UNIVERSITY OF GLASGOW

DIETARY EFFECTS ON MILK PROTEIN IN DAIRY COWS

by

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being

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ABSTRACT

The nutritional and non-nutritional factors influencing dairy cow milk production were reviewed, with particular attention to the effects of diet. Three experiments were undertaken to study further the effects of diet on milk production, especially milk protein content and yield.

The initial experiment examined the effects of three complete, but differing ME diets, pre and post-partum, on animal performance. The higher ME diets significantly increased TDMI, and animals offered these diets showed a lower weight loss and an earlier peak milk yield. No significant effects on milk fat or lactose contents were observed, but milk protein content was consistently higher in the higher energy fed cows. Milk casein contents were depressed from normal lactational values, but were higher in the higher energy fed cows. The conclusions from this trial were that the costs of the higher energy diets outweighed the small increases found in milk yield and constituents.

Experiment 2 examined the effects of a high crude protein concentrate, fed at different stages of lactation, on milk production. The substitution rate of silage with concentrates was lower and silage DMI was significantly higher in the high protein fed cows. Milk analysis results (with the exception of the casein and NPN results) were adjusted by covariance. Milk yield was significantly higher in the high protein fed animals up to 18 weeks of lactation, with no significant differences being recorded in milk fat or lactose content. Significant increases were found in NPN content in the high protein diets, but with no effect on milk casein content. The conclusion of this work was that with a good quality silage, including a low degradability animal protein in the supplement had no beneficial effect on milk production.

The final experiment was a short-term changeover trial examining the effects of two energy sources (starch and sugar) at two crude protein levels on milk production. There were no significant differences in forage intake. Milk results (excepting casein and NPN) were adjusted by covariance. Average milk yield and constituent contents were higher with the starch diets although the sugar diets showed a greater response in milk yield to increased dietary protein. Milk casein content was unaffected by the diets, but NPN content was significantly higher in the starch diet at the higher protein level.

The conclusions from these experiments are that future work should be centred around: dietary effects on milk protein constituents, and development of an accurate method for calibrating milk analysis machines for milk casein; more long term trials should be used to examine total lactational changes and effects of cow condition manipulation prepartum on body reserves utilisation, and, trials should be designed to examine the effect of feeding frequency of sugar based supplements on forage intake.

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ABBREVIATIONS

AA	Amino acids
ADP	Adenosine diphosphate
AHEE	Acid hydrolysed ether extracts
ATP	Adenosine triphosphate
BHBA	Beta-hydroxybutyrate
CP	Crude protein
CTP	Cytidine diphosphate
DCP	Digestible crude protein
DM	Dry matter
DMI	Dry matter intake
DOMD	Digestible organic matter in the dry matter
FCM	Fat corrected milk
GOT	Glutamic oxaloacetic transaminase
GPT	Glutamate pyruvate transaminase
GTP	Guanosine triphosphate
IVD	*In vitro* digestible organic matter
LWT	Liveweight
ME	Metabolisable energy
mRNA	Messenger ribonucleic acid
N	Nitrogen
NCD	Neutral Cellulase Digestibility
NEFA	Non-esterified fatty acid
NPN	Non-protein nitrogen
RDP	Rumen degradable protein
RNA	Ribonucleic acid
SAC	Scottish Agricultural College
SNF	Solids-not-fat
rRNA	Ribosomal ribonucleic acid
TDMI	Total dry matter intake
tRNA	Transfer ribonucleic acid
UDP	Undegradable protein
UTP	Uridine triphosphate
VFA	Volatile fatty acids
WSAC	West of Scotland Agricultural College

For Jamie, and Jonathan.

CHAPTER 1

INTRODUCTION

The nutritional importance of milk in the human diet is widely recognised. Cows' milk provides a substantial percentage of the vitamins, minerals and protein required by a child or adult, as shown in table 1.1 (from Ling, Kon and Porter 1961):

Table 1.1: The contribution of 0.5 litres of milk/day to the nutrient requirements of a child, and man doing moderate work

	4 yr old child		Adult man	
	Daily Requirement	% from milk	Daily Requirement	% from milk
Energy kJ	6400	25	12600	13
Protein g	56	30	87	20
Calcium g	1	60	0.8	75
Iron mg	7.5	2	12	1
Nicotinic acid mg	6	7	12	3
Riboflavin mg	0.9	85	1.8	45
Vitamins: A iu	3000	30	5000	15
B iu	0.6	35	1.2	20
C iu	15	70	20	50
D iu	400	2	-	-

An essential introduction to any work examining the effect of diet on milk production in dairy cows, is a knowledge of the synthesis of milk and the nutritional and non-nutritional factors affecting it.

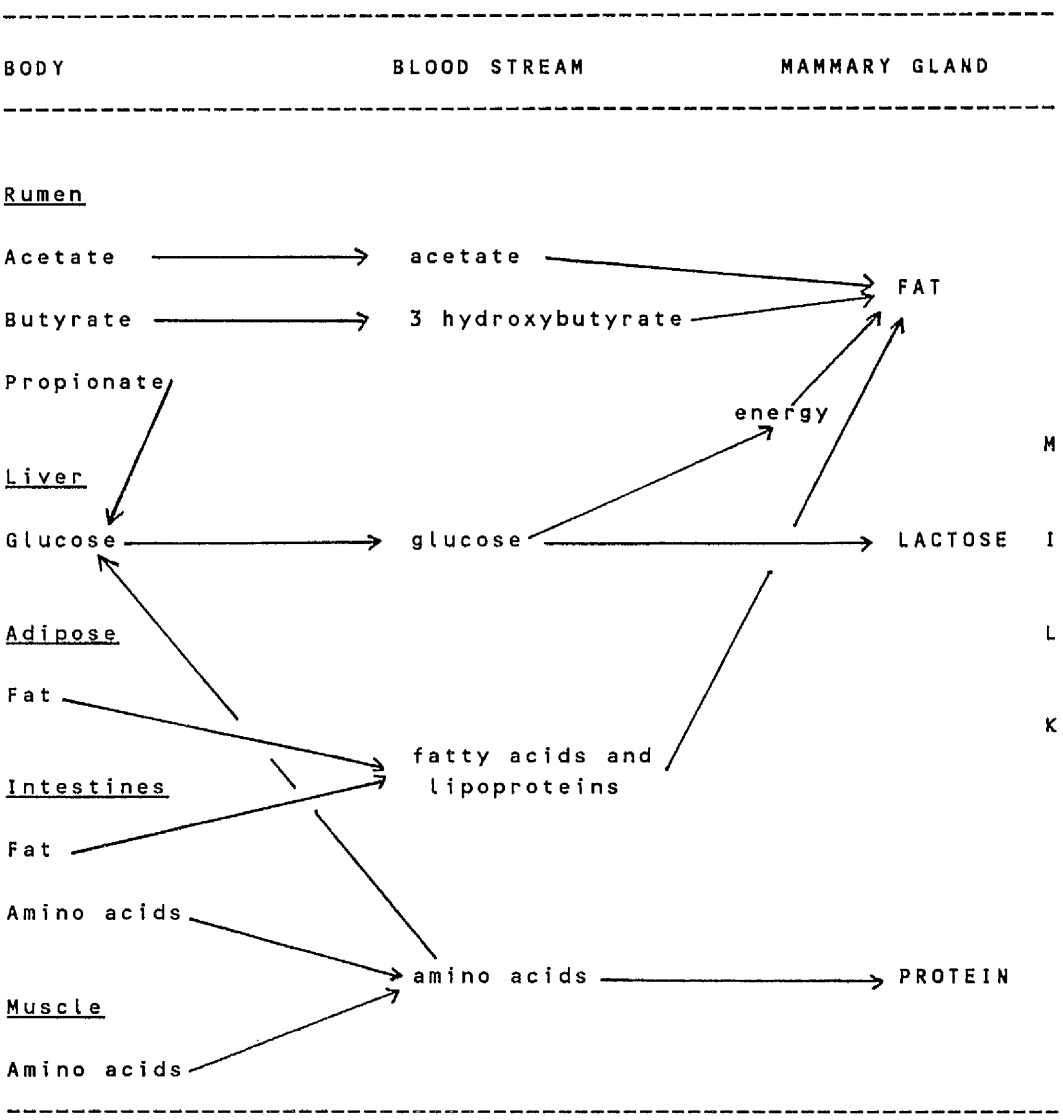
MILK SYNTHESIS

1. Introduction

The materials required for milk synthesis are found in the diet and changes in feed quality and/or quantity can ultimately affect milk yield and composition. For a dairy cow to attempt to achieve its potential therefore, it should be offered an adequate supply of required materials (i.e. fibre, protein, energy), otherwise it may suffer short or long term effects on milk synthesis.

