empty cow" rates in the supplementation trials reported in both Chapter 3 and 4 were substantially improved by supplementation, suggesting that reduced milk urea was improving conception. Another more likely explanation is that an improved metabolisable energy (ME) and readily fermentable energy supply to the herds occurred in late September/October/November at a time when pasture quality is reduced (the reproductive phase of ryegrass growth). This either improved rumen fermentation or increased ME intake at mating time (mid October-late November mainly). Evidence supporting this reasoning is that production responses to supplements were greatest in October/November and that the lower "empty cow" rates must have resulted from differences in "actual conception", ecause the returns to heat were not detected within the 60 days. Presumably some unsupplemented cows returned to the bull for mating later because these cows had gone back into anoestrus or possibly aborted.

Relatively few trials have examined the effects of high energy supplements on cow fertility in the New Zealand pastoral system. The results in this thesis suggest that gains in herd reproductive performance will be made in many herds and that this response should be included in economic evaluation of supplementation.

In retrospect some of the experimental work in this thesis would have been better done in more controlled circumstances (eg Chapters 4 and 5), but other work presented required larger numbers (especially the reproductive data) and was only possible in a commercial environment (eg Chapters 1 and 3).

7.13 CONCLUSIONS

- This thesis has provided clear evidence of association between high dietary protein in fresh pasture, reduced milk production and herd fertility, and elevated milk and blood urea levels, but has relied on other published evidence showing that a "protein penalty" to milk production occurs (Chapter 1, 2, 3, 4, 5, 6, 7.1-7.3). Bulk milk urea can be used to monitor dietary protein excess (Chapter 7.4). There were other less strong associations between blood, pasture nutrients, milk production and herd reproductive performance (Chapter 1).

- The seasonal pattern of variation in the major nutrients in dairy pasture in New Zealand was established and the likely consequences for milk production and lactation curves discussed (Chapter 2, 7.4, 7.7). There was large between sample variation on each farm but also a clear seasonal pattern for most nutrients on each farm. The excess pasture protein and lack of readily fermentable carbohydrate (especially in summer) were the most notable features. There appear to be numerous opportunities to enhance milk production from pasture based diets based on known nutrient requirements for lactation.

- Supplementation with relatively low levels of readily fermentable carbohydrate (less than 1.5 kg DM high energy concentrate or molasses) in a 1200-cow controlled field trial from September-November increased milk production mainly in October-November and halved empty cow percentage at the end of the mating season (Chapter 3, 7.3, 7.6). Supplementation also reduced milk urea levels. The late response in October-November suggests current spring-summer supplementation strategies may need to be reassessed.

- A specially formulated concentrate added to a maize silage/pasture diet was compared with an isoenergetic maize silage/pasture diet in a 240 cow commercial dairy herd in spring (Chapter 4, 7.6). Supplementation led to improved milk production and reduced empty cows. Measurements suggested the extra production came from increased pasture consumption and increased tissue mobilisation. A more "balanced" diet improved cow productivity in this trial.

- After ryegrass/clover dairy pasture was grazed, SOLCHO was reduced for 1-2 weeks (Chapter 5, 7.9). Increasing maturity in spring reduced CP and DOMD, but increased ADF and NDF. There was an increase in herbage accumulation rate near 1800-2200 kg DM/ha herbage mass which continued to near 3200-3500 kg DM/ha herbage mass.

- Nitrogen application to pasture in the winter/spring period raised CP, reduced ADF and NDF initially, and reduced SOLCHO and DM% for 2-8 weeks after application. These effects increased in cooler conditions with shorter daylength. The effects on fibre components in pasture were unexpected and shed new light on reasons for variation in cow responses to nitrogen fertilised pasture and ways to counteract potential negative effects. The consequences for pasture fed cows are discussed in Chapter 6 and 7.8.

- Knowledge of the dietary nutrients present provided useful information that was able to explain the responses to supplementation seen (especially in Chapter 3, 7.4, 7.6, 7.7) or likely responses based on knowledge of nutrient requirements (Chapter 2). Of particular importance was the improved response to supplement later in the experimental period, when pasture quality (DOMD) was poorer. This illustrates the value of knowing more about the pasture based diet than just dry matter intake, and that further progress in researching pasture related dairy nutrition should follow once this kind of information is obtained and used.

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APPENDICES

Appendices A.1 - A.11	Analytical Methods (Copies of laboratory methods used in this thesis)
Appendices B.1 - B.5	Selected publications resulting from (or associated with) work undertaken in this thesis

APPENDIX A.1

Extraction of carbohydrates ("Nelson's method) - used in Chapters 2, 3 and 5 of this thesis.

A colormetric determination of water soluble carbohydrate as total reducing sugar as used by the Nutrition Laboratory, Department of Animal Science, Massey University.

CARBOHYDRATE ANALYSIS

Serial Extraction of Carbohydrates

PRINCIPLE:

Carbohydrates (CHO) are sequentially extracted from plant material, animal digesta and faeces by hot water (water soluble CHO [WSC]) and 0.5% ammonium oxalate (pectins). Not all of each CHO is extracted in the described fraction, notably some pectin being present in the hemicellulose extract. The monosaccharide present in the water soluble CHO and cellulose fractions are estimated as glucose, hemicellulose monosaccharide as xylose, and pectin as D - galacturonic acid.

SAMPLE PREPARATION:

Samples must be freeze dried and ground through a 1mm sieve.

EQUIPMENT -

4 decimal place balance
thermolyne hot plate
Ralta personal fan
2 foil trays
12 x 250 ml quickfit conical flasks
12 quickfit filter / condenser stalks
50 ml quickfit measuring cylinder
glass boiling beads
straight through delivery side suction quickfit adaptor
100 ml measuring cylinder
40 ml dispenser
vacuum extraction tap

REAGENTS -

1. 0.5% ammonium oxalate
Weigh 10g ammonium oxalate into a 2L volumetric flask, make up to volume with d.$H_2$0

2 2N H_2SO_4
Wear **safety goggles and gloves**, add 56ml concentrated H_2SO_4 to 750ml d.H_2O in a large beaker in sink. When cool make up to 1L.

LABORATORY PROCEDURES:

1. Accurately weigh approximately 0.4 - 0.5 g sample into a tared 250ml quickfit conical flask, record the weight. Add 40ml of just boiled d.$H_2$0 and a glass anti-bumping bead into the flask, attach the filter condenser stalk (with the filter furthest from the flask).

2. Reflux samples for 1 hour with the cooling fan on. Initially put flasks on cold hot plate, turn up to 400°C until just boiling, turn down to about 350°C - simmer to maintain currents in the suspension, allow some bumping of beads and whistling of stalks, with perhaps a little fluid above the filter, but without excess foaming or leaking at the joint. Swirl occasionally to wash residue down.

3. After refluxing, filter samples through the stalk by inverting it into a 100ml quickfit measuring cylinder connected to the delivery suction adaptor and vacuum. Holding the conical flask firmly, disconnect the vacuum hose and wash the residue with a little $d.H_20$ (<10ml) by adding it to the top of the stalk and swirling the flask to wash down the sides as the $d.H_20$ is sucked into it. Refilter into the measuring cylinder. **Retain residue for Pectin analysis.**

4. When cool make the filtrate up to 50ml with $d.H_20$. **Record the volume and retain it for HWSC (hot water soluble CHO) analyses.**

5. To the residue from Step 3, add 40ml 0.5% ammonium oxalate back through the stalk to ensure that all residue is washed off the filter, and reflux for 2 hours as in step 2. Be especially careful about fast boiling and excess bumping at this stage for digesta and faeces samples.

6. Filter as in steps 3 and 4 above and **retain filtrate frozen for uronic acid (pectin) analysis.**

7. Take 20ml filtrate from HWSC (from step 4), put into a 150 ml quickfit conical flask, add 20ml 2N H_2SO_4, insert filter condenser stalk (with filter furthest from flask) Reflux 1 hour. when cool make up to 50ml with $d.H_20$ using measuring cylinder. Record the volume, store in labelled bottle and freeze. **This solution is ready for the reducing sugar analysis.**

DATA HANDLING:

Worksheet as follow:

Date	Sample No.	Flask No.	S.Wt (g)	VOL. HWSC	VOL. AMOX	V.HWSC.HYD

Nelsons determination of reducing Sugars

PRINCIPLE:

This photometric method is used to determine the amount of reducing sugar as the amount of soluble carbohydrate in the plant materials, digesta and faeces. Under suitable conditions copper reagents and arsenomolybdate react with reducing sugar to give molybdenum blue. The optical density of the colour developed is proportional to the amount of reducing sugar present, and is stable over long periods of time. Using glucose or xylose as standards, substituting neutralised water for the sugar solution as the blank, the absorbance of the molybdenum blue colour is measured at 520nm. The amount of sugar in the sample may then be determined by reference to a standard curve.

SAMPLE PREPARATION:

The acid hydrolysed solution of HWSC was frozen and stored for analysis. Before analysis, take the samples out of the freezer, leave them at room temperature overnight. If some containers have cracks, put bottles inside beakers.

One batch consists of 7 standards plus 12 or 16 samples in duplicate.

EQUIPMENT:

25ml beakers
small magnetic flea and stirrer
5 ml pipette and tips
vortex mixer
1 x 50ml plastic tube with lid for each sample
1 x 25ml volumetric flask for each sample
2 x 10 ml glass Kimax test tubes for each sample, standard and blank
1 ml micropipette
Boiling water bath
Spectrophotometer at 520nm
Test tube racks

REAGENTS

Except for solution C, the reagents are kept prepared. All the chemicals are Analytical grade. Use a fume cupboard, safety goggles and gloves where necessary.

1. **5 M NaOH**
   Weigh 50g NaOH (A.R.) into a larger beaker, add 250ml d.$H_2$0, stir until dissolved.

2. **0.5M H_2SO_4**
   Add 200 ml distilled water to a 400ml beaker, add 12.24g (–6.7ml) concentrated H_2S0_4, stir a make up to 250ml with d.$H_2$0.

3. **0.5M NaOH**
   Weigh 5g NaOH into a 400ml Beaker, add 250ml d.$H_2$0. stir until dissolved.

4. **Solution A**

25 g	Na_2CO_3 anhydrous
25 g	K Na tartrate (Rochelle salt)
20 g	$NaHCO_3$
200g	Na_2SO_4 anhydrous

To a 1L conical flask add 800 ml H_20 and a large magnetic stirrer, weigh and dissolve sequentially the amounts of each salt, then make up to 1L with $d.H_20$. Filter if necessary, into a stoppered, labelled bottle, store in 37°C incubator.

5. **Solution B**

Weigh 15g $CuSO_4 . 5H_20$ into a 100ml volumetric flask, make up to volume with $d.H_20$ and add 1 – 2 drops of concentrated H_2S0_4.

6. **Solution C**

Make fresh just prior to use. Pipette 25ml Solution A (if necessary, filter first) to a 50ml conical flask, add 1ml of solution B and stir.

7. **Arsenomolybdate Solution**

(Make at least two days before use)

Caution: Wear gloves and dust mask at all times when handling Sodium Arsenate.

(a) Add 450ml $d.H_20$ and a large magnetic stirrer into a 1L conical flask, add 25g ammonium molybdate, dissolve, carefully add 21 ml concentrated H_2S0_4 and mix.

(b) Dissolve 3.0g $Na_2HA_s0_4 . 7 H_20$ in 25ml distilled H_20.

(c) Add solution (b) to solution (a), mix well, store in a brown bottle, incubate at 37°C for at least 48 hours prior to use.

8. **1% Phenolphthalein**

Weigh 1g phenolpthalein into a 200ml beaker, add 60ml drum alcohol, 40ml $d.H_20$, mix.

LABORATORY PROCEDURES:

This assay will take one day. Standards should be analysed with samples in the same batch. After using, the standards and samples should be stored in the refrigerator.

1. **Standards**

(a) Place 0.2g glucose in a beaker, put it in a 105°C oven for two hours, cool in desiccator.

(b) 'Neutralised' water
To a 2L beaker, add 80ml 2N H_2S0_4, 120 ml distilled water, 80 drops (about 2.7ml) phenolphthalein, and a magnetic stirrer. Put on stirring plate and add 5M NaOH dropwise until the water turns just pink. Add 0.5M H_2S0_4 dropwise, carefully, until the pink just clears and then make it just pink again with 0.5M NaOH. Make to 1L.

(c) Standard stock solution - 400 ppm
Weigh 0.1g dried glucose accurately into a 250ml volumetric flask, dissolve it in 'neutralised' water and make up to 250ml. Mix well.

(d) Standard dilutions
Prepare 7 x 100ml volumetric flasks, label with the appropriate standard concentration. Using the table below, add the standard stock solution into each flask using a pipette, make up to 100ml with neutralised water. Mix well.