The site of biosynthesis is the vast number of secretory cells which line the alveoli in the mammary gland, and discharge milk into the alveolar lumen. An important feature of these cells is the high number of mitochondria present, emphasising the high energy demand of biosynthesis, and the marked hypertrophy at the milk constituent synthesis sites in the Golgi apparatus and endoplasmic reticulum (Rook and Thomas 1983). The alveoli are well supplied with blood capillaries from which required metabolites are drawn. Arterio-venous difference studies have shown that glucose (Wood et al 1965; Linzell 1974), amino acids (Barry 1961; Mepham 1979), free fatty acids (Bickerstaffe 1971; Stead and Welch 1975), proteins, acetate, and 3-hydroxybutyrate are all withdrawn from the blood and act as precursors in milk synthesis, figure 1.1 (adapted from Bourne and Orr 1988):

Figure 1.1: Milk synthesis in the dairy cow



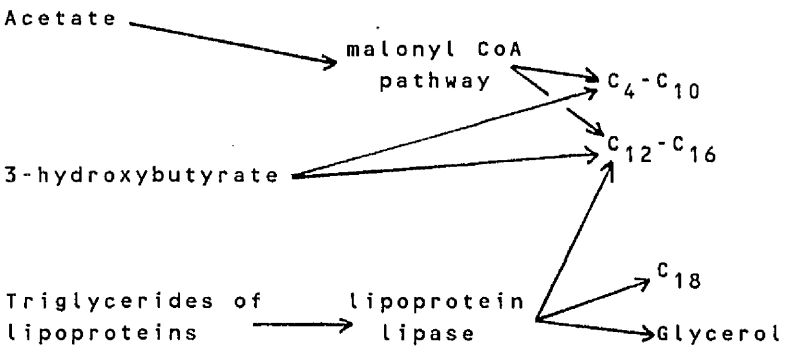
2. Milk fat synthesis

Milk fat consists predominantly of triglycerides. The fatty acids present in the triglycerides are derived from two sources:

1. either, directly from the fatty acids of plasma constituents,
2. or, synthesised de novo in the gland.

The fatty acids range from C_4-C_{18} in chain length, and are characterised by having a relatively high proportion of short chain fatty acids (Bourne and Orr 1988). Research by Hartmann and Lascelles (1964), and Palmquist et al (1969) has shown that the fatty acids in milk fat with carbon chains from C_4-C_{14}, and part of C_{16} are derived primarily from de novo synthesis. Thus, short and medium chain fatty acids are synthesised using acetic acid and 3-hydroxybutyrate as precursors whereas the long chain fatty acids and some medium chain are derived directly from circulating lipoproteins in the plasma, figure 1.2:

Figure 1.2: Milk fat triglyceride synthesis

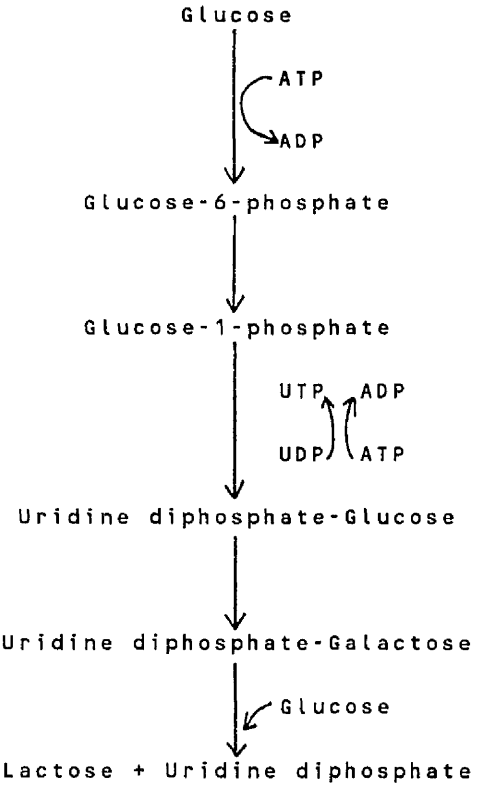


The lack of glucose utilisation in the ruminant for fatty acid synthesis is linked with evolutionary adaptation to feedstuff digestion (Bauman and Davis 1974). Fermentation of ingested carbohydrates in the rumen to acetate, butyrate and propionate is extensive, resulting in minor absorption of glucose from the gut. Thus, acetate and butyrate are the predominant carbon sources for fatty acid synthesis in the cytosol of the secretory cells.

3. Milk lactose synthesis

Milk lactose is chemically the simplest of all the major milk constituents. It is synthesised from glucose, as shown in figure 1.3:

Figure 1.3: Pathway of lactose synthesis in the dairy cow

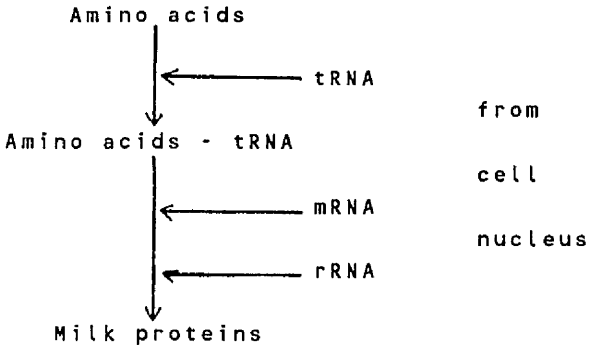


Lactose is unique in that, apart from a few plants, the mammary gland is its only natural source. Secretion from the epithelial cells in the mammary alveoli appears to be in conjunction with the milk protein secretion in that they are released from vesicles, together with water, water-soluble materials and ions (Rook and Thomas 1983).

4. Milk protein synthesis

In recent years the milk constituent of greatest importance, both economically and nutritionally, is undoubtedly milk protein. The milk proteins synthesised are derived mainly from blood amino acids, although the imbalance between absorbed amino acids and those present in the milk proteins suggests that some amino acid synthesis must be taking place (Barry 1961; Larson 1979; Thomas 1983). Protein synthesis involves the assembly of amino acids, and peptide bonds into a polypeptide chain, with the sequence being determined on the ribosome of the cell by ribonucleic acid (RNA) through the process of transcription, figure 1.4 (from Thomas 1983):

Figure 1.4: Pathway of protein biosynthesis



Milk 'total' protein is estimated as 6.38 x total nitrogen content, which assumes that milk protein contains 158.9gN/kg (Thomas 1983). Approximately 95% of the nitrogen is 'true' protein with the remaining 5% being non-protein nitrogen which is mainly urea, and in equilibrium with blood urea (Thomas 1980). The components of the 'true' protein fraction have been studied for many years with early reports

showing casein, a-lactalbumin, and B-lactoglobulin to be the main constituents (Rowland 1938; Rook 1961b). Later studies, using advanced separation techniques, have determined a plethora of constituents which make up the three basic components mentioned above, and which have been reviewed in a variety of papers (Jenness 1974; Davis and Law 1980; Brunner 1981; Eigel et al 1984). In general, the main components of the casein fraction are a_{S1}-, a_{S2}-, B-, y-, and k- caseins, whilst the whey proteins consist of B-lactoglobulin, a-lactalbumin, bovine serum albumin, immunoglobulins and a proteose-peptone fraction (Thomas 1983).

NUTRITIONAL FACTORS AFFECTING MILK PRODUCTION

1. Introduction

Feed represents the major cost in animal production; Bax et al (1989) in a study of two systems of producing a milk quota estimated that the percentage of total costs (including labour etc.) that feed represented was 21% (SD = 4.66; range 14 - 28%). Chemistry has basically provided the matrix upon which nutritional factors affecting milk composition have been based. The fractionation of feedstuffs by Liebig in 1842 into proteins, carbohydrates and lipids marked the beginning of the proximate analysis of feeds. This in turn enabled descriptions to be made of the nutritive value of feedstuffs, with later energy balance and respiratory exchange studies in the mid 19th century allowing precise values to be calculated. From these early works much research has been carried out on the nutritive values of feeds and their effects in relation to milk production in the dairy cow.

2. Type of diet and effect on products of digestion

The diet of a dairy cow is a major factor affecting milk composition, and has consequently been widely studied (Rook 1976; Thomas 1976; Van Horn *et al* 1979; Laird *et al* 1981; Thomas 1983; Mayne and Gordon 1985).

2.1 Forage

2.1.1 Grazed grass

Grazed grass is the most common forage used in dairy cow nutrition. However, the British climate limits winter months' supply, and this necessitates the conservation of the grass forage either as hay or silage as a feed for in-wintered animals. Producing a good quality grass in sufficient quantity has been reviewed by Raymond (1964) and Castle (1982) who concluded that efficient management was the key factor. Assessments of the nutritive value of conserved forages used in dairy cattle diets have also been made (Campling and Milne 1972; Thomas *et al* 1981; Thomas and Rae 1988).

2.1.2 Hay

When conserving grass as hay, the material is partially or wholly dried in the field to prevent the microbial growth which can cause deterioration. Owen and Wilman (1983) examined the effects of field drying herbage on sward regrowth and found that when the cut forage was removed after a period of 5 or 10 days on the sward, the dry matter of the herbage harvested during the remaining growing season decreased by 8 and 16% respectively. They further concluded that this method of forage conservation led to later cuts of the grass being low in leaf

blade area, and generally that the next cut of grass had a much darker environment in which to grow.

2.1.3 Straw

Untreated straw has virtually no significant role to play in dairy cow feeds because of its low energy value and crude protein content (approximately 5.7 MJ/kg DM and 38 g/kg DM respectively for long barley straw). Research involving straw in diets tends to be centred around treated straw or strawmixes. Techniques have evolved for treating straw with chemicals to disrupt the lignified structure of the cell walls and subsequently make more cellulose available for fermentation. Two main methods are available for making nutritionally improved straw:

1. applying sodium hydroxide - increases the ME to 8.5 MJ/kg, which is comparable with moderate hay.
2. creating an airtight seal around stacks of straw, and admit ammonium hydroxide - increases ME and NPN concentrations to levels sufficient for optimal microbial synthesis.

However, both methods produce a straw too low in ME to merit inclusion in dairy cow diets at high levels, and usually compounders of concentrates incorporate it on a least cost basis if the economics justify it.

2.1.4 Silage

Maize silage, because of its ease of ensiling, suitability for manure and slurry and its high feed value, has prompted interest among farmers as a forage crop. Phipps (1990), citing unpublished work by Brabander in Belgium, reported that as the maize silage dry matter

increased, the maize silage dry matter intake of the cows increased (from 12.7 kg/d at 220 g/kg DM to 14.6 kg/d at 340 g/kg DM). Phipps related this to energy requirements in the dairy cow and suggested that this increased intake was equivalent to a theoretical improvement in milk yield of 6 kg/d. These results were similar to those obtained in France (Demarquilly 1988; Pflimlin 1990) and Britain (Phipps et al 1988; Gerring 1990).

Despite the advantages of feeding maize silage, a number of problems are associated with the forage particulary in view of its requirement of warm weather for efficient growth. Cool and moist areas of Britain have unreliable harvests of maize especially in Scotland where farmers have attempted to periodically grow maize as a forage (see Potts et al 1979). As a result of the high dry matter contents associated with maize silage, the forage is difficult to consolidate in the clamp with high aerobic instability, and intakes decrease at high levels of dry matter content. Consequently, because of these problems and especially the weather conditions, the main forage in Scotland is primarily grass silage.

Much research has been focussed on grass silage as the main forage in winter dairy cow diets (see Thomas and Chamberlain 1982). Conserving the grass as silage is achieved by ensiling wilted grass (24 hours in the field in good weather), and sealing to prevent aerobic degradation. Reducing the pH of the herbage, either by application of an acidifier or a water soluble carbohydrate which encourages the formation of lactic acid by herbage bacteria, avoids spoilage by anaerobic organisms (Frame et al 1987). Jackson and Forbes (1970) examined four silages of

different dry matter contents, achieved by altering the period of wilting. They found that the longer wilted, higher dry matter content forages increased dry matter and ME intake through improved voluntary intake. Forbes (1983) stated that the intake of silage was usually lower than that of hay made from the same grass, and attributed it to the high lactate content, low pH, slow rate of passage and low availability of protein. However, different types of silage have been studied since, and the effect of silage preference was examined by Weller and Phipps (1986).

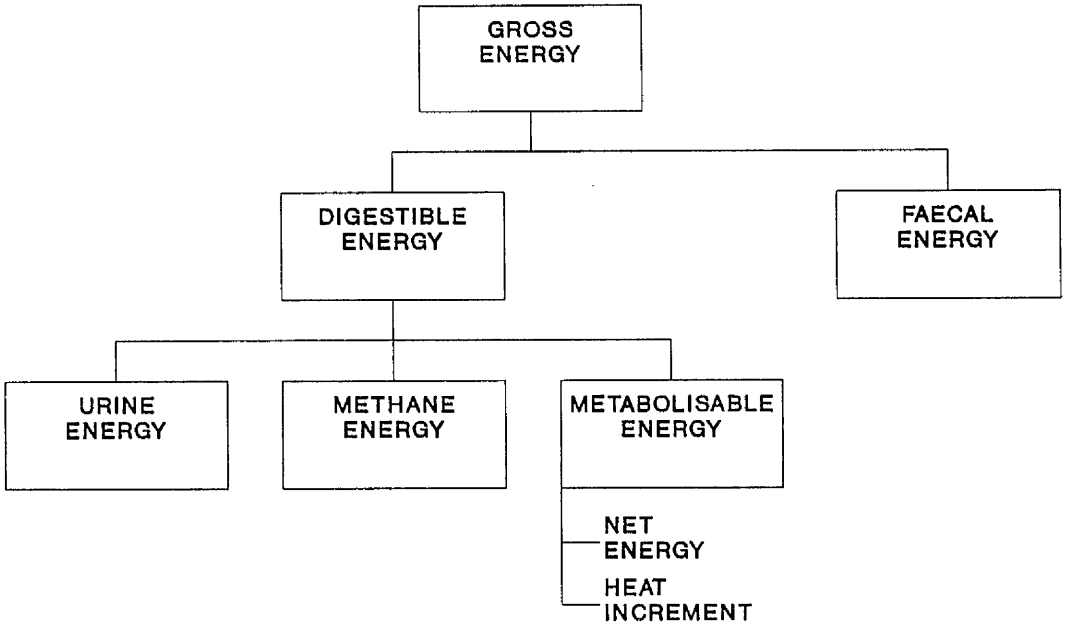
2.2 Concentrates

The problems of insufficient dietary energy associated with forage only diets have been controlled by supplementing the herbage with high energy, high protein concentrates, the levels of which are determined by animal productivity. The energy requirements of a milking cow are detailed in MAFF (1984), and the feeding values of feedstuffs used are currently available in the 'UK tables of nutritive value and chemical composition of feedstuffs' (1990). The cereals rich in starch which are in common use as supplements are barley, wheat, oats and maize (Campling and Lean 1983), although some crops such as fodder beet have shown potential in milk production (Roberts and Martindale 1990).