Conc. of Standard (ppm)	Volume (ml) of stock solution (400pm)
0	0
20	5.0
50	12.5
100	25.0
150	37.5
200	50.0
300	75.0

Label 7 x 50ml plastic graduated centrifuge tubes. Pour some of each standard solution into each tube, cover with lid, put in the rack. Keep the rack and flasks with standard solutions in the refrigerator. The standard solution can be stored for two months.

2. **Assays**

(a) **Sample neutralisation**

Into a 25ml beaker with a small magnetic stirrer, pipette 5 ml of sample (hydrolytic solution of HWSC) and add 2 drops phenolphthalein. Place on stirring plate and add 5M NaOH dropwise until the sample turns just pink. Keeping stirring, add 0.5M H_2S0_4 dropwise until the pink just clears, make back to just pink with 0.5M NaOH. Transfer the neutralised sample into a 25ml volumetric flask, rinse beaker and stirrer with distilled water, make up to 25ml. Mix well and then put it into a 50ml plastic labelled tube, cover with lid, put them in racks.

(b) **Analysis**

(i) Put 10ml glass Kimax text tubes with lid in rack, two for each standard, and three for each sample.

(ii) Pipette 1 ml of standard solution or sample into each corresponding tube, add 1.0ml solution C to each standard tube and 2 of the 3 sample tubes. Add 1.0 ml distilled water to the third sample tube as the sample blank, cover with lids. Mix on vortex mixer, and heat in boiling water bath for 20 minutes.

(iii) Cool in sink of cold water. **Wearing gloves**, add 1.0ml arsenomolybdate solution to each tube. Mix on vortex mixer, add 7ml d.H_20 to make volume up to 10ml.

(iv) Mix tubes by inverting 3x. Read o ppm standard at 520nm and zero on the highest absorbance of the two blanks. Read the absorbances of all standards and samples at 520nm, record the results on the worksheet.

(v) Wash all the used glassware and dry them.

APPENDIX A.2

Uronic Acid Determination ("Blumenkrantz Method") - used in Chapters 2, 3 and 5.

Pectin Analysis method as developed and used by the Nutrition Laboratory, Department of Animal Science, Massey University.

Blumenkrantz Method for Uronic Acid (Pectin) Determination

PRINCIPLE:

Pectin occurs in plant materials as a polymer of uronic acid. The polymer is routinely hydrolysed by refluxing with 0.5% ammonium oxalate, after which the released uronic acid is determined by a calorimetric procedure. The calorimetric assay is based upon the appearance of a chromogen when uronic acid, heated to 100°C in concentrated sulphuric acid / tetraborate, is treated with meta-hydroxydiphenyl.

SAMPLE PREPARATION:

Ammonium oxalate extraction solution is stored frozen in freezer until analysis. Leave the solution at room temperature overnight before assay.

One batch consists of 6 standards plus 8 or 16 samples in duplicate.

EQUIPMENT:

Spectrophotometer at 520nm
Boiling water Bath
Vortex mixer
4 x 10ml Kimax glass test tubes for each sample and standard
5µl and 0.5ml micropipette
3 ml acid dispenser
test tube racks

REAGENTS:

All reagents to be made-up fresh before hand. All the chemicals are Analytical grade. Use a fume cupboard, safety goggles and gloves where necessary.

1. **H_2SO_4/Di-Na tetraborate (0.0125M in Conc. H_2S0_4)**

   Wear full face mask and gloves. Weigh 4.767g $Na_2B_40_7$. $10H_20$ into a 1L volumetric flask. Add about 500 ml conc. H_2S0_4 and leave overnight in fume cupboard for large pieces to dissolve. Make up to volume with conc. H_2S0_4 and mix well.

2. **0.5% NaOH**

   Weigh 0.5g NaOH powder into a 100ml volumetric flask, make up to volume with d.H_20 and store in a plastic container.

3. **Meta - OH diphenyl solution (Store 1 month in fridge) (0.15% in 0.5% NaOH)**

   Weigh 0.075 g meta-phenyl phenol accurately into a 50ml volumetric flask, add 0.5% NaOH solution to the volumetric, stir until dissolved (pink), make up to volume.

4. **0.4% ammonium oxalate**

   Weigh 8g ammonium oxalate into a 2L volumetric flask, make up to volume with d.H_20.

LABORATORY PROCEDURES:

This assay will take one day. Standards should be analysed with samples in the same batch. After analysis, the standards and samples should be stored in the fridge.

1. **Standards**

(a) Standard stock solution - 200ppm
Weigh accurately 0.04g (40mg) galacturonic acid into a 200ml volumetric flask, make up to volume with 0.4% ammonium oxalate solution, mix well.

(b) Standard dilutions
Prepare 6 x 100ml volumetric flasks, label with the appropriate standard concentration. Using the table below, add the standard stock solution into each flask using a pipette, make up to 100ml with 0.4% ammonium oxalate solution. Mix well.

Conc. of Standard (ppm)	Volume (ml) of stock solution (200ppm)
0	0
25	12.5
50	25.0
75	37.5
100	50.0
125	62.5

Label 6 x 50ml plastic graduated centrifuge tubes. Pour some of each standard solution into each tube, cover with lid, put in the rack. Keep the rack and flasks with standard solutions in the refrigerator. The standard solution can be stored for 2 months.

2. **Assays**

(a) **Sample dilution**

Dilute the ammonium oxalate extracts of the samples to an appropriate concentration with 0.4% ammonium oxalate. The following dilution factor is for reference only.

chicory ~ 1 in 8
herbage ~ 1 in 2 ~ 1 in 6
hay or grass ~ 1 in 3

(b) Analysis

(i) Put 10ml Kimax glass test tubes with lid in rack, four for each standard and each sample.

(ii) Pipette 0.5ml of standard solution or diluted sample into each corresponding tube, add 3.0ml concentrated H_2SO_4 / Na tetraborate carefully down the side of all tubes. Cool in sink of cold water for a few minutes. Mix carefully but thoroughly on vortex. cover with lid. Put all the tubes in waterbath at 100°C for 5 minutes. Cool in a cold waterbath until ready, e.g. overnight if necessary.

(iii) If time allows absorbances to be read immediately continue. Add 50µl M-OH diephenyl to 2 of the 4 standard or sample tubes. Add 50µl 0.5% NaOH to the other 2 of the 4 standard or sample tubes as the standard or sample blank. Mix on a vortex thoroughly in at least 4 short bursts. Pink colour will develop.

(iv) After at least 5 minutes pour each solution into a separate cuvette and read the absorbances at 525nm.

Read the 0 ppm standard with M-OH diphenyl at 525nm and zero on the highest absorbance of the two. Read the absorbances of all standards and samples with M-OH diphenyl at 525nm, record results in 'A row B' column.

Read the 0 ppm standard with 0.5% NaOH at 525nm and zero on the highest absorbance of the two. Read the absorbance of all standards and samples with 0.5% NaOH at 525nm as standards and samples' blanks, record results in 'A row A' column. Finish readings within 1 hour as the sample colour is unstable.

(v) Wash all the used glassware, and dry.

DATA HANDLING:

1. Worksheet

Data	Tube No.	Sample No.	Dilution	A row B	A row A	Ā row A	ΔA (ArowB - ĀrowA)	Conc. (ppm)	Pectin (g/100 g.DM)
	1A	1							
	1B	2							
Standard									
	0								
	25								
	50								

2. Calculation

Using computer to calculate the result:-

Name of Data handling file: Basica spec. bas
Name of assey: Pectin
Results from computer: ppm/tube

$$\text{Pectin (g/100g.DM)} = \frac{\text{ppm x extract volume (ml) x 0.83 x 0.90 x dilution}}{\text{Sample Wt (g) x 100 x DM\%}}$$

0.83 : for anhydrous standards
extract volume = VAMOX
0.90 = for uronic acid condensation

If the CV of concentration (from computer) of duplicates of the sample is more than 5%, repeat it from the ammonium oxalate extracts, if relative error of duplicates of the sample for pectin content is more than 20%, repeat if from extraction of sample.

Reference

New method for Quantitative Determination of Uronic acids. Nelly Blumenkrantz and G. Asboe - hansen. Ana. Biochem. 54, 484-489 (1973)

Prepared by Maggie Zou March 1995

[carbohydrate analysis - serial extrac.fj - tech disk]

APPENDIX A.3

Protein determination (used in Chapters 1-6) by Kjeldahl digestion method as used by the Nutrition Laboratory, Department of Animal Science Department, Massey University. (Protein determined by analysis of N x 6.25).

DETERMINATION OF KJELDAHL NITROGEN CONTENT WITH A KJELTEC AUTO SYSTEM

PRINCIPLE

The Nitrogen atoms in protein and other compounds react with boiling concentrated sulphuric acid in the presence of a selenium catalyst (Se) to form ammonium sulphate. The acid mixture is cooled, diluted with distilled water, and made strongly basic with sodium hydroxide. The ammonia is released and distilled into a boric acid solution. The ammonia in the boric acid solution is titrated with standardarized hydrochloric acid.

N-analysis is performed on feed, digesta and faecal and urine samples.

SAMPLE PREPARATION

Forage samples, seeds, chopped straw etc is freeze-dried then ground to 1mm mesh. The sample size for this type of sample is approximately 0.1g (semi micro) /0.2g-0.3g (Macro).

Semisolid samples, such as meat, should be fat-extracted and ground through a 0.5mm mesh. The sample size should be approximately 0.5g (semi-micro) - 2.0g (Macro).

Solutions such as milk, rumen fluid or urine should be well mixed. The sample size is approximately 2g.

Micro analysis is used when the available sample size is less than 0.1g e.g. rat illeal digesta.

For Macro N-determination, 10 samples are weighed out in duplicate per batch for analysis and 6 samples in duplicate for micro N-analysis.

EQUIPMENT

Kjeltec Auto 1030 Analyser and accessories
Tecator Macro and Micro Digestive systems and accessories
Fume Hood and sink
Macro or Micro Digestion Tubes
Analytical balance (4 decimal place)
1 x 50ml dispenser
1 x 30ml dispenser

REAGENTS

1. Sulphuric acid,

Concentrated A.R. grade, N free. Store in a winchester with a 30ml dispenser attached. Use in fume hood at all times! Wear gloves and face shield.

2. Kieltab (Se)

Macro or micro.

3. 35-40% Sodium hydroxide

Weigh 1.6kg NaOH (AR Grade) into a beaker, cover with tin foil or glad wrap. Pour 4l (measuring cylinder) of distilled water into a 5 l conical flask containing a magnetic flea, place on magnetic stirrer. Pour the NaOH pellets gradually (approximately 100g each time) through a large paper funnel into the conical flask containing the water, mix thoroughly. Allow the solution to cool before adding more NaOH When all the NaOH is added and the solution is clear and cool, add the solution to the alkali container.

4 Receiver Solution

Place 200g Boric acid in a 5l beaker containing a magnetic flea and 4l d.$H_2$0 Heat to dissolve the Boric acid by stirring continuously (approx 40 mins) on a magnetic hot plate. Then using 2 separate measuring cylinders add 200ml Bromocrescol green solution (0.1g in 100ml methanol) and 140ml methyl red solution (70mg in 70ml methanol).

Pour the dark red solution into the 20l receiver solution container and make up to 20l with a further 16l of distilled water. Mix thoroughly.

Add approximately 1ml 1M (4%) sodium hydroxide (2mg in 5ml) dropwise to the solution in the container until greeny black in colour.

5. Hydrochloric acid

Prepare the standard solution from the appropriate ampoule. For Macro analysis, empty two ampoules of 0.5MHCL into a 5l volumetric flask and make up to volume with distilled water. This gives an approximately 0.1M HCL solution.

For Micro analysis, an appropriately sized aliquot can be taken and diluted ten fold by making up into another suitable volumetric flask. This gives an approximately 0.01MHcl solution. Check titrant concentration by manual titration against a known weight of Sodium Carbonate (see manual appendices).

6. Distilled Water Solution (0.8g $Na_2B_4O_4$/l d.$H_2$0) (7.59/10 l H_2O)

The water tank will need re-filling after every three or four batches of Nitrogens. Dissolve the required quantity of $Na_2B_4O_4$ in d.H_2O ie, if 10l are required dissolve 8g $Na_2B_4O_4$ in 1l d.H_2O when dissolved add to the tank (rinse $Na_2B_4O_4$beaker with some of this d. $H_2$0 and add to the tank. Shake tank to mix.

Laboratory Procedures

1. Digestion tubes must be clean and dry.

2. Dry samples are weighed in duplicate directly into tubes or onto a weighing boat and quantitatively transferred to the digestion tube. Semi-solid samples are preferably weighed in duplicate directly into the tube or on a weighing paper (Nitrogen free) and transferred together with this paper into a digestion tube. Wet samples (i.e. urine) must be weighed directly into the tube. Record tube number and weight of sample. For each new batch of acid a blank should also be analysed to check that all reagents are N free. Digest acid and Kjeltab as per sample procedure. If weighing papers are used, digest and analyse the weighing paper as per sample procedure.