Whilst cereal grains are the main energy supplying concentrate feeds, other products are routinely used to supply energy to livestock: fat, oils, molasses. Animals use energy for a variety of bodily functions ranging from muscular activity to tissue synthesis. The result of this is the fact that energy intake determines the level of performance an animal can sustain. The pathways of energy utilisation

are shown in figure 1.5:

Figure 1.5: Pathway of ruminant energy utilisation



The common sources of protein for dairy cows are either vegetable based: soya bean, rapeseed, groundnut when all oil has been removed, or animal based: fishmeals, meat and bone meals. The composition and nutritive value of these protein sources depends upon processing techniques in the former and composition of the raw materials used in the latter. An added advantage of feeding animal proteins as high quality supplemental sources is that they can remedy deficiences in the essential amino acid composition of the remainder of the diet.

2.3 Effects on products of digestion

The assessment of nutrient supply in relation to feedstuffs has provided a necessary first step in the study of the relationship between diet and milk production. The next stage is the process of digestion itself, a detailed account of which is available in Lewis and Hill (1983). The products of digestion vary according to the constituents offered in the feed (reviewed earlier in this chapter), although all are a potential source of energy. The complexity of the relationship between diet and the products of digestion, and the uncertainties of the role of nutrient supply in milk secretion regulation (Rook and Thomas 1983), make a quantitative summary of any interrelationships between diet and milk production unreliable (Thomas and Rook 1983). Nevertheless, Chalmers (1979) examined factors which influenced the products of digestion in terms of dietary carbohydrate, lipids and protein. The growth of ruminal bacterial populations is influenced by the amount of ATP made available from principally dietary carbohydrate. Sufficient energy enables efficient utilisation and increases the microbial and undigested dietary protein flowing from the rumen, subsequently improving small intestine absorption of amino acids. The contribution that dietary protein makes to the amino acid absorption in the intestine depends upon the rumen degradability, amino acid composition and digestibility of the material passing from the rumen (Chalmers 1979). Due to the adverse effects of surplus ammonia when increasing dietary protein supply, rumen protected proteins or 'undegradable proteins' are utilised in dairy cow feeds to improve the amino acid supply to the small intestine, reduce stress on liver metabolism and generally improve body condition (Kaufmann and Lupping 1982).

3. Lactation experiments

An inherent problem when designing lactation experiments is the lack of nutritional-management or nutritional-environmental awareness (Thomas and Rook 1983). Initial experiments on dairy cows took no account of udder infections which led Waite *et al* (1963) to suggest that milking should be carried out on individual quarters. Undoubtedly, this is too time consuming and efficient management is the chosen policy (Smith 1983). Generally, in later lactation experiments, accurate records are kept of dairy cow health, and treatment where necessary is administered as soon as possible (Dodd 1983).

Thomas and Rook (1983) suggested that experiments should ideally be carried out during mid-lactation when the milk yield is starting to steadily decline. Small groups of animals can be used in short-term trials with adjustments being made for any between-animal differences. Nonetheless, some experiments are designed to investigate if diet can affect the standard curves quoted for milk yield and constituents (see Rook and Campling 1965). Large numbers of animals are necessary to overcome the substantial between-cow differences in milk production, and variations in food quality should be taken into account.

4. Dietary effects on milk production

4.1 Introduction

The influence of diet on the yield and composition of bovine milk has long been recognised and investigated. Variations in milk composition attributed to diet have been linked with dietary effects on the products of digestion (see earlier; Rook and Thomas 1983; Thomas

and Rook 1983). However, the gross composition of milk reflects the relative rates of constituent secretion and consequently changes in the amount of one or more of these components leads to a change in the proportions of the others.

There are several detailed reviews on the effects of diet on milk production (Rook 1961a; Thomas 1976, 1980; Castle and Gunn 1984); attention here will be focussed on two main headings - dietary protein and dietary energy, with some discussion being made on their interaction.

4.2 Dietary protein

Dairy cows require protein to provide them with the amino acids necessary for body maintenance and secretion of products containing proteins, such as milk. The composition and quantity of the amino acids available for absorption in the small intestine determines the protein value of a feedstuff (Miller 1982). The crude protein content of a feed contains the main nitrogen component required by an animal, and the difference between this and faecal nitrogen represents the amount of 'digestible crude protein' (DCP) in the diet (the quantity of nitrogen absorbed during digestion).

The site of microbial fermentation activity in the digestive tract varies between species. As a result of its accessibility and obvious importance, more research has been carried out on ruminal fermentation activity and metabolic implications than on the microbial activity sites in non-ruminants. However, from studies on fibre digestibility between species (Keys et al 1969) it is evident that the microbial contribution to the non-ruminant digestive process is less than that

for the ruminant. In practice, the efficiency with which digested protein is utilised in monogastrics is largely dependent upon the supply of essential amino acids; the amino acid that is present in least supply relative to requirement determines the extent of protein synthesis (the limiting amino acids - usually lysine and methionine in pigs and poultry).

Although ruminants, like monogastrics, must necessarily have requirements for specific amounts of amino acids, the nature of the digestive system ensures that the amino acid composition of absorbed protein is less variable and less influenced by the amino acid composition of dietary protein. The composition of ruminal microflora is largely determined by the equilibrium established in relation to diet (see Lewis and Hill 1983), and many organisms require anaerobic conditions, making dietary requirements for ammonia, some vitamins and carbon dioxide a prerequisite. Efficient bacterial protein synthesis is dependent upon an adequate supply of dietary energy and rumen degradable protein (RDP). With sufficient dietary energy, microorganisms can hydrolyse dietary protein to yield peptides, amino acids and ammonia from which they can synthesise microbial protein. However, bacterial protein alone is insufficient to meet total requirement (Kaufmann and Lupping 1982) and a supply of undegradable dietary protein (UDP) is necessary to maximise animal performance. The digestion of the microbial protein in the lower gut yields amino acids which increase the efficiency with which UDP is utilised. Thus, the significance of ruminal activities lie essentially in the extent to which dietary protein is converted into bacterial protein and therefore upon the relative nutritional values of ingested feed protein and that

synthesised in the rumen.

The most commonly used sources of protein in concentrate feeds have already been reviewed. Developments ten years ago suggested that non-protein nitrogen (NPN) instead of plant protein could be used under certain conditions, although within the first two months of lactation there is little opportunity in a high yielding cow for effective NPN utilisation (Tamminga and van Hellemond 1977). In view of this difficulty, Tamminga and van Hellemond suggested that the use of 'protected' proteins could improve the situation. The search for proteins which have a good quality (amino acid pattern), and are available in sufficient amounts at acceptable prices and which are naturally and consistently of low degradability has had little success so far. Additionally, some protein-rich feeds which are claimed to have a low degradability, such as blood meal, fish meal, maize gluten, brewer's grains etc. have only in most cases been tested by <u>in vitro</u> methods (ammonia liberation, solubility in buffers) and not by direct measurements of the flow of UDP to the duodenum of cows (Kaufmann and Lupping 1982). Artifical protection therefore provides a way of producing protein with consistently low degradability as a suitable feed for high yielding dairy cows. Two main methods of protecting dietary protein are:

1. by chemical treatment: treating soya protein with formaldehyde reduces its degradability

2. by physical methods (usually heat treatment: dry heating or heating in an autoclave): heat treating fish meal, casein or

soyabean meal in an autoclave usually results in proteins becoming less soluble with lower ammonia concentrations in the rumen (Kaufmann and Lupping 1982).