3. Add one of the appropriate Kjeltab (macro or micro) to each digestion tube containing a sample to be analysed.

4. **Wear gloves and a face shield**. Add concentrated sulphuric acid from a dispenser, 10ml for macro; 4mls for micro and mix carefully by gently swirling the tube by hand or by using a test tube mixer. The larger the sample weight that has to be heated and digested, the more chemicals will be needed, the longer time it will take to reach decomposition temperature of the digest, and the longer time will be needed for complete digestion. Therefore, do not use larger amounts of sample than is necessary to obtain a repeatable result.

5. Place the digestion tubes and stand with the prepared samples beside the digestor and fit the exhaust manifold on top of it. Turn on the vacuum source (water aspirator) to maximum airflow.

6. Place stand, tubes and exhaust manifold in the preheated digestor (420°C). Hook the heat shields onto the stand.

7. Digest for 3-5 minutes with maximum airflow through the exhaust manifold. Then adjust the flow until fumes are just contained. A piece of wood about 12 mm thick placed underneath one side of the digestor prevents condensate from dropping straight down into the boiling sample mixture which can result in sudden and irregular changes in pressure.

8. Continue digestion until the mixture is clear and colourless (usually 20-45 minutes). Do not over digest. Some types of sample material are far more easy to digest than others. When digesting a new type of material check digestion regularly. While samples are digesting start up the auto analyser as in Step 11.

9. When digestion is complete remove the stand with tubes and exhaust manifold and place the entire assembly on the wooden sheet beside the digestor to cool.

10. Cool sample solutions to hand temperature and add distilled water (30ml for macro and 10ml for semi-micro and micro). Swirl to mix. **Wear heat resistant gloves and a face shield** for protection. If the digest is too hot when water is added the reaction will be too violent and ammonia might be lost. On the other hand if the digest is too cold when water is added salts may precipitate which are difficult to redissolve. Solidification should be avoided but in case precipitation does occur, dissolve it by placing the tubes in the digestor for a few minutes.

11. Start up the "Kjeltec Auto 1030 Analyser" as follows:

(a) Check the steam generator is empty or the water level is below the electrodes. Close the drain valve.

(b) Switch on "POWER" and check the display reads "HELP". If any refill lamp is on fill the corresponding tank. If "cycle over" is flashing the "no alkali" mode is on.

(c) Check the titrant flask is filled and the burette is free from air bubbles (see Manual Section VII.19).

(d) Press the "REC-SOL" button several times to check the receiver solution is dispensed into the titration vessel.

(e) Open the cold water tap (¼ turn).

(f) Connect a digestion tube (macro or micro) and close the safety door.

(g) Press steam button to ON. Check water is flowing into the expansion vessel. If necessary, fill water tank (add 0.8g $Na_2B_4O_7$/l d.H_2O)

(h) Open the feed valve and fill steam generator until water level just touches the electrodes then quickly close feed valve.

(i) Open the feed valve while watching the steam meter and quickly close the feed valve as soon as meter moves.

(j) Wait until the water in the steam generator is boiling and water and steam appear in the tube. ~~Repeat (i).~~

(k) Open the feed valve and leave open. After 2 minutes or an audible click from the machine, the steam is now heated up. **Turn off steam button**.

(l) Check that Kjeldahl is selected and alkali is on. Select macro or micro alkali with the switch behind the top front panel.

(m) Open the safety door and remove the tube. Press "AUTO/RESET" to "AUTO".

(n) Place a digestion tube containing concentrated sulphuric acid in the quantity as used in the digestion and 10ml (micro and semi-micro) or 30ml (macro) of distilled water (= blank) in position. If it has been shown recently that the present batch of acid and catalyst are nitrogen free just use 10 (micro and semi-micro) or 30ml (macro) of distilled water.

(o) If "cycle over" light is on close the safety door.
Set A = 00.00; B = 1.000; Blank = .00

(p) When the "cycle over" light comes on again open the safety door and remove the tube. The display value is automatically printed at the printer when the safety door is opened. Repeat until the digested acid catalyst and distilled water give the same values as the undigested acid. Be careful that the acid blank does not receive any backflow of condensed acid during the digestion which may contain nitrogen from the samples. If weighing papers were used digest these, add 10 or 30ml of distilled water and run as the blank.

(q) Set Blank = constant value. When a new titrant solution has been made or if the auto analyser has not been used for a while, test for the recovery of ammonia as follows:

(i) Weigh out 0.500 g ammonium iron (II) sulphate ($(NH_4)_2Fe(SO_4)_2.6H_2O$ into a macro tube.

(ii) Connect tube to the analyser and close the safety door.

(iii) For Standard Ammonium Iron Sulphate Analysis

Set A = 00.00; B = 1.0000; Blank - distilled water blank value

(iv) $$\%\ N = \frac{(ml - blank) \times M \times 1.401}{0.500\ (g\ sample)}$$

$$ml - blank = \frac{\%\ N \times 0.500}{M \times 1.401}$$

where M = titrant molarity

%N = 7.145 (ammonium iron (II) sulphate)

For 0.5 M HC1 ml - blank = 5.10
0.2 M HC1 ml - blank = 12.75
0.1 M HC1 ml - blank = 25.50

OR enter B value for Recovery

$$B\ Value = \frac{1.401 \times M}{wt\ (g)}$$

Analyse in duplicate If B value is entered on Kjeldahl analyser the result displayed is %N.

$$\%\ Recovery = \frac{mean\ (\%N\ of\ ammonium\ standard)}{7.145} \times 100$$

For Sample Analysis
Set A = 00.00; Blank = constant value above; B = value in Table 1 for appropriate titrant concentration and divided by the weight of sample.

Table 1: Kjeldahl Nitrogen Determination B Values

HC1 concentration (M)	B Value
0.0100	0.014
0.0500	0.070
0.1000	0.140
0.2000	0.280
0.5000	0.701

If constant weight of sample = 1 g use B Values in Table 1. If any other sample weights are used the B value in Table 1 must be divided by the actual sample weight in grams.

$$\text{B Value} = \frac{1.401 \times M}{\text{wt (g)}}$$

For macro analysis the recommended titrant concentration is 0.2 or 0.5M HC1. For semi-micro analysis use 0.1M HC1, and for micro analysis use 0.01M HC1.

If B value is entered as 1.000 for samples use the following calculation to determine %N.

$$\%\ N = \frac{14.01 \times M \times f \times 100 \times (\text{ml titrant - ml blank})}{\text{mg sample}}$$

$$= \frac{1.401 \times M \times f \times (\text{ml titrant - ml blank})}{\text{g sample}}$$

where 14.01 = the atomic weight of nitrogen
M = the molarity of titrant HC1 (mole/litre)
f = standard Kjeldahl factor = 1.00 for %N

Connect and remove tubes in order as they are analysed. Remove the tubes only when the "cycle over" light comes on. If the %N for the duplicates of each sample differ by more than 2% repeat their analysis.

N.B. Remember to enter the new B value for each sample as the cycle is started.

12. When finished close down the auto analyser as follows:

(a) Close the feed valve.

(b) Open the safety door and remove tube if you have not already done so.

(c) Switch "POWER" off.

(d) Open the drain valve and ensure the steam generator empties completely.

(e) Remove the drain basin and clean. Wipe safety door,steam tube and holder foot. Also wipe the outside of the machine and the surrounding bench area.

(f) Close the cold water tap.

(g) Clean the digestor exhaust manifold drip tray and rinse exhaust manifold. **Be careful - concentrated acid!**

(h) Wash tubes in tap water and detergent, rinse at least twice in tap water and once with distilled water, and leave to drain.

Reference: Tecator Manual (Kjeltec Auto 1030 Analyser)

b:\nitrogen.FLI

APPENDIX A.4

Determination of acid detergent and neutral detergent fibre (used in Chapters 1-6).

Modified van Soest method as developed and used by the Nutrition Laboratory, Department of Animal Science, Massey University.

FIBRE DETERMINATION METHOD

PRINCIPLE

For the determination of NDF (neutral detergent fibre), ADF (acid detergent fibre) and lignin hence Hemi Cellulose and Cellulose percentages in plant materials and digesta. A gravimetric procedure, which is an alternative to full carbohydrate fractionation. Although this detergent system is much faster, it tends to be less specific.

Through the sequential treatment with Neutral Detergent, Acid Detergent, 72%. H_2SO_4 and ashing, cell contents are washed out (NDF), cell walls are broken up (ADF) and cell walls are digested (H_2SO_4 and ash).

SAMPLE PREPARATION

The samples are freeze dried and ground (1mm mesh). The size of the hot plate determines the size of a batch, i.e. 6 samples in duplicate.

Before use, the crucibles need to be ashed in the furnace at 500°C for 1 hr. and cooled in desiccator.

EQUIPMENT

12 x 400 ml beakers
12 x 600 ml beakers
12 x 150 ml beakers
12 gooch crucibles (porosity 1) 40 mn
36 glass beads (small)
1 pair of tweezers
12 x 10 cm watchglasses
12 x 12.5 cm watchglasses
12 glass stirring rods - 10-12 cm long
furnace tray
wash bottle
water vacuum filtering system + Guho adaptors
500°C furnace
105°C oven
hotplate
desiccator
balance (4 decimal place)

2

REAGENTS

Except for the α-amylase solution, the reagents are kept prepared.

(a) Neutral detergent solution:

120 g Na Lauryl sulphate (or Na dodecyl sulphate)
74.44 g Na_2 EDTA (EDTA-disodium)
27.34 g Na tetraborate decahydrate
18.24 g Na_2 hydrogen phosphate anhydrous (Na_2HPO_4)
40 ml ethylene glycol
Approx. 4 l RO water

1. Weigh first 4 ingredients into a 4l conical flask.
2. Dissolve in 3l RO water on stirrer.
3. Add the ethylene glycol.
4. Make the solution up to 4 l.
5. Check the pH is between 6.9 - 7.1 and adjust with NaOH or HCl if necessary.
6. Store in incubator, 37°C, for further use.

(b) α-Amylase solution:

1 g α-amylase (serva alpha amylase Rohalase A3 from Bacillus subtilis 38 U/mg)
10 ml 2-ethoxyethanol
approx 40 ml RO water

1. Weigh 1g α-amylase into a 50 ml volumetric flask
2. Dissolve in 30 ml RO water
3. Add 2-ethoxyethanol
4. Make solution up to 50 ml with RO water
5. **Store in fridge for no more than 1 week**

(c) Acid detergent solution (5%):

112 ml conc. $H2SO_4$
80 g CTAB (Cetyltrimethylammonium bromide)
Approx 4 l RO water

1. Put 2 l RO water into a 4 l conical flask
2. Carefully add the conc. acid. Stir.
3. Make up to 4 l with RO water
4. Weigh CTAB into a 4 l beaker, **WEAR A DUST MASK AND GLOVES**
5. Add the acid solution and stir to dissolve
6. Store at room temperature

(d) 72% H_2SO_4 (w/w):

TO BE MADE UP IN A FUME CUPBOARD WEAR FACE MASK AND GLOVES, SLOWLY ADD ACID TO WATER IN SMALL AMOUNTS

340 ml RO water
652 ml conc H_2SO_4

1. Put RO water into a 4 l conical flask
2. Whilst swirling carefully add 100 ml conc. H_2SO_4. Leave to cool.
3. When cool, repeat step 2
4. When solution is cool make up to 1 l with RO water. (approx. 100 ml)

(e) Acetone

LABORATORY PROCEDURES

Caution should be exercised with over-drying (105°C), where accurate lignin analysis are required on some feeds, as heating can cause "artefact" lignin formation from soluble sugars and protein. It would be safer to dry under vacuum at 45°C.
The procedure takes 5 consecutive days, because of the overnight drying after every stage. See Appendix (1) for lay-out of record sheet.
When filtering and rinsing the samples and crucibles, end the procedure with a rinse of the inside of the crucible, so all sample flows onto the filter. This way some sample doesn't get lost when transferring to desiccator.