The advantages of using protected protein in dairy cow diets was shown by Kaufmann and Lupping (1982), who found that they improved milk yield by 1.5-2 litres per day in both cows and heifers with no effect on milk protein content. Attempts have been made to 'protect' silage protein, but in general they are largely unsuccessful because of the technical difficulty in controlling the application rate of formaldehyde, which usually results in excess treatment. Spain et al (1990) recently investigated the effect of different types of protein source on milk composition in mid-lactation dairy cows using fishmeal, corn glutenmeal and soybean meal. They concluded that fishmeal contained some factor that affected milk fat production because results indicated a drop in milk fat in these diets (a result also found by Oldham et al 1985), but ruminal volatile fatty acids (VFA) were not significantly affected thus suggesting no ruminal fermentation alteration. In general they found that the soybean meal supported better total production, although work by Thomas et al (1985) revealed that soyabean meal produced a lower response in milk protein yield to duodenal non-ammonia crude protein, as a result of a reduction in the efficiency of transfer of non-ammonia crude protein into milk protein. The effect of fishmeal on milk production found by Spain and colleagues is supported by the earlier work of Pennington and Davis (1975) who found that ruminal and abomasal infusions of fish oil reduced milk fat content and by Rae et al (1986) who found depressions in milk fat in animals offered a high proportion of fishmeal in the supplement as

compared with milk fat contents of animals offered no supplement or a cereal based supplement. Fish meals are excellent sources of protein with high proportions of lysine, methionine and minerals. The composition of fish meals is dependent upon the type of fish used, demersal species (cod) produce low oil content meals (2-6%) whereas pelagic species (herring) produce meals with fairly high oil contents (7-13%). The effects of fish meal in dairy cow diets have been examined in many experiments with contradicting results (Miller et al 1983; Small and Gordon 1985; Oldham et al 1985b; Thomas et al 1985). Thomas et al (1985) found that observed duodenal non-ammonia nitrogen was in excess of predicted values and that there was a considerable range in microbial efficiency, however, causes of the variation are still unclear. In a comparison of the latter two experiments mentioned above, Thomas and Rae (1988) concluded that the composition of the diet influenced not only digestion but also the efficiency of duodenal protein utilisation by the dairy cow for milk protein.

Examination of the effects of infusing animals with different proteins and amino acids, although not directly related to natural dietary effects, enables an understanding of the processes which affect milk production through nutrient absorption. With the increase in milk yield due to better management nowadays, the dairy cow is finding it increasingly difficult to meet nutrient requirements because of the limits of appetite, physical capacity, and the inability to generate sufficient quantities of precursors to meet mammary gland demands for maximum milk synthesis. Clark (1975) reviewed the effects of postruminal infusions of proteins and amino acids on milk production and found that casein, because of its position as a major milk protein

and consequently its ideal pattern of amino acids, was the main source of protein used in postruminal infusion studies (see Broderick et al 1974; Clark et al 1977; Rogers et al 1984; Cohick et al 1986). The postruminal infusion of casein was found to be of greatest effect in high producing cows where increases of up to 4kg were evident in milk yield and an increase of 10-15% was apparent in milk protein percentage. Milk fat content decreased as a result of improved yield and not because of a suppression effect from the casein. Although Vik-Mo et al (1974) reported a lack of response to casein infusions thought to be due to feed intake fluctuations, the increase in protein yield resulting from an abomasum infusion was suggested to be a result of increases in 'true' protein and NPN.

The move of todays' dairy products market towards high quality milk has returned research to examine the make-up of milk protein which has been improved by dietary factors. A substantial review by Thomas (1983) on milk protein investigated dietary effects on milk protein composition, which led to the conclusion that dietary changes produced only small effects in milk protein composition, and those mainly in the minor protein components. Thus, if dietary effects on milk protein content are measured in terms of total nitrogen, then increases or decreases in the NPN content can confound the interpretation of changes in 'milk protein' secretion (Thomas 1980). Unfortunately, relatively few papers examine changes in milk protein composition, unless they specifically investigate dietary nitrogen or blood urea, and consequently, any claimed increases in milk protein content as a result of dietary manipulation could simply be an elevation or decline in milk urea. In terms of dairy product manufacture, paying for urea (in total

protein milk) and not true protein is uneconomical. Subsequently, reviews of papers quoting 'total protein' content should be read with this in mind.

The effect of dietary protein on milk production has therefore been the subject of many reviews. Woodman (1957) proposed a number of standards of dietary protein content above or below which little beneficial effect was evident on milk yield or composition. Rook and Line (1962) examined the influences of dietary protein on milk production at levels above and below the recommended Woodman standards. In two consecutive yearly experiments using cows in mid-lactation they found that the general belief that Woodman was unduly generous in his margin of safety was unfounded since animals that were high yielders showed a significant loss in milk yield within a short period on the lower, revised standards (see Evans 1960). They further concluded that feeding protein above the Woodman standard was unlikely to improve milk quality. Lindell (1983) examined the effects of three protein levels over three lactations in dairy cows and found that animals changing from the medium level of 60g DCP/kg FCM to the low level of 45g DCP/kg FCM showed a significantly higher fat content in the milk than the medium and high protein level fed animals. No significance was found between protein contents but lactose was significantly lower in the low protein fed cows. Lindell (1983) suggested that increased protein in the diet significantly increases milk yield although persistency was low, and a drop in protein level resulted in a drop in yield. An advantage of this type of work is that the experimental period was long, allowing effects to occur and be interpreted in dietary terms. A problem associated with many experiments on lactation effects is the

fact that the experimental period is too short, and that true effects are probably hidden by environmental or normal lactation effects.

The general opinion regarding dietary protein effects on milk production is that milk yield is usually affected (Thomas 1980; Sloan et al 1988); milk fat is affected only under severe conditions (Sutton 1984), and that milk protein is rarely affected unless severe undernutrition of crude protein occurs (Thomas 1983). Paquay et al (1973) took into account the fact that milk production capacity was dependent upon the stage of lactation and found that when crude protein content was fed below the limiting value, no effect was found in milk protein but milk yield was reduced. They further reported that feeding nitrogen above the limiting factor resulted in the supplement of nitrogen being lost in the urine. An interesting point in this work would have been the changes in the NPN component of the milk protein, and subsequently dietary effects on milk 'true' protein. Paquay et al concluded that milk protein effects were evident at only very low levels of DCP, an effect observed by Gordon and Forbes (1970). Thomas (1983) suggested that responses in milk protein content were related to the effect of dietary protein change on the passage of amino acids to the small intestine, which is consistent with the fact that milk protein effects usually occur when dietary protein supplements are of low rumen degradability. In contrast with these findings, recent experiments by Chalmers and Thomas (1978), Gordon and McMurray (1979) and Mayne and Gordon (1985) have shown that milk protein can be improved by manipulating the concentrate-to-forage ratio and increasing the concentration of crude protein in the concentrates. Nevertheless, Mayne and Gordon concluded that the greater responses to protein

supplementation found in the low-concentrate diets may be attributable only in situations where total food intake is restricted.