Dav 1:

1. Weigh the crucibles accurately and record on record sheet (c wgt)
2. Weigh approx 1 g of sample into the 400 ml beakers in duplicate and record on sheet. (s wgt)
3. Add 3 glass beads to each beaker
4. Add 50 ml Neutral Detergent to each beaker
5. Place beakers on the hotplate at 300°C, covered with a watchglass (handle with heat proof gloves)
6. Bring to the boil; be careful of foaming/overboiling
7. Simmer samples for 30 minutes
8. Whilst still on the hotplate, add another 50 ml Neutral Detergent to each beaker
9. Add 2 ml α-amylase solution to each beaker
10. Bring back to the boil, then simmer 30 minutes
11. Boil jugs of RO water; fill wash bottle with warm/hot RO water
12. Take beakers off the hotplate; and place on the trolley
13. Filter sample through correspondingly numbered crucibles while sample is still hot, on vacuum filtering system.
14. Use a low vacuum, increasing it if necessary, be aware of clogging of the crucibles with a strong vacuum.
15. Rinse sample 3-4 times with warm-hot RO water, until no Neutral Detergent is present (i.e. no more bubbling of liquid passing through the filter).
16. Dry crucible + sample overnight in 105°C oven

4

Day 2:

1. Cool crucibles with samples in desiccator to room temperature
2. Weigh crucibles and record weight on sheet (CNDF)
3. Put crucibles sideways into 600 ml beakers
4. Cover crucible with acid detergent
5. Cover with watchglass, boil solution for 1 hr at 300-320°C on hotplate
   Be aware of "jumping" crucibles in the beaker when solution heats up.
6. Boil jugs of RO water; fill wash bottle with warm/hot RO water
7. Take beakers off the hotplate
8. Lift the crucible out of the solution with tweezers and rinse outside of crucible back into beaker
9. Filter acid detergent solution through crucible on low suctioning vacuum system
10. Wash the sample several times with warm/hot RO water until all detergent is removed.
11. Place crucibles on an oventray and dry overnight in 105°C oven

Day 3:

WEAR SAFETY GOGGLES AND GLOVES WHEN WORKING WITH 72% H_2SO_4

1. Cool crucibles with samples in desiccator to room temperature
2. Weigh crucibles and record weight on sheet (CADF)
3. Put crucibles upright in 150 ml beakers on a tray
4. **Carefully** dispense approx 10 ml 72% H_2SO_4 into the crucible; **be careful of splashing acid!**
5. Leave the sample soaking in the acid for approx 10 minutes
6. With a glass rod **carefully** stir the sample and acid to a smooth paste: watch out not to scratch the crucible too much with the rod
7. Add a further 20 ml of 72% H_2SO_4 and stir gently to mix
8. Leave glass rod in each crucible
9. Keep the samples covered in acid for 3 hours; occasionally stir and refill crucibles with acid from their respective beakers
10. Fill wash bottle with hot RO water
11. With the help of tweezers, transfer crucible to vacuum system; be careful not to splash or spill acid
12. Rinse outside and inside of crucible for acid
14. Place crucibles on oventray and dry overnight in 105°C oven.

Day 4:

1. Cool crucibles with samples in desiccator to room temperature
2. Weigh crucibles and record weight on sheet (CSAR)
3. Place crucibles and samples on furnace tray and ash overnight in 500°C furnace

Day 5:

1. Cool crucibles with ash in desiccator to room temperature. Be careful when handling the ashed samples not to lose any
2. Weigh crucibles and record weight on sheet (CASH)
3. Wash and dry crucibles

5

DATA HANDLING

See Appendix (2) for lay-out of data sheet.

		Acceptable error between duplicates
% NDF =	$\frac{\text{CNDF - C Wgt}}{\text{S wgt}} \times 100$	$\leq$ 3%
% ADF =	$\frac{\text{CADF - C Wgt}}{\text{S wgt}} \times 100$	$\leq$ 5%
% lignin =	$\frac{\text{CSAR - CASH}}{\text{S wgt}} \times 100$	$\leq$ 8%

% Hemi cellulose = % NDF - % ADF
% Cellulose = % ADF - % lignin

Before collating the results on summary sheet, convert the results to a dry matter basis by dividing the original results with the percentage dry matter for the respective sample. Give results of % NDF, ADF, lignin, Hemicellulose and Cellulose on summary sheet.

REFERENCES

Robertson, J.B., Van Soest, P.J. (1981). "The Detergent System of Analysis and its Application to Human Foods, in the Analysis of Dietary Fibre in Food." Vol. 3. Chapter 8, Ed. W.P.T. James and O. Theander. Marcel Dekker, Inc.: New York.

This method was written by M. Hendriks, May 1994

APPENDIX A.5

Determination of In Vitro Digestibility using cellulase (used in Chapters 2, 3 and 5) as developed and used by the Nutrition Laboratory, Department of Animal Science, Massey University.

IN VITRO DIGESTIBILITY

LABORATORY PROCEDURE

Nutrition Laboratory
Animal Science

November 1988

Principle

Laboratory determination of *in vitro* digestibility involves comparing samples of unknown digestibility with samples of known *in vivo* digestibility (standards). Known amounts of freeze-dried, ground (1 mm mesh) samples and standards are first subjected to a hot neutral detergent solution to remove soluble cell contents. The samples are then washed once in hot distilled water and twice in cold distilled water. The cell wall is then hydrolysed with fungal cellulase solution (derived from *Trichoderma* sp.) for 5 hours at 50°C initially, and then a further 16 hours at 50°C. The remaining undigested material is filtered, weighed and ashed. The total percentage ash by weight of the samples and standards is determined simultaneously. Laboratory results for the *in vivo* standards are used to derive a regression relating the laboratory *in vitro* digestibility to their known *in vivo* digestibility values. This regression is then used to estimate *in vivo* digestibility for the unknown samples. The procedure is able to estimate DMD, DOMD, and OMD. The procedure follows closely that of Roughan and Holland (1977).

Equipment

72	30 ml Pyrex beakers
72	20 ml culture tubes with screw caps
1-2	rack(s) to hold 72 tubes above
72	Gooch crucibles 40 mm i.d. porosity 1
1	muffle furnace
1	1 litre wash bottle
1	10 ml dispenser 1 litre reservoir
1	1 litre measuring cylinder
3	2 litre erlenmeyer flasks
5	boiling beads (5 mm diameter)
1	bunsen burner
1	tripod
1	gauze mat
1	pH meter
1	centrifuge heavy duty general purpose
1	filter pump (aspirator)
2	large glass dessicators 250 mm i.d.
2	dessicator plates 200 mm i.d.
1	powder funnel
1	2 litre filter flask
1	filter adapter rubber to fit filter flask above
1	stirring rod
1	100 ml beaker
1	analytical balance accurate to 0.1 mg

1 drying oven 40-200°C
1 incubator 10-120°C with rotisserie motor attached
1 rotating stage with 72 20 ml tube capacity
1 10 ml weighing boat

Reagents

Neutral Detergent Solution

sodium dodecyl sulphate	30.0 g
ethylene diamine tetra acetic acid - disodium salt (EDTA)	18.6 g
di-sodium tetraborate (Borax)	6.8 g
di-sodium hydrogen orthophosphate	4.6 g
sodium sulphite	10.0 g
2-ethoxyethanol	10.0 ml

Make up fresh before use. Place all of the above reagents in a 2 litre erlenmeyer flask. Add 5 boiling beads. Add 1 litre of distilled water to the above and (heat to) dissolve. The pH should be between 6.9-7.1. Add concentrated HCl (about 2.5 ml) to achieve this.

Buffered Cellulase Solution

sodium acetate ($C_2H_3NaO_2$ AR grade)	2.04 g
acetic acid glacial	0.9 ml
cellulase T-2 (Trichoderma derived) powder	18.0 g
distilled water	1.5 l

Make the acetate buffer (pH 4.8) solution first. Weigh the sodium acetate into a weighing boat. Transfer to a 2 litre erlenmeyer flask and dissolve in 500 ml of distilled water. Add the acetic acid followed by 1 litre of distilled water.

Weigh cellulase powder into a 100 ml beaker. Add approximately 30 ml of the buffer solution to the cellulase powder and mix to a paste. Add another 30 ml and mix well. Pour off the dissolved cellulase into a clean 2 litre erlenmeyer flask. Add another 60-70 ml of buffer solution to the cellulase and repeat the process until all the cellulase is dissolved. This solution can be made up the day before and stored covered in a refrigerator overnight.

The acetate buffer (pH 4.8) is used to keep the cellulase T-2 powder in its optimum pH range 2.5-7.0.

Cellulase T-2 powder has an optimum temperature range of 30-60°C. Be careful to keep the cellulase solution within this range during the laboratory procedure.

NOTE: Cellulase T-2 powder contains mainly cellulase (Cl-ase 20,000 u/g), and hemicellulase (36,000 u/g xylanase). Cellobiase, avicelase, CMC-ase, amylase, and protease are also present in small quantities. Cellulase T-2 powder is available through Pfizer Chemicals Division, a division of Pfizer Laboratories Limited, Auckland.

Procedure:

This laboratory procedure requires 6 working days to complete. Days 3, 4 and 5 must be run consecutive with no break between them. Consecutive runs of this proce can be overlapped if a large no. of samples are to be analy

DAY ONE (MONDAY OR THURSDAY)

1. Choose at least 6 in vivo standards over the greatest range possible of those available. It is necessary to choose standard types (ie hay, lucerne, silage, fresh pasture, etc) to correspond with those of the unknowns (Goto and Minson, 1977; McLeod and Minson, 1982). The standards are placed in the last available spaces in the run, eg no.s 61-72 for 6 in vivo standards.

2. Label 72 30 ml pyrex beakers from 1 to 72. Place beakers on a tray and into alternate beakers (starting with beaker no. 1) place approximately 2.0 g (half fill the beaker). Write the sample or standard name next to the appropriate numbers on the result sheet. Next to the name of each standard record the in vivo DMD, DOMD, and OMD values in the appropriate columns.

3. Place all the beakers in oven at 105°C overnight.

DAY TWO (TUESDAY OR FRIDAY)

1. Remove beakers from the oven and transfer to a dessicator to cool. Cool to room temperature.

2. Label 72 20 ml screw cap test tubes from 1-72. Place them in order in the rack(s). Accurately weight out 0.19 - 0.21 g of each sample or standard into a weighing boat. Record the weight (to 0.1 mg) on the result sheet in the column labelled 'WS' (Weight of Sample). Quantitatively transfer the contents of the weighing boat into the appropriate test tube. Duplicate this for each sample and standard.

N.B. This section of the procedure is determining the total percentage ash of the samples and standards.

3. Accurately weigh (to 0.1 mg) the alternate empty beaker (starting with beaker no. 2) and record on the result sheet in the column labelled 'WB' (Weight of Beaker). Transfer the remainder of the dry sample from the beaker immediately prior into the weighed beaker. Weigh the beaker and sample (to 0.1 mg) and record in the column labelled 'WBND' (Weight of Beaker and Non-Digested dried sample).

4. Place weighed beakers containing the dry sample in a muffle furnace at 500°C overnight. (This will have to be done Monday night for a Thursday start batch).

5. Make up neutral detergent solution as given in the section on 'Reagents' above. Cover and leave to cool overnight.

6. Check the pH of the neutral detergent solution is 6.9-7.1. If not, add concentrated HCl to get the right pH (about 2.5 ml).

DAY THREE (WEDNESDAY OR MONDAY)

1. Turn rotisserie oven on at 100°C.

2. Remove ashed samples from the furnace and transfer to a dessicator to cool. Cool to room temperature.

3. Weigh beaker and ashed sample (to 0.1 mg) and record in the column labelled 'WBNA' (Weight of Beaker and Non-Digested Ashed sample). Discard ash, wash and dry beakers.

4. Heat the neutral detergent solution to boiling.

5. Add 10 ml of just-boiled neutral detergent solution to each test tube containing weighed sample. Cap each tube and shake vigorously to thoroughly mix sample and detergent solution.

6. Place tubes on the rotating stage for the rotisserie oven. Be careful to balance the rotating stage to reduce wear on the rotisserie motor. The rotating stage does not balance, so the test tubes are arranged to compensate for this.

7. Place rotating stage and test tubes in the rotisserie oven for 1 hour at 100°C.

8. Remove rotating stage and test tubes from the oven and quickly transfer the tubes to the centrifuge tube holders. Centrifuge while hot at 2000 rpm for 15 mins.

9. Aspirate the supernatant, being careful not to remove any of the sample or standard.

10. Heat 1 litre of distilled water to boiling (use white jug).

11. Add 10 ml of boiled distilled water to each test tube, cap each test tube and shake vigorously to thoroughly mix sample and water.

12. Place rotating stage and test tubes in the rotisserie oven for 30 mins at 100°C.

13. Remove rotating stage and test tubes from the oven and quickly transfer the tubes to centrifuge holders. Spin at 2000 rpm for 15 mins.

14. Aspirate the supernatant, being careful not to remove any of the sample.

15. Add 10 ml of cold distilled water to each test tube, cap each tube and shake vigorously to thoroughly mix.

16. Centrifuge the tubes at 2000 rpm for 15 mins.

17. Repeat steps 14 and 15.

18. Centrifuge the tubes at 2000 rpm for 30 mins.

19. Aspirate the supernatant, being careful not to remove any of the sample.

20. Make up the cellulase solution as given in the 'Reagents' section. Cover, and place in the refrigerator overnight.

DAY FOUR (THURSDAY OR TUESDAY)

1. Turn rotisserie oven on to 50°C.

2. Add 10 ml of cellulase solution to each test tube. Cap each tube and shake vigorously to thoroughly mix sample and cellulase solution.

3. Do (2) and (3) of Day Three if not already done.

4. Place tubes on the rotating stage as in Step 3, Day Three.

5. Place rotating stage and test tubes on rotisserie oven for 5 hours at 50°C. 10.45 → 3.45. 9.45 → 2.45

6. Number 72 Gooch crucibles porosity 1 from 1-72. Place on a tray in order and dry in a furnace for 3 hours (500°C).

7. Remove crucibles from furnace and transfer to dessicators to cool. Cool to room temperature.

8. Accurately weigh (to 0.1 mg) the dried crucibles and record on the result sheet in the column 'WC' (Weight of Crucible).

9. Remove the rotating stage and test tubes from oven. Quickly transfer the test tubes to centrifuge tube holders while still warm.

10. Centrifuge the tubes at 2000 rpm for 15 mins.

11. Aspirate the supernatant being careful not to remove any sample.

DO 12, 13, 14 4.00 PM ONWARDS

12. Add 10 ml of cellulase solution to each test tube. Cap each tube and shake vigorously to thoroughly mix sample and cellulase solution.

13. Place tubes on the rotating stage as in Step 3, Day Three.

14. Place rotating stage and test tubes in rotisserie oven overnight (15 hours - 5 pm to 8 am) at 50°C.

DAY FIVE (FRIDAY OR WEDNESDAY)

1. Remove the rotating stage and test tubes from the oven.

2. Place tubes in centrifuge holders, spin at 2000 rpm for 15 mins.

- 4 -

3. Aspirate cellulase off. Resuspend in room temperature distilled water, 10 mls.

4. Place crucible no. 1 in filter adapter in filter flask. Filter contents of test tube no 1 through crucible. Rinse screw cap and test tube with distilled water until no fine pieces of sample remain. Repeat until all samples are filtered.

5. Place tray of crucibles and filtered samples in oven at 105°C overnight.

6. Wash and dry test tubes and lids.

DAY SIX (MONDAY OR THURSDAY)

1. Remove crucibles from oven and transfer to dessicators to cool Cool to room temperature.

2. Accurately weight (to 0.1 mg) the crucibles and dried filtered samples and record on result sheet in column 'WCUD' (Weight of Crucible and Undigested Dried sample).

3. Place the dried crucibles in muffle furnace at 500°C overnight.

DAY SEVEN (TUESDAY OR FRIDAY)

1. Remove the crucibles with ash from the furnace and transfer to dessicators to cool. Be careful not to lose any of the fine ash when moving the crucibles. Cool to room temperature.

2. Accurately weigh (to 0.1 mg) the crucibles and the ashed samples and record on result sheet in column 'WCUA' (Weight of Crucible and Undigested Ashed sample).

3. Wash and dry crucibles rinsing them well.

Run computer programme to calculate regressions

Formulae used:- $\%\ \text{ash} = \frac{WBNA - WB}{WBND - WB} \times 100\%$ (DM basis)

$\%\ \text{ash of undigested sample } (\%Ash) = \frac{WCUA - WC}{WCUD - WC} \times 100\%$

in vitro $DMD = \frac{WS - (WCUD - WC)}{WS} \times 100\%$

in vitro $DOMD = \frac{WS \times (100 - Ash\%) - (WCUD - WCUA)}{WS} \times 100\%$

in vitro $OMD = \frac{DOMD}{(100 - Ash\%)} \times 100$

APPENDIX A.6

Non-structural carbohydrate determination (used in Chapter 1).

Individual carbohydrate identified and quantified using gas-liquid chromatography technique as used at Dairying Research Corporation, Hamilton, New Zealand.

BACKGROUND TO TOTAL SOLUBLE NON-STRUCTURAL CARBOHYDRATE DETERMINATIONS

In plant tissue, the most predominant carbohydrates are the monosaccharides glucose and fructose, the disaccharide sucrose (a glucose-fructose molecule) and the polysaccharides starch and fructosan (Butler & Bailey 1973).

The carbohydrates that are sweet, water-soluble and crystalline compounds are known collectively as sugars. The sugars are the metabolic intermediates of the polysaccharides and usually occur at low concentrations. The polysaccharides starch and fructosans are storage carbohydrates and vary widely in concentration in field-grown herbage. The storage carbohydrates are usually separated from the sugars using specific concentrations of ethanol.

The choice of extraction used in carbohydrate determinations depend on two factors. These factors are the plant tissue type and the carbohydrates expected to be present in the herbage, and the information required. The extraction solution and the carbohydrates extracted by the procedure is as follows:

(i) Hot water extracts sugars, fructosans, and the water soluble component of starch, amylose.

Solutions Required

0.05% Amyloglucosidase
To prepare 600 ml: Weigh 0.30 g of enzyme and make up to 600 ml (measuring cylinder) using acetate buffer at pH 4.5. Solution stable for 1 day.

Acetate Buffer for Amyloglucosidase
Prepare 2.5 M acetate acid (acetic acid). Prepare 2.5 M sodium acetate. By measuring cylinder aliquot 50 mls of each solution into a 1 litre volumetric flask and make up to the mark using distilled water. Check pH. Solution is stable for 1 month.

Inositol Solution
Take ~0.2 g (4 dp) Inositol and dissolve in water. Transfer to 100 ml volumetric flask and make up to the mark.

2

Total non-structural carbohydrates including starch (Freeze dried material - autoclaved water extraction plus amyloglucosidase).

Stage 1

1. Weigh 2.0 g (4 dp) of freeze dried ground sample into 250 ml conical flask.
2. Add 100 mls of distilled water. Cap with foil.
3. Autoclave at 121^C for 1 hour (Allow pressure to drop on 'slow exhaust').
4. When cool, below 60°C, add 50 mls of amyloglucosidase (0.05% on pH 4.5 acetate buffer).
5. Incubate at 55^C for 1 hour.
6. Wash quantitatively from flask with distilled water and filter through glass fibre filter paper.
7. Transfer filtrate to a pre-labelled and weighed 2 dp 500 ml plastic jar, washing with approximately 100 mls of water. (Note: The jar must be labelled before weighing).
8. Re-weigh plastic jars to obtain total filtrate weight. Store in the fridge. Freeze if unable to begin Stage 2 within 24 hours.

Stage 2

Within 24 hours a sub-sample should be hydrolysed using the following procedure. N.B. A run comprises 11 samples and one correction factor sample. The Stage II hydrolysation destroys ~3% of Glucose and ~30% of Fructose in the sample.

1. For the 11 samples transfer ~20 g (2 dp) aliquot of filtrate into a 50 ml round flat bottom flask with a ground glass neck.

2. For the correction factor sample (CFS). Thaw the CFS and transfer it quantitatively to a 50 ml round flat bottom flask with a ground glass neck. The final weight made up with distilled water is ~20 g (2 dp) $\pm$ 0.2 g.

3. Take samples from steps 1 and 2 and add 0.5 ml of conc H_2SO_4 (SG 1.84 g ml^{-1}) using a calibrated automatic pipette (Caution: wear safety glasses).

4. Add anti bumping granules.

5. Reflux for 1 hour. Remove from hot plate and cool.

6. Add 2 g (4 dp) of internal standard (2.0 mg/ml Inositol - ie. total of 4 mg Inositol/20 g aliquot).

7. Neutralise with solid calcium carbonate to pH 5.5-7.0. This involves adding small amounts of calcium carbonate until fizzing stops.

8. Filter through Whatman #44 paper or Toya 5C.

9. Half fill 4 G.C. auto sampler vials with filtrate; cap with tin foil and freeze samples as soon as possible. Run 2 samples through G.C. and keep 2 samples in reserve.

3

Samples are stored in the freezer until G.C. analysis is possible. When appropriate samples and standards are freeze dried using the procedure below. Samples should be assayed within 72 hours of drying.

* Prepare shelf freeze drier so that the vacuum may be applied as soon as the samples and standards are placed in the drier.
* Remove duplicate samples and appropriate standards from the freezer and put a single hole in the tin foil caps. this allows vapour to escape, but ensures the sample powder remains in the auto sampler vial. Place samples and standards in freeze drier.
* Apply vacuum and leave overnight (Note: Shelf temperature should not rise above 40°C).
* When dry, the following morning, remove the samples and standards and place them in a desiccator. Also place in the desiccator the equivalent of 3 X 100 ml beaker containing hot sand (105°C - this step ensures that the samples are kept dry, before the TMS procedure. The beakers of sand aid this process by removing excess air.

Derivitization of the sugar extracts

* Sample vials are removed from the desiccator (Note: A release of vacuum should be heard. If not, this fact should be recorded on the sample sheets including the calibration and correction factor sheets for the G.C.).
* Place samples and standards in the fume cupboard. Add 0.4 ml of TriSil reagent to each vial. Cap with auto sampler lids.
* Shake the tray containing the samples from side to side for about 30 sec.
* Place the samples and standards in a plastic bag and place on top of the oven (29-33°C). Leave samples for 18 hours, then analyse using the capillary G.C. ie. for Samples TMS'ed at (Day 1) 4 pm the G.C. analysis starts at (Day 2) 8 am.

  There is a maximum of 40 hours before TMSed sugars start to deteriorate so all samples should be analysed by (Day 3) 8 am.

  * using a HP-1 capillary column, call up the SUGAR method.
  * input the correct ALS information ie. First sample, last sample etc. Run G.C.
  * solvent A and B for the washes is Dichloromethane (Methylene Chloride).

APPENDIX A.7

Urea determination in milk/blood (used in Chapters 1-4) - enzymatic test for Hitachi autoanalyser.

UREA

SYS 1
BM/Hitachi 704/911
Urea

Kinetic UV test

816 361

for 6 x 50 ml solution 1 (diluent/enzyme/coenzyme/substrate) and 2 x 42 ml starter reagent

Contents
1 Diluent (6 x 50 ml)
1a Enzyme/coenzyme/substrate (6 bottles of granulate)
2 Buffer (2 x 42 ml)
2a Urease (1 x 12 reagent tablets)

Also required:
Calibrator for automated systems

Method
Neumann, U. and J. Ziegenhorn. (1977). *Scand. J. clin. Lab. Invest.* **37**: Supplement 147, Abstract 97.

Test principle

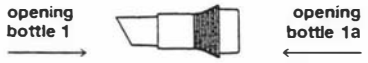
$$\text{urea} + H_2O \xrightarrow{\text{urease}} 2\ NH_4^+ + CO_2$$

$$2\ \alpha\text{-ketoglutarate} + 2\ NH_4^+ + 2\ \text{NADH} \xrightarrow{\text{GLDH}} 2\ \text{L-glutamate} + 2\ NAD^+ + 2\ H_2O$$

Preparation and stability of solutions

R 1

1 Diluent/Enzyme/Coenzyme/Substrate
Connect one bottle 1 to one bottle 1a using one of the enclosed adapters (see diagram), and completely dissolve the granulate in the diluent.



In the instrument (at appr. +10°C) stable for three weeks. In the instrument without cooling stable for four days

R 2 (Starter reagent)

2 Buffer/Urease
for 42 ml
Add six reagent tablets from bottle 2a into bottle 2. Dissolve by gently swirling.

The following table applies to smaller series:

ml buffer from bottle 2	reagent tablets (bottle 2a)
14	2
21	3
28	4
35	5

In the instrument (at appr. +10°C) stable for three weeks. In the instrument without cooling stable for four days.

Sample material
Serum, heparinized plasma or EDTA-plasma (do not use ammonium heparinate).
At +4°C stable for three days.

Calibration
Use Calibrator for automated systems.

BM/Hitachi 704

Instrument settings
Temperature: 25°C/30°C/37°C

PROGRAM 2 CHEMISTRY PARAMETERS	
TEST	..... (UREA)
ASSAY CODE (KINETIC-A)	5–20–25
SAMPLE VOLUME (µl)	4
R 1 VOLUME (µl)	320–50–0
R 2 VOLUME (µl)	80–50 or 20–0
WAVELENGTH (nm)	376–340
CALIBRATION	1–0–0
STD. (1) CONC.-POS. (mg/dl)	0–1
(mmol/l)	0.0–1
STD (2) CONC.-POS.	Target–2
STD (3) CONC.-POS.	0–0
STD (4) CONC.-POS.	0–0
STD (5) CONC.-POS.	0–0
STD (6) CONC.-POS.	0–0
UNIT	MG/D or MMOL/L
SD LIMIT	0.1
DUPLICATE LIMIT	100
SENSITIVITY LIMIT	0
ABSORPTION LIMIT (INC/DEC)	5000–1
PROZONE LIMIT	0–0
EXPECTED VALUE (mg/dl)	10–50
(mmol/l)	1.7–8.3
INSTRUMENT FACTOR	1.00

..... Data entered by operator

Dilution threshold
If the urea concentration in serum or plasma exceeds 300 mg/dl (50 mmol/l), dilute 50 µl of sample with 100 µl of 0.9% NaCl solution (result x 3).