Both the type and level of dietary protein offered to cows are therefore factors which influence the yield and composition of milk, due to their effects on the supply of amino acids to the udder. However, in diets where the animals are offered varying amounts of different protein sources, interpretation of the effects of dietary protein is difficult due to the limited information concerning the amino acid mixture taken up by the small intestine. Rook (1976) supports this by stating that a shortage of dietary protein depresses milk yield, but the mechanism of the effect is unknown. Although much work has been carried out on fistulated animals, ruminal activity in digestion of the diversity of feedstuffs consumed by the cow is still not well understood (Legates 1990). Thomas (1984) supports this view in the report on feeding and protein production. A number of papers were cited in which positive and negative responses were found in milk production to changes in dietary protein, and it was suggested that the problems associated with accurately assessing rumen-degradability and precise amino acid requirement in dairy cows may be the key factor. It was concluded that inconsistencies between feeding and infusion experiments may be due to the amino acid balance of the protein mixture entering the small intestine. Further work is therefore necessary on ruminal activity, amino acid composition in the rumen and assessment of rumen degradability in feeds in this area of milk production research, while the effects of NPN, stage of lactation and dietary crude protein concentration requires further work in general lactation experiments.

4.3 Dietary energy

The requirement of adequate energy supplies for efficient protein synthesis makes it virtually impossible to separate the effects of dietary energy and protein supply on milk production. As a result, it is more prudent to examine how changes in the main energy source of the diet (usually carbohydrate or lipid) can influence milk production.

A limiting factor in diets formulated for improved milk production is the level or 'plane' of energy nutrition: a term coined specifically to describe an improvement in energy supply achieved through additional starch concentrates in the diet (see Rook and Line 1962). This is usually achieved through reducing dietary forage, which questions any effects reported since they may be confounded by dietary compositional changes and a decline in fibrous content. Rook (1961a) reported depressions in milk fat contents in diet lacking physical fibre and related the effect to the change in the physical nature of the ruminal contents associated with the change in the proportions of ruminal VFA. Thus effects associated with changes in the plane of energy nutrition should be interpreted carefully, bearing any variations in the dietary composition in mind.

The level of energy nutrition of a cow can be increased in a variety of ways:

1. increasing the intake of a diet of constant composition
2. replacing poor quality herbage with forage of good quality
3. incorporating a lipid supplement of high ME value
4. changing energy source
5. increasing the energy density of a diet, by changing the

proportion of high energy concentrates relative to low energy forage.

An obvious problem with an attempt on maintaining a diet of constant composition is the actual quality of the feedstuffs used. Although a constant ratio of forage to concentrates may be attained, changes in dry matter, degradability, environmental and management factors are more difficult to control. In relation to frequency of feeding, an experiment by Thomas and Kelly (1976) and a review by Gibson (1984) revealed that attempts have been made on increasing dietary intake of constant feed composition. Thomas and Kelly found a reduction in milk fat and protein when dietary intake was increased from 80 - 100% of energy requirement, but concluded that this was probably a reflection of premature lactational changes brought on by the underfeeding. Gibson (1984) stated that all the statistically significant responses recorded in milk fat only occurred when the milk fat content was originally depressed and concluded that cows already producing commercially acceptable milk fat concentrations were unlikely to benefit from increased frequency feeding.

Another method of increasing the level of energy nutrition is the use of a good quality forage, since both the type and nature of the forage offered to dairy cows can influence milk secretion. Thomas (1984) reviewed the effects of forages on milk compositional quality and summarised that the digestibility of the forages was the key factor. Legumes showed increases in milk yield (compared with grass of similar digestibility) through improvements in the solids-not-fat content eventhough milk fat content was lower. Thomas (1984) concluded

that although changes in milk composition were small they were unlikely to be the result of the high proportion of forage in the diets used, and consequently more attention should be given to type and level of concentrate fed and the composition of the forage.

Energy source therefore is an important factor in dietary effects on milk production. The effect of energy source on dairy cow performance has been widely studied (Sloan and Rowlinson 1986; Thomas and Rae 1988; Miettinen and Huhtanen 1989; Spain et al 1990). Casper et al (1990) examined the effects of two cereal sources of non-structural carbohydrates (maize and barley) on milk production in early lactation dairy cows and found that the barley fed animals had lower percentages of milk fat and SNF, but protein content was similar in both diets. They concluded that the alternative source (barley in this case) did not increase utilisation of the readily available crude protein source (urea), thus making it less economical in milk production. Broster et al (1970) earlier examined two sugar sources (sucrose and glucose monohydrate) and found that sucrose had a beneficial effect on milk yield; depressed milk fat content and increased SNF content and yield. Glucose had little effect on milk yield; variable effects on milk fat content, but also increased SNF content and yield. In their continuous treatment experiment, Broster et al found that milk fat content recovered on both sugar diets eventhough milk yield and milk fat did not change from the previous values; rumen contents showed that both sugars increased propionic acid and decreased acetic acid, sucrose having the greatest effect.

. Glucose, of all the metabolites supplying carbon, nitrogen and energy for milk synthesis, is the premier nutrient because of its

central role in udder metabolism. It supplies a significant portion of the energy required for milk synthesis, contributes significantly to milk fat and protein synthesis, and supplies the carbon necessary for lactose synthesis. In early lactation, shortages of glucose result in the metabolic disease ketosis, in which condition 85 - 90% of the body glucose is used by the mammary gland. In these cases the animals would benefit from an increased supply of glucose, primarily from the availability of precursors from the diet for increased gluconeogenesis (Clark 1975). Work by Vik-Mo et al (1974) has shown that mixtures of glucose and casein administered post-ruminally significantly increased milk production although the increase in milk yield may have been due to the casein (see earlier section). In general, infusions of glucose or mixtures of saccharides had no significant effect on milk yield (Clark 1975). Fisher and Elliot (1966) and Vik-Mo et al (1974) both found that postruminal infusion of glucose increased milk protein yield, whereas milk fat yield was lowered from between 1 - 10% if casein was infused in addition to the glucose.

Infusing a cow with glucose is perhaps an unrealistic way to investigate effects on milk production since ruminant animals absorb little glucose from the digestive tract (carbohydrates are generally extensively degraded to VFA in the rumen: see figure 1.1), but obtain the majority of the required sugar via gluconeogenesis. However, it has been reported that glucose absorption from the gut has been increased when feeding high concentrate diets (see Armstrong and Beever 1969). Propionic acid (figure 1.1) is a major precursor of gluconeogenesis, in addition to amino acids, thus establishing the strong link between dietary protein and energy supply effects. Energy sources which provide

large amounts of amino acids and propionic acids upon degradation in the rumen consequently increase the availability of glucose to the mammary gland, although high concentrations of propionic acid tend to depress milk fat content (see Sutton 1984).

The effect of the amount and composition of dietary fat on milk composition has been reviewed by Rook (1976). Thomas and Rook (1983) made a comprehensive study of the dietary lipid effects on milk secretion and suggested that the observed response in milk fat secretion was the net result of many independent but related processes (see Thomas and Rook 1983 for detailed accounts of processes and effects). Emery (1978) found that increasing dietary energy with fat usually decreased the concentrations of protein in the milk while Sutton (1984) concluded that supplementing diets with fats and oils generally increased milk yield, but with wide variations in the milk fat content. Sutton advised that when examining milk fat production, the level of intake, feeding pattern and diet composition were all important factors controlling fat synthesis.