Normal values
Serum/Plasma[1]: 10▶50 mg/dl (1.7–8.3 mmol/l)

Quality control
Accuracy: Precinorm® U, Precinorm® S, Precipath® U, Precipath® S
Precision: Precinorm® UPX

Please note
Instructions for the determination of urea in urine are available upon request.
Do not leave bottle 1a open.
Solutions 1 and 2 contain sodium azide as stabilizer. Do not swallow. Avoid contact with the skin and mucous membranes.

Initial concentrations of solutions
1 Tris/HCl buffer: 15 mmol/l, pH 7.5; ADP ≧ 1.3 mmol/l; NADH ≧0.19 mmol/l; GLDH ≧ 0.90 U/l; α-ketoglutarate ≧ 5.5 mmol/l
2 Tris/HCl buffer: 31 mmol/l, pH 7.2; urease ≧ 17.5 U/ml

Final concentrations in the test
Tris/HCl buffer: 18 mmol/l, pH 7.4; ADP ≧ 1.0 mmol/l; NADH ≧ 0.15 mmol/l; GLDH ≧ 0.71 U/ml; α-ketoglutarate ≧ 4.4 mmol/l; urease ≧ 3.5 U/ml

Reference
1 MacKay, E. M., and L. L. Mackay. (1927), *J. Clin. Invest.* 4:295.

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BOEHRINGER MANNHEIM

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APPENDIX A.8

Determination of Beta-Hydroxy Butyrate in blood (used in Chapters 1, 3 and 4).

ALPHA SCIENTIFIC LTD.	CHEMISTRY	HITACHI METHODS

Beta-Hydroxy Butyrate

Computer code: BOH

Principle

D-3-hydroxybutyrate is the primary meatbolic product of ketosis . This is oxidised to acetoacetate by 3-hydroxybutyrate dehydrogenase (BHDH)

3-OHB + NAD ---------> NADH + AA + H

Measurement of the D-3-hydroxybutyrate is used as a biochemical marker of ketosis.

Reagents

1) R1- Buffer

100 mmol Tris, 2 mmol EDTA, 20 mmol Oxalic acid

Dissolve the following in 500 ml of deionised water

Tris 12.114 g

EDTA 0.7445g

Oxalic acid 2.5214g

Adjust the pH to 8.5 with 5M NaOH and dilute to 1L. Stable for 1 month at 4 C

(5M NaOH made by dissolving 200g of NaOH in 1L deionised water, store on shelf)

2) R2- Reagent solution

To 20ml of buffer add,

0.0532g NAD+ (stored in Hitachi fridge)

12.5 U of b-hydroxybutyrate dehydrogenase. To calculate this: The activity of the enzyme is 5.6 units/mg protein. In 1X 10mg bottle there are 56 KIUof enzyme.

To get 12.5 U, $\frac{12.5}{56}$ = 223 ul BHDH

BUFFER	NAD+	BHDH
20 ml	0.0532g	223 ul
40 ml	0.1064g	446 ul
60 ml	0.1596g	669 ul
80 ml	0.2128g	892 ul
100 ml	0.2660g	1115 ul
120 ml	0.3192g	1338 ul

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Standards

Prepare 20 mmol/L stock solution of BOHB by dissolving 307 mg of DL-hydroxybutyrate-Na in 50 ml of normal saline.

Prepare diluted standards by diluting stock standard with normal saline as follows.

Prepare freshly each week

Conc of std mmol/L	ml of stock	ml of normal saline
10	5	5
6	3	7
4	2	8
3	1.5	8.5
2	1	9

Sample

Use non-haemolysed serum or heparinised plamsa. Minimum volume 100 ul.

Method

See overleaf for Hitachi parameters

Method is linear only to 3mmol/L. Dilute any values over this level.

Quality Control

When either R1 or R2 are replaced perform an S2 calibration. Check PARAMETERS page, menu 2, test code 24 to confirm the position of the S1 and S2 standards. Use the 2mmol/l standard as the S2.

The 2 and 3 mmol/L standards are run to check the calibration as well as the current control sera. See the QC charts for the acceptable limits.

Clinical Significance

Where there is a deficiency of intracellular glucose metabolism, fatty acids are metabolised. The end products of this are acetoacetate, B-hydroxybutyrate, and acetone. In prolonged periods of starvation the peripheral tissues cannot metabolise all the ketone bodies and so the levels rise in the blood.

Ketosis is of major economic importance in farm animals. In dairy cows loss of milk production and possible infertility may result. In sheep, ketosis before parturition can threaten the lives of both the ewe and the foetal lambs.

References

Automated Kinetic Method for D-3-hydroxybutyrate in Plasma or Serum.
Cecil.H.Murray, W.J. Blanchflower, Desmond A. Rice: Clinical Chemistry Vol 30 No.3 1984

APPENDIX A.9

Albumin determination in blood (used in Chapters 1, 3 and 4) - Colorimetric test for Hitachi autoanalyser.

ALB

Albumin (BCG)

2
1
6

SYS 2
BM/Hitachi 717/911
1040 782

10 x 100 ml solution 1

Contents
1 Bromocresol green

Also required:
Calibrator for automated systems

Method[1]
Bromocresol green (BCG) method

Test principle
Formation of an albumin/bromocresol green complex at pH 4.2 and photometric measurement of absorbance.

Preparation and stability of solution
R 1
1 **Bromocresol green (BCG)**
Use contents as supplied.
In the instrument (at appr. +10°C) stable for three months.

Sample material
Serum, EDTA-plasma
If stored at +4 to 25°C stable up to six days.

Calibration
Use Calibrator for automated systems.

Dilution threshold
First run: 5 g/dl (50 g/l)
In the event of a rerun the dilution threshold is extended to 10 g/dl (100 g/l).

BM/Hitachi 911
The required application number for "dialling" on the diskette is given on the barcode label alongside the catalogue number. If the barcode is not read by the BM/Hitachi instrument the numerical sequence on the barcode label can be entered manually via the keyboard.

BM/Hitachi 717

Instrument settings
Temperature: 25°C/30°C/37°C

PROGRAM 2 CHEMYSTRY PARAMETERS	
TEST	..... (ALB)
ASSAY CODE (1 POINT)	1–8–0
SAMPLE VOLUME (µl)	3–1
R 1 VOLUME (µl)	350–100–0
R 2 VOLUME (µl)	0–20–0
WAVELENGTH (nm)	700–600
CALIB. METHOD	1–0–0
STD 1 CONC.-POS. (g/dl)	0.0–1
(g/l)	0–1
STD 2 CONC.-POS.	expected value–0
STD 3 CONC.-POS.	0–0
STD 4 CONC.-POS.	0–0
STD 5 CONC.-POS.	0–0
STD 6 CONC.-POS.	0–0
SD LIMIT	0.1
DUPLICATE LIMIT	100
SENSITIVITY LIMIT	0
ABS. LIMIT (INC/DEC)	0–0
PROZONE LIMIT	0–0
EXPECTED VALUE (g/dl)	3.5–5.0
(g/l)	35–50
PANIC VALUE (g/dl)	.....–.....
INSTRUMENT FACTOR	1.0

..... Data entered by operator

Reference values[2]
Adults: 3,5–5,0 g/dl or 35–50 g/l

Quality control
Accuracy: Precinorm® U, Precinorm® Protein, Precipath® U, Precipath® Protein
Precision: Precinorm® UPX

Concentrations in the test
Succinate buffer: 75 mmol/l, pH 4.2; bromocresol green: 0.14 mmol/l;

References
1 Doumas, B. T., et al. (1971). *Clin. Chim. Acta* **31**: 87.
2 Tietz, N. W. (1986), p. 589 in *Textbook of Clinical Chemistry*, W. B. Saunders Company, Philadelphia.

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Albumin

Computer code: ALB

Principle

See kit insert for principle of test.

Batch number of kit in use is 653506-01 Date 1/8/95 Signed;
656344-01 Date 7/11/95
658969-01 Date 1/7/96
666058-01 Date 30/10/96
Date

Reagent

1) R1- Bromocresol green, BCG

Use contents as supplied. Stable up to the expiry date when stored at 4-12 C.

Sample

Serum, EDTA plasma

Serum or plasma can be stored at 4-25 C for up to 6 days

Method

See kit insert for method. Hitachi parameters overleaf.

Quality Control

When R1 is replaced perform an S1 calibration. If the control values fall outside the acceptable limits, perform an S2 calibration. Check the PARAMETERS page, menu 2 , test code 11. Ensure the S2 value is correct for the calibrator in current use.

Clinical Significance

Albumin has several functions. Transportation of organic anions, binding of toxic heavy metal ions, hormone transport and the maintenance of colloidal osmotic pressure to name a few.

In dehydration total serum protein may be raised but the A:G ratio usually remains unchanged.

Hypoalbuminemia may result from impaired synthesis, increased metabolism, protein loss or an alteration the the distribution between intravascular and extravascular compartments.

APPENDIX A.10

Glucose determination in blood (used in Chapter 1) - enzymatic test for Hitachi autoanalyser.

GLU

SYS 1
BM/Hitachi 704/911
Gluco-quant® Glucose

2
1
6

Hexokinase method in serum and plasma

UV-test

1 447 513
for 12 x 50 ml reagent 1 and 6 x 22 ml starter reagent

Contents
1 Buffer/ATP/NADP (12 x 50 ml)
2 Buffer/enzymes (6 x 22 ml)

Also required:
Calibrator for automated systems

Method[1]
Hexokinase/G6P-DH assay

Test principle

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

$$\text{G-6-P} + \text{NADP}^+ \xrightarrow{\text{G6P-DH}} \text{gluconate-6-P} + \text{NADPH} + \text{H}^+$$

Preparation and stability of solutions

R 1

1 **Reagent** (buffer/ATP/NADP)
Use contents as supplied.
In the instrument (at approx. +10°C) stable for a minimum of four weeks.

R 2 (Starter reagent)

2 **Buffer/enzymes**
Use contents as supplied.
In the instrument (at approx. +10°C) stable for a minimum of four weeks.

Sample material
Serum, plasma.
Serum or plasma should be separated from cellular constituents immediately if possible, and no later than one hour after collecting the blood specimen. The sample can be stored up to 24 hours at +15 to 25°C after addition of a glycolysis inhibitor (NaF, KF)[2]. In serum/plasma stable for up to seven days in a closed vessel at +4°C.

Calibration
Use Calibrator for automated systems.

Dilution threshold
If concentrations exceed 900 mg/dl (50 mmol/l), dilute 100 µl of sample with 200 µl of 0.9% sodium chloride solution (Result x 3).

Quality control
Accuracy: Precinorm® U, Precinorm® S, Precipath® U, Precipath® S
Precision: Precinorm® UPX

BM/Hitachi 704

Instrument settings
Temperature: 25°C/30°C/37°C

PROGRAM 2 CHEMISTRY PARAMETERS	
TEST	..... (GLU)
ASSAY CODE (2 POINT)	2–15–32
SAMPLE VOLUME (µl)	3
R 1 VOLUME (µl)	350–50–0
R 2 VOLUME (µl)	70–20–0
WAVELENGTH (nm)	415–340
CALIB. METHOD	1–0–0
STD 1 CONC. POS. (mg/dl)	0–1
(mmol/l)	0.00–1
STD 2 CONC. POS.	specified value–2
STD 3 CONC. POS.	0–0
STD 4 CONC. POS.	0–0
STD 5 CONC. POS.	0–0
STD 6 CONC. POS.	0–0
UNIT	MG/DL or MMOL/L
DD LIMIT	0.1
DUPLICATE LIMIT	200
SENSITIVITY LIMIT	0
ABS. LIMIT (INC/DEC)	0–0
PROZONE LIMIT	32000–1
EXPECTED VALUE (mg/dl)	76–110
(mmol/l)	4.22–6.11
INSTRUMENT FACTOR	1.00

..... Date entered by operator

Normal values in serum and plasma[2] (fasting)
76–110 mg/dl or 4.22–6.11 mmol/l.

Please note
BM/Hitachi 911 analyzer
Prepare the reagent solutions as directed in the package insert.
For selection of the appropriate assay data stored on the application disk enter the application number printed in the box alongside the Catalogue Number on the bar-code label.
If the bar code cannot be read into the analyzer, enter manually the series of numbers given beneath the bar code.
Manual instructions for the determination of urinary glucose are available upon request.

Solutions 1 and 2 contain sodium azide as stabilizer. Do not swallow. Avoid contact with the skin and mucous membranes.