In an attempt to increase the level of energy nutrition, the majority of experiments have involved the supplementation of a basal forage with concentrates (either cereal, sugar or fat), thereby increasing energy intake but reducing the forage to concentrate ratio. Thus, effects relating to energy intake may be confounded by those relating to diet composition (Thomas 1980). Milk yield has usually shown a curvilinear response to additional concentrate as the energy intake increases; the effect being more pronounced in high yielders and early lactation cows (Rook 1961a). The lactation peak has been found to

be increased if generous feeds (i.e. high levels of concentrates) are offered in early lactation with total yield for the lactation being improved if adequate diets are fed for the remainder of the milking period (Thomas 1980). Undernutrition can lead to a decreased yield through a poor forage to concentrate ratio, and if particulary severe the effect on milk yield is profound (Thomas and Kelly 1976). The effect of protein underfeeding on milk protein content however is negligible until severe undernutrition of protein occurs. Milk protein effects are usually evident in mid-lactation if the plane of energy nutrition is increased, and Rook and Line (1962) suggested that the effect was related to the increased energy intake and decreased ratio of forage to concentrate. Thomas and Rook (1983) quoted values of 2 to 3g/kg increase in milk protein content in relation to plane of energy nutrition effects. Effect of dietary energy on milk fat has been reviewed by Sutton (1984) who concluded that sucrose, unsaturated fats, starch components and high intakes all contributed to a reduction in milk fat content, while long forage, fodder beet, saturated fats, lactose and general roughages were found to maintain milk fat levels.

4.4 Interaction between dietary energy and protein

The associative effects of dietary energy and protein on milk production were recognised twenty years ago.

Gordon and Forbes (1970) in a factorial experiment involving two levels of dietary protein and dietary energy, reported that the milk yield response to increases in either energy or protein was usually greater when the other variable was at the higher level. The diets had

no effect on milk lactose, but milk fat was found to be reduced at both protein levels when the energy intake was high. The effects found in milk protein were more complex but pointed to a greater response when both variables were at the higher level, table 1.2 (from Gordon and Forbes 1970):

Table 1.2: The effect of two levels of dietary protein and energy on milk protein content (g/kg)

Energy	Protein Low	Protein High	Effect of increasing dietary protein
Low	33.2	32.7	-0.05
High	33.2	33.8	+0.06
Effect of increasing dietary energy	0.00	+0.11	

Later work by Broster (1973) provided evidence to suggest that the response to protein supplementation was greater at high rather than low levels of energy intake.

It is evident therefore, from previous work and earlier sections of this chapter that dietary energy and protein are linked through the provision of ATP for protein metabolism.

5. Mineral nutrition

The importance of minerals in the diets of lactating dairy cows has long been recognised in relation to nutritional disorders incurred through deficiency.

All animal tissues and feeds contain widely varying proportions of inorganic or mineral elements. At present twenty-two mineral elements are believed to be essential for the high forms of life (Underwood 1981), and comprise seven macronutrient minerals - calcium, phosphorus, potassium, sodium, chlorine, magnesium and sulphur. Three functions for minerals exist:

1. structural components of body organs and tissue
2. catalysts in enzyme and hormone systems
3. constituents of the body fluids and tissues as electrolytes concerned with osmotic pressure maintenance, membrane permeability etc.

High yielding dairy cows require much more dietary calcium and phosphorus than low yielding cows because of the richness of milk in these elements. However, the necessary percentages in the dry ration do not rise to the expected extent because total dry matter intakes increase with rising productivity of the cow almost as rapidly as do mineral requirements (Underwood 1981). Therefore, minimum mineral intakes must be sufficient to ensure the long term maintenance of the mineral reserves of the body tissues and the amount of those minerals in the edible products of the cow. Nevertheless, the dairy cow body has the capacity to adjust to suboptimal intakes by reducing the amount of mineral in the milk.

NON-NUTRITIONAL FACTORS AFFECTING MILK PRODUCTION

1. Introduction

Rook (1961a) highlighted the variety of non-nutritional factors affecting milk production. These included season, age, breed, disease, stage of lactation and the actual methods used in milking, and are discussed below. The Milk Marketing Boards' 'Dairy Facts and Figures' report yearly on the trends in milk yield and constituents for dairy herds as a result of non-nutritional effects.

2. Seasonal effects

The seasonal trends shown in the mean composition of bulk milk throughout the United Kingdom are well defined in the Milk Marketing Board's publications of 'Dairy Facts and Figures'. The compositional qualities of milk applicable to this work are shown in table 1.3, from the Scottish Milk Marketing Board's records for 1988-1989 (Dairy Facts and Figures 1989):

Table 1.3: Compositional quality of seasonal milk

Month	Content (g/kg) Fat	Protein	Payments for protein (pence per litre)
April	36.8	30.3	+0.11
May	36.0	33.3	+0.22
June	36.1	33.9	+0.22
July	38.2	33.9	+0.22
August	38.8	34.4	+0.33
September	39.8	34.9	+0.33
October	40.8	33.1	+0.22
November	39.9	31.7	Pool price
December	38.9	31.1	+0.22
January	38.3	30.2	+0.11
February	38.1	30.4	+0.11
March	38.6	30.5	+0.11

The Scottish Milk Marketing Board has producer payment schemes whereby a pool price is paid for milk quality within a defined band with higher cash incentives being paid for milk with a better compositional quality. Therefore, in the period from December to April a higher price is paid for a lower protein content milk than that found in the summer and autumn periods. A main explanation of these seasonal changes is the change in diet: cows at grass tend to produce more milk of a higher protein content than animals which are housed (Rook 1961a). An alternative explanation is the effect of stage of lactation (discussed later). Figures 1.6, 1.7 and 1.8 show the seasonal trends in milk constituents, where MMB - England and Wales milk marketing board; SMMB - Scottish MMB; AMMB - Aberdeen and district MMB, and NSMMB - North of Scotland MMB).

Figure 1.6: Protein content of milk by board area (1988/9)

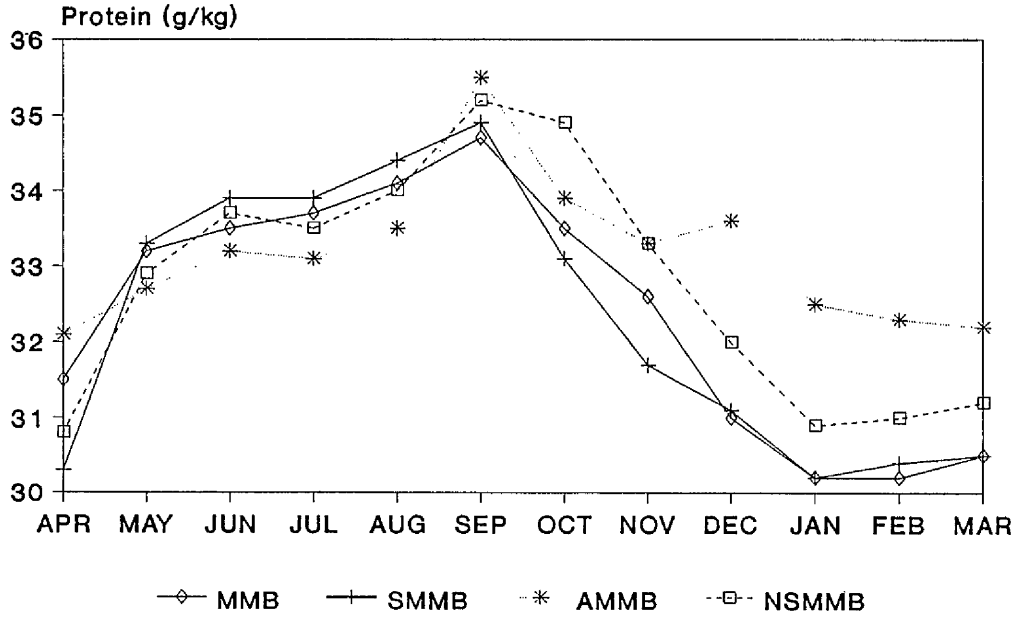


Figure 1.7: Fat content of milk by board area (1988/9)