Initial concentrations of solutions
1 Tris buffer: 100 mmol/l, pH 7.8; Mg^{2+}: 4 mmol/l;
ATP ≧ 1.7 mmol/l; NADP ≧ 1.0 mmol/l
2 Hepes buffer: 30 mmol/l, pH 7.0; Mg^{2+}: 4 mmol/l;
HK ≧ 8.3 U/ml; G6P-DH ≧ 15 U/ml

Concentrations in the test
Tris buffer: 83 mmol/l, Hepes buffer: 5 mmol/l, pH 7.7; Mg^{2+}: 4 mmol/l; ATP ≧ 1.4 mmol/l; NADP ≧ 0.83 mmol/l; HK ≧ 1.4 U/r G6P-DH ≧ 2.5 U/ml

References
1 Schmidt, F. H. (1961). *Klin. Wschr.* **39**:1244.
2 Schmidt, F. H. (Boehringer Mannheim). Pers. communication.
3 Hoffmeister, H. and B. Junge. (1970). *Z. klin. Chem. u. klin. Biochem.* **8**:613.

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APPENDIX A.11

Determination of Non-esterified (or free) fatty acids (NEFA C) in serum (used in Chapter 1) - enzymatic test for Hitachi autoanalyser.

Wako

NEFA C

Code No. 990-75401

ACS-ACOD Method

For the quantitative determination of non-esterified (or free) fatty acids in serum

INTENDED USE

The Wako NEFA C test kit utilizes an *in vitro* enzymatic colorimetric method for the quantitation of non-esterified (or free) fatty acids (NEFA or FFA) in serum.

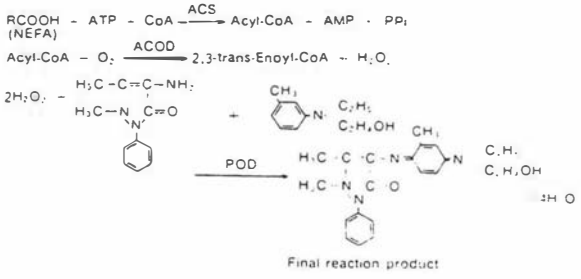
SUMMARY AND EXPLANATION OF TEST

Extraction methods are widely used for the colorimetric determination of non-esterified fatty acids (NEFA) in serum. NEFA are converted to their copper salts which are extracted into an organic solvent. The salts are then complexed with a dye for purposes of colorimetric measurement (1, 2, 3, 4). Alternatively, extracted NEFA are titrated with standard alkali to an acid-base indicator endpoint (5, 6). These approaches are time consuming, hazardous and not easily automated. Wako has made extensive studies of NEFA quantitation and has succeeded in developing an original enzymatic method which is available in kit form. This enzymatic method is accurate, precise, simple and fast. The need for an extraction step has been eliminated and the method can be automated. The Wako enzymatic method relies upon the acylation of coenzyme A (CoA) by the fatty acids in the presence of added acyl-CoA synthetase (ACS). The acyl-CoA thus produced is oxidized by added acyl-CoA oxidase (ACOD) with generation of hydrogen peroxide. Hydrogen peroxide, in the presence of peroxidase (POD) permits the oxidative condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA) with 4-aminoantipyrine to form a purple colored adduct which can be measured colorimetrically at 550nm.

CHEMICAL PRINCIPLES OF TEST

Non-esterified fatty acids (NEFA) in serum, when treated with acyl-CoA synthetase (ACS) in the presence of adenosine triphosphate (ATP), magnesium cations and CoA, form the thiol esters of CoA known as acyl-CoA as well as the byproducts adenosine monophosphate (AMP) and pyrophosphate (PP_i). In the second portion of the procedure, the acyl-CoA is oxidized by added acyl-CoA oxidase (ACOD) to produce hydrogen peroxide which in the presence of added peroxidase (POD) allows the oxidative condensation of 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA) with 4-aminoantipyrine to form a purple colored adduct with an absorption maximum at 550nm. Hence, the amount of NEFA in the sample can be determined from the optical density measured at 550nm.

Ascorbic acid (vitamin C) existing in the sample would be expected to cause significant interference due to its biological role as an antioxidant and known ability to react with hydrogen peroxide. Therefore, ascorbate oxidase (AOD) is added to the reaction mixture at the outset to conveniently and completely remove all ascorbic acid from the sample.



Final reaction product

REAGENTS

Reagent supplies for at least 50 tests are provided with each kit. All reagents and diluents provided are stable until the stated expiration date on each container when stored between 2°C and 10°C.

6 Vials	COLOR REAGENT A. This reagent is provided in dry form. Each vial contains the following ingredients.	
	ACS (acyl-coenzyme A synthetase)	3 U/vial
	AOD (ascorbate oxidase)	30 U/vial
	CoA (coenzyme A)	7 mg/vial
	ATP (adenosine triphosphate)	30 mg/vial
	4-Aminoantipyrine	3 mg/vial
1 Bottle	DILUENT FOR COLOR REAGENT A. This bottle contains 65 mL of an aqueous solution with the following ingredients.	
	Phosphate buffer, pH 6.9	0.05 mol/L
	Magnesium chloride	3 mmol/L
	Surfactant	
	Stabilizers	
6 Vials	COLOR REAGENT B. This reagent is provided in dry form. Each vial contains the following ingredients.	
	ACOD (acyl-coenzyme A oxidase)	132 U/vial
	POD (peroxidase)	150 U/vial
1 Bottle	DILUENT FOR COLOR REAGENT B. This bottle contains 130 mL of an aqueous solution with the following ingredients.	
	MEHA (3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline)	1.2 mmol/L
	Surfactant	
1 Bottle	NEFA STANDARD SOLUTION. This vial contains 10 mL of an aqueous solution with a known concentration of a non-esterified fatty acid:	
	Oleic acid	1.0 mmol/L (1.0 mEq/L)
	Surfactant	
	Stabilizers	

Warnings and precautions

A. The Wako NEFA C is *for in vitro* diagnostic use only.
B. The reagents and supplies provided with this kit are not to be used internally in humans or animals.
C. The reagents and supplies provided with this kit have been optimized for use with one another. Do not mix reagents and supplies from test kits bearing different lot numbers.
D. Do not use the reagents and supplies provided with this kit after the date of expiration which appears on each container label.
E. Do not use the reagents, supplies and/or derived solutions for any other purpose than is herein described for the NEFA C.
F. Please follow all instructions exactly for best results.

Preparation of reagent solutions

COLOR REAGENT A SOLUTION. Sufficient for Ten Tests.
Add exactly 10mL of Diluent for Color Reagent A to one vial of dry Color Reagent A. Mix gently by inverting the vial until the contents are completely dissolved. See notes below.

COLOR REAGENT B SOLUTION. Sufficient for Ten Tests.
Add exactly 20mL of Diluent for Color Reagent B to one vial of dry Color Reagent B. Mix gently by inverting the vial until the contents are completely dissolved. See notes below.

NOTES

A. Color reagent solutions should be stored between 2°C and 10°C under which conditions they are stable for five days after preparation. Be sure to date the solutions as they are prepared.
B. Be sure to return all reagent solutions and diluents to the refrigerator promptly after use.
C. Reagent solutions must not be frozen.
D. Reagent solutions must not be exposed to direct sunlight.
E. If more than ten tests are to be performed in one run, two (or more) vials of each reagent solution will have to be prepared. It is recommended that you combine the Color Reagent A Solutions in a separate glass or plastic bottle (pvc, PE and silicon, etc.) and mix gently prior to starting the run. It is similarly recommended that the Color Reagent B Solutions be combined.
F. Color reagent solutions may be combined only if they have identical lot numbers and have been prepared on the same date. Other combinations are not recommended.
G. Color reagent solutions which develop a precipitate at some time after preparation should not be used.

SPECIMEN COLLECTION AND PREPARATION

1. Blood should be collected in the early morning after the patient has fasted for at least 12 hours. Collection of only a few mL of blood from the antecubital vein into a plain evacuated tube will be quite satisfactory.

TABLE IV SERUM NEFA ADDITIVE STUDY

Additive		Serum with Additive		NEFA Standard Solution (1.00mEq/L) Net Absorbance
Name	Final Concentration	Absorbance	Final Concentration (mEq/L)	
GLUCOSE	None	0.091	0.35	0.262
	100mg/dL	0.091	0.35	
	200mg/dL	0.091	0.35	
	500mg/dL	0.091	0.35	
URIC ACID	None	0.091	0.35	0.262
	5mg/dL	0.091	0.35	
	10mg/dL	0.091	0.35	
	20mg/dL	0.091	0.35	
ASCORBIC ACID	None	0.091	0.35	0.262
	5mg/dL	0.091	0.35	
	10mg/dL	0.091	0.35	
	20mg/dL	0.091	0.35	
HEMOGLOBIN	None	0.091	0.35	0.262
	50mg/dL	0.097	0.37	
	100mg/dL	0.101	0.39	
	200mg/dL	0.112	0.43	
BILIRUBIN	None	0.091	0.35	0.262
	5mg/dL	0.086	0.33	
	10mg/dL	0.081	0.31	
	20mg/dL	0.074	0.28	
GLUTATHIONE	None	0.091	0.35	0.262
	20mg/dL	0.091	0.35	
	50mg/dL	0.091	0.35	
	100mg/dL	0.091	0.35	
CYSTEINE	None	0.091	0.35	0.262
	5mg/dL	0.091	0.35	
	10mg/dL	0.091	0.35	
	20mg/dL	0.091	0.35	
Anticoagulants	(Final Conc.)	(Ordinary Amount)	(NEFA Conc.)	(Reference Serum)
(1)Sodium Citrate	1.0%	0.5%	0.32mEq/L	0.32mEq/L
(2)Heparin	0.01%	0.01%	0.36mEq/L	
(3)Ammonium Oxalate	0.5%	0.2%	0.32mEq/L	
(4)EDTA	0.5%	0.1%	0.32mEq/L	
Glycolitic Inhibitor				
(1)Sodium Fluoride	1.0%	1.0%	0.32mEq/L	

Sensitivity

The sensitivity of the Wako NEFA C colorimetric method was calculated according to the following equation

$$a = \frac{A}{CL}$$ Where a = absorptivity
A = measured absorbance or optical density
C = concentration in mEq/L
L = path length of light in centimeters

Using a Hitachi Model #556 Spectrophotometer at 550nm, the sensitivity of the method expressed as absorptivity is 52 L/mEq·cm for fresh reagent and 52 L/mEq·cm for reagent after one year's refrigerated storage

Precision

The precision of the Wako NEFA C was established by analyzing a series of standards in replicate. The results of this precision study are presented in Table V.

TABLE V: SERUM NEFA REPLICATE ANALYSIS STUDY

REPLICATE NO	NEFA CONCENTRATION (mEq/L)		
	SERUM I	SERUM II	SERUM III
1	0.32	0.62	0.98
2	0.33	0.63	0.99
3	0.34	0.62	1.00
4	0.34	0.62	1.00
5	0.32	0.64	1.01
6	0.32	0.63	1.00
7	0.32	0.62	1.00
8	0.33	0.63	0.98
9	0.34	0.63	1.00
10	0.34	0.62	1.00
11	0.32	0.62	0.98
12	0.33	0.63	1.00
13	0.33	0.63	0.98
14	0.33	0.64	0.99
15	0.34	0.63	1.02
16	0.35	0.63	1.00
17	0.34	0.62	0.99
18	0.33	0.64	0.98
19	0.33	0.63	1.01
20	0.34	0.62	1.00
$\bar{X}$	0.33	0.62	0.99
S.D.	0.009	0.007	0.01
CV	2.7%	1.1%	1.1%

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2 Itaya, K., and Ui, M.: J. Lipid Res. **6**, 16 (1965)
3 Novak, M.: J. Lipid Res. **6**, 431 (1965)
4 Elphick, M. D.: J. Clin. Pathol. **21**, 567 (1968)
5 Trout, D. L., Estes, E. H. and Friedberg, S. J.: J. Lipid Res. **1**, 199 (1960).
6 Dole, V. P. and Meinertz, H.: J. Biol. Chem. **235**, 2595 (1960)

ORDERING INFORMATION

Code No.	Product	Package
994-75409	NEFA C	50 Tests

Distributed by

Wako Pure Chemical Industries, Ltd.

1-2, Doshomachi 3-Chome, Chuo-ku, Osaka 541, Japan
Telephone (06) 203-3741
Facsimile (06) 222-1263

Wako Chemicals USA, Inc.

1600 Bellwood Road
Richmond, VA 23237 U.S.A.
Telephone (804) 271-7677
Telex 293208 WAKO UR (RCA)
Facsimile (804) 271-7791

Wako Chemicals GmbH

Neuss, D-4040 Neuss
Germany
Telephone (02131) 311-0
Telex 8517001 wako d
Facsimile (02131) 311100

950101 K

Quality control

A quality control program is recommended for all clinical laboratories. The analysis of control sera in both the normal and abnormal ranges with each batch of samples is recommended for monitoring the performance of the procedure. The values obtained for the controls should fall within the manufacturer's stated acceptable range.

If values are to be established for unassayed control sera, the laboratory should assay each serum a sufficient number of times to generate a valid mean and acceptable range.

RESULTS

The NEFA values are obtained from the calibration curve or calculation.

1. From the calibration curve
   The NEFA content corresponding to the measured absorbance can be read directly from the previously prepared calibration curve.
2. By calculation:
   The NEFA content is calculated from the following equation:

$$\frac{A\ Sample}{A\ Standard} \times C\ Standard\ (mEq/L) = C\ Sample\ (mEq/L)$$

Where A = Absorbance at 550nm
C = NEFA Concentration (mEq/L)

Sample calculation:

1. For normal serum
   Absorbance of sample $A_s = 0.184$
   Absorbance of the NEFA standard solution $A_{std} = 0.262$
   Value of the standard solution is 1.0 (mEq/L)
   NEFA value (mEq/L)

$$C_{Sample} = \frac{0.184}{0.262} \times 1.0\ (mEq/L) = 0.70\ (mEq/L)$$

2. For icteric, hemolyzed or lipemic serum
   Absorbance of sample $A_s = 0.224$
   Absorbance of the NEFA standard solution $A_{std} = 0.262$
   Absorbance of sample blank against reagent blank $A_{SB} = 0.022$
   NEFA value (mEq/L)

$$C_{Sample} = \frac{0.224 - 0.022}{0.262} \times 1.0\ (mEq/L) = 0.77\ (mEq/L)$$

LIMITATIONS OF PROCEDURE

1. Blood for the NEFA C procedure should be collected after an overnight fast because the level of circulating non-esterified fatty acids is strongly influenced by food ingestion. If the patient has not properly fasted, the determined level of NEFA will be elevated and not directly comparable to a normal range derived from fasting normal controls.
2. Heparin is known to stimulate the activity of lipoprotein lipase which acts upon triglycerides associated with blood lipoproteins to release free or non-esterified fatty acids. For this reason, blood collected from patients receiving therapeutic heparin or blood collected in heparinized containers is not suitable for the NEFA C test.
3. The anticoagulants sodium citrate, ammonium oxalate and EDTA, as normally encountered in evacuated blood collection systems, do not interfere with the NEFA C procedure. See Table IV, Serum NEFA Additive Study.
4. Sodium fluoride, a glycolytic inhibitor, does not interfere with the NEFA C procedure in the amount normally encountered in evacuated blood collection systems. See Table IV, Serum NEFA Additive Study.
5. The level of NEFA will increase in serum as it is allowed to stand at room temperature due to enzymatic action. Serum specimens should be frozen (−20 C) for up to 24 hours if immediate analysis is not possible.
6. Bilirubin interference has been documented with the NEFA C method. Please refer to the Table IV, Serum NEFA Additive Study. For practical purposes, total bilirubin levels below 10 mg/dL have a negligible depression of the measured NEFA result. Visibly icteric specimens with total bilirubin levels above 10 mg/dL can be accommodated by running a serum blank, as described in the "Details of procedure".
7. Hemoglobin interference has been documented with the NEFA C method. Please refer to the Table IV, Serum NEFA Additive Study. For practical purposes, hemoglobin levels below 100 mg/dL show a negligible increase of the measured NEFA result. Visibly hemolyzed specimens with a hemoglobin level above 100 mg/dL can be accommodated by running a serum blank, as described in the "Details of procedure".
8. Ascorbic acid present even at levels ten times above normal has no effect on the measured NEFA level due to its decomposition by ascorbate oxidase present in Color Reagent A.
9. The measured optical density of the final reaction mixture corresponding to the standard solution (1.0 mmol/L or 1.0 mEq/L), measured relative to the reagent blank, must exceed 0.180. The analysis must be repeated with fresh reagent solutions if this test is failed.
10. The measured optical density of the final reaction mixture corresponding to the reagent blank, measured relative to water, must not exceed 0.100. The analysis must be repeated with fresh reagent solutions if this test is failed.
11. Quality control specimens must yield results within the acceptable ranges, as determined by the specimen manufacturer or within the laboratory by repeat previous analyses. Otherwise the test should be repeated with fresh reagents.
12. Interference from lipemia has been documented in the NEFA C method. Visibly lipemic specimens may be accommodated by running a serum blank, as described in the "Details of procedure".
13. The range of linearity of this method is up to 2.0 mEq/L. It is recommended that specimens of higher NEFA concetration be diluted with water to 25% of original concentration (dilute one part of patient serum with three parts of reagent grade water) and reanalyzed in a subsequent run on the same day along with the control and a reagent blank.

EXPECTED VALUES

The expected normal range for serum NEFA from fasting patients is 0.1 − 0.6 mEq/L. It is strongly recommended that each laboratory determines an expected normal range in the particular population of patients in its locality.

PERFORMANCE CHARACTERISTICS

Accuracy

The accuracy of the Wako NEFA C test method was determined by recovery studies in which 1 mEq/L of NEFA were added to serum pool. The relative activity of the Wako NEFA C test method was examined by analysis of each fatty acid solution. The results are presented in Table III below.

TABLE III: SERUM NEFA RECOVERY STUDY

FREE FATTY ACID (NEFA)	ACTIVITY %	RECOVERY %
Acetic acid	0	0
Propionic acid	0	0
Butyric acid	2	2
Valeric acid	4	3
Caproic acid	98	99
Caprylic acid	97	94
Capric acid	99	96
Lauric acid	98	97
Myristic acid	103	93
Palmitic acid	97	94
Stearic acid	92	
Arachidic acid	94	
Palmitoleic acid	102	103
Oleic acid	101	102
Linoleic acid	99	100
Linolenic acid	102	100
Arachidonic acid	94	85
Citric acid		0
Oxalic acid		0

Specificity

The specificity of the Wako NEFA C test method was examined by analysis of serum samples containing additions of known amounts of potential interfering substances. Of the substances tested, only heparin, hemoglobin and bilirubin had some documented interference. There is no method for correcting the interference from heparin insofar as heparin stimulates the production of authentic NEFA and hence must be avoided. Elevated hemoglobin and bilirubin in specimens can be accommodated by including a specimen blank in the test run, as described in the "Details of procedure" section. Table IV presents the experimental data to support the specificity of the NEFA C test.

2. After the blood has been allowed to clot, the serum should be separated by centrifugation as soon as possible. If immediate analysis is not possible, the serum may be frozen for up to 24 hours prior to analysis. Please note that serum is the specimen of choice, but alternate collections may be acceptable. Please refer to the section on the "Limitations of procedure".
3. Any specimen containing heparin is unsuitable for this analysis. Hence any patient receiving heparin therapy, or any specimen collected in a heparinized collection vessel is unsuitable for this analysis.
4. Specimens that are noticeable icteric, hemolyzed or lipemic may yield inaccurate results unless a specimen blank is also analyzed. Instructions for preparing a specimen blank are included in the "Details of procedure" section, and some criteria for determining whether or not a blank is necessary are included in the section on the "Limitations of procedure".

PROCEDURE

Materials required but not supplied

Micropipette: 50μL
Test tubes
Transfer pipettes: 1.0, 2.0, 10.0 and 20.0mL
Water bath: Set to maintain 37°C
Spectrophotometer or colorimeter: Capable of measuring optical density at 550nm.
If double wavelength photometry is used, absorption at 546nm may be used relative to 660nm.

Details of procedure

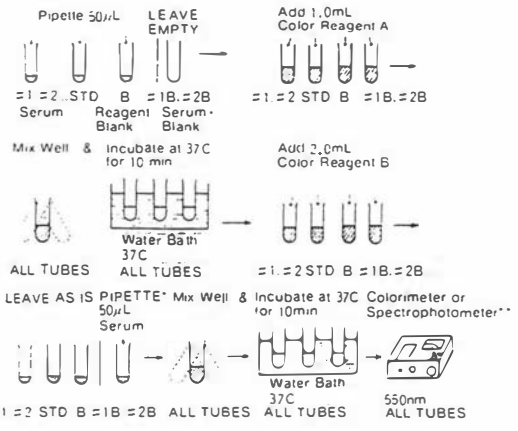
1. Label a series of glass test tubes as follows:
   Specimen: #1, #2, #3, etc.
   Specimen Blanks: #1B, #2B, #3B etc. (see note below)
   Standard: STD
   Reagent Blank: B
   Note: Specimen blanks are not required for specimens of normal clarity and color. Please refer to the section of "Limitations of procedure" to determine whether a specimen blank is appropriate.
2. Accurately pipette 50 μL of the appropriate patient or quality control serum into the appropriate Specimen Tubes (#1, #2, #3, etc.). Introduce no samples into the Specimen Blank Tubes at this time. Accurately pipette 50μL of reagent grade water into the Reagent Blank Tube (B).
3. Accurately pipette 1.0mL of Color Reagent A Solution into all test tubes.
4. Mix all test tubes well and place them in a 37°C incubator bath for ten minutes. For purposes of reproducibility, it is important that this ten minute incubation be accurately timed.
5. Accurately pipette 2.0mL of Color Reagent B Solution into all test tubes. If any specimen blanks have been set up, accurately pipette 50μL of the appropriate patient serum into the appropriate Specimen Blank Tubes (#1B, #2B, and #3B etc.).
6. Mix all test tubes well and place them in a 37°C incubator bath again for ten minutes. Once again, timing of the second incubation is critical for good reproducibility.
7. Remove the test tubes from the incubator and allow them to equilibrate with room temperature for five minutes. Then read the optical density of all tubes at 550nm versus the Reagent Blank (B). Also, record the optical density of the Reagent Blank (B) versus water at 550nm.

TABLE I : PROCEDURE OUTLINE

	Specimen (#1, #2, #3 etc.)	Standard (STD)	Reagent Blank(B)	*Specimen Blank(#1B, #2B etc.)
Sample	Serum 50μL	Std Sol 50μL	Distilled or Deion water 50μL	
Color Reagent A	1.0mL	1.0mL	1.0mL	1.0mL
Mix well and incubate at 37C for 10 minutes				
Color Reagent B	2.0mL	2.0mL	2.0mL	2.0mL
(Sample)	—			Serum 50μL
Mix well and incubate at 37C for 10 minutes				
Spectrophotometer or colorimeter with filter of around 550nm	Measure the optical density of all tubes versus the Reagent Blank(B) at 550nm			

* Note that Specimen Blanks are not required for serum specimens of normal clarity and color. Please refer to the section on the Limitations of procedure to determine whether a specimen blank is appropriate.

Procedure diagram



NOTE * For normal serum no serum blank is needed, but it is essential when the serum samples are deeply colored.
** If a double wavelength photometry is used, λ_1 = 660nm, λ_2 = 546nm.

Calibration

The Wako NEFA C test follows Beer's Law and is linear over the range from 0.0 to 2.0 mEq/L. It is recommended that the linearity of the test system be confirmed for each new lot of reagents at the time of receipt or during the first usage of the reagents. First, verify that the spectrophotometer or colorimeter is in proper calibration as delineated in the manufacturer's instrument manual. Then proceed to prepare three standard solutions in the analytical tubes according to the following simple protocol and analyze them by the normal procedure for the NEFA C test including the usual reagent blank.

TABLE II

Tube No.	Name	1.0mEq/L NEFA Std	Reagent Water	Reagent A	Incubation	Reagent B	Incubation	Optical Density	NEFA Conc
#1	BLANK	—	50μL	1.0mL		2.0mL		0.000	0.00mEq/L
#2	LO STD	25μL	25μL	1.0mL		2.0mL		(read)	0.50mEq/L
#3	MID STD	50μL	—	1.0mL		2.0mL		(read)	1.00mEq/L
#4	HI STD	100μL		1.0mL		2.0mL		(read)	1.97mEq/L

NOTE * The final volume of the High Standard is slightly increased due to the fact that twice the normal volume of standard is used. However, in this case, the final NEFA concentration is corrected to be slightly above 2.00.

Using linear graph paper, prepare a calibration graph as is exemplified in Figure 1 and plot the recorded optical densities versus NEFA concentration in mEq/L on the abscissa. The straight line in Figure 1 will be similar to but not identical with the line that will be determined from laboratory results. For purposes of example the line in Figure 1 was determined from the following optical density readings on a Model 550 Hitachi Spectrophotometer:

OPTICAL DENSITY AT 550 nanometers

Tube #1 BLANK	0.000	
Tube #2 LO STD	0.131	plot versus 0.50 mEq/L
Tube #3 MID STD	0.262	plot versus 1.00 mEq/L
Tube #4 HI STD	0.516	plot versus 1.97 mEq/L

FIGURE 1 : Typical NEFA calibration graph