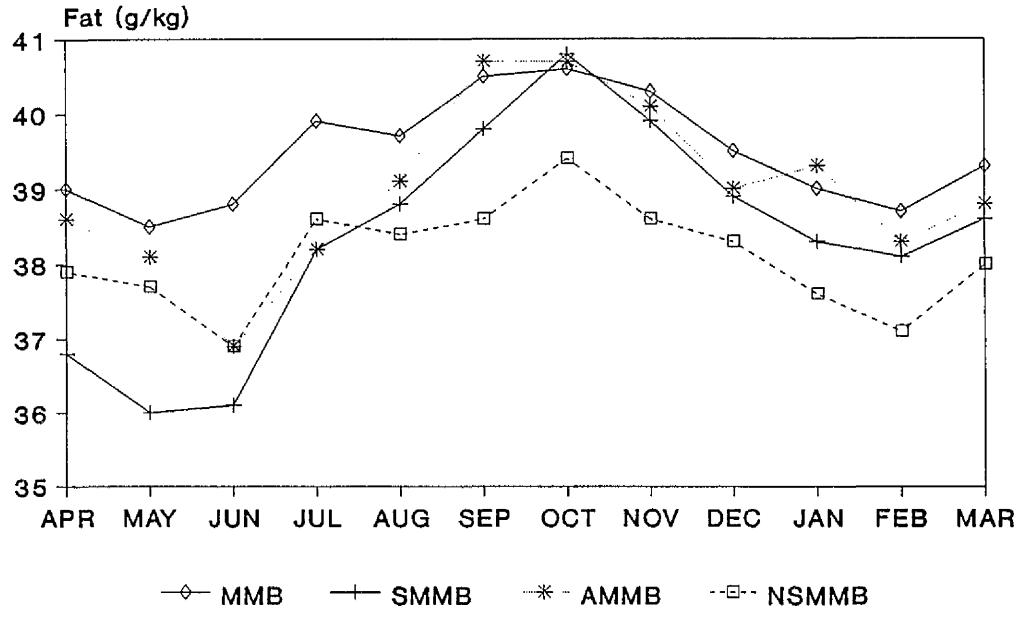
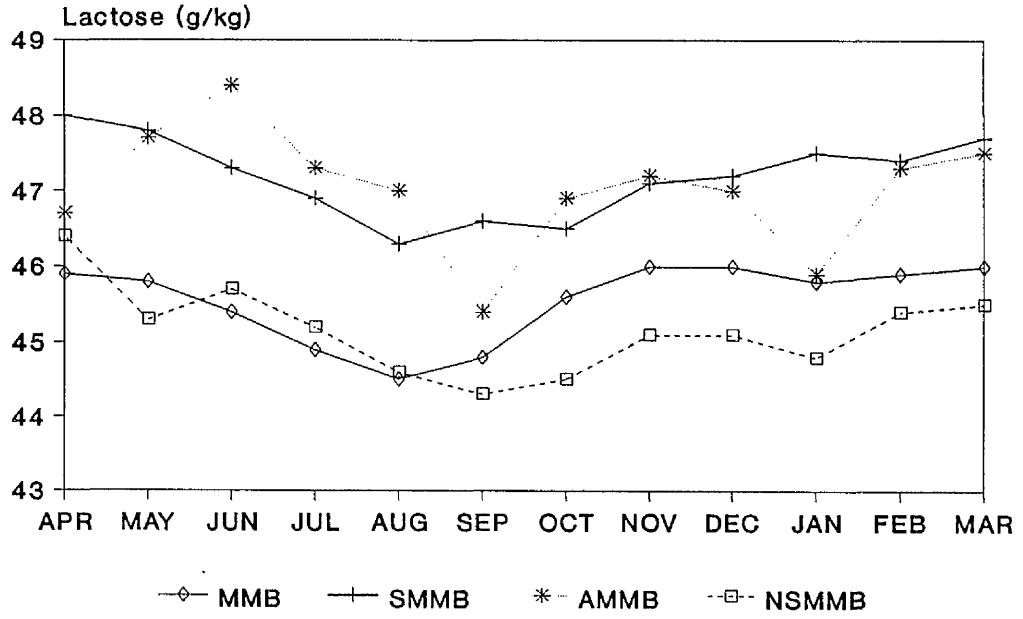


Figure 1.8: Lactose content of milk by board area (1988/9)



3. Stage of lactation

Through hormonal control, lactation and reproduction are coordinated and interrelated. Lactation is initiated when parturition occurs as the final stage of the reproductive cycle. As a result of improved milking techniques and diets the period of lactation has been extended further than that required by a calf for development.

Rook and Campling (1965) made an extensive study of lactation and found that in general fat and protein contents fall as yield increases to a peak, whereas milk lactose declines steadily throughout the lactation. Detailed studies, however have shown that many small but significant changes in milk yield and constituents occur throughout the lactation (Dawson and Rook 1972; Wood 1976; Clapperton et al 1980).

At the onset of lactation, immediately following calving, the milk synthesised is rich in colostrum which increases total solids in the milk to approximately 25g/kg, the majority of which is protein (Rook 1961a). The amount of protein synthesised in this intial milk is twice that found in milk from mid-lactation and is composed of casein, a-lactalbumin and B-lactoglobulin. In these mammary-synthesised proteins there is an exceptionally high proportion of globulins, particulary immunoglobulins (Thomas and Rook 1983). The high concentration of protein falls rapidly within the first few weeks of the lactation but then progressively increases until the end of the lactation. Rook (1961a) stated that the milk constituents protein and fat fell to a minimum at about 6 and 8 weeks respectively with large increases occurring at the end of the lactation as a result of pregnancy. Consequently, at peak yield, the fat and protein contents of the milk

are at their lowest values.

Milk lactose concentration shows the opposite effect of the previous two constituents: levels at the onset increase to peak values at approximately 5 weeks, which remain steady for 5 to 6 months before dropping to a minimum at the end of the lactation. Milk yield follows a similar pattern to that of milk lactose. A dramatic increase following parturition gradually slows to a progressive decline until the completion of the lactation.

These changes in constituents are thought to be the result of two factors:

1. hormonal control: Wilcox et al (1959) found that in Holstein and Guernsey cows towards the end of the lactation, protein and solids-not-fat contents increased in pregnant animals but not in barren cows. Thus, hormonal factors are probably largely responsible for the lactational trends.

2. dietary effects: under commercial conditions it is apparent that animals are often underfed in mid-lactation during peak yield and overfed in later lactation in preparation for calving.

4. Breed, individual characteristics and genetics

The Friesian breed has dramatically replaced other breeds at an increasing rate since the turn of the century, with 8 of the EEC countries (UK, Irish Republic, Greece, Spain, France, Italy, Luxemborg and Germany), having Friesian cows as the main milk producers while the Netherlands, Belgium and Denmark have their own indigenous breeds.

Scottish milk marketing board figures show that 59.8% of total dairy cows are Friesian, with Ayrshires following at 16.8%. Although the introduction of the Holstein revealed a higher yielding animal when compared with the Friesian, its poor beef conformation has resulted in low prices being paid for unwanted male calves which are less acceptable to the beef market. Schutz et al (1990) found large differences between breed (Holstein, Jersey and Guernsey) in protein yield curves and milk fat contents. The Jersey cow produces a good quality milk but at a lower yield - approximately 1500kg less per 305 day yield than the average Friesian. Thus, the breed of the cow has a defined effect on milk composition, table 1.4 (from Dairy facts and figures 1989):

Table 1.4: Milk yields and composition for British dairy cows

Breed	1988 Milk yield (kg)	Fat (g/kg)	Protein (g/kg)
Friesian	5721	38.2	32.4
Holstein	6405	38.2	31.5
Ayrshire	5312	38.8	33.0
Jersey	4108	54.2	39.9
Other	5505	38.9	32.7

Although breed tends to put a cow's milk into a certain range, there is a wide variation both between herds and between individual animals. The variation between herds is suggested to be the result of different environments and management techniques. However, the between

cow differences are thought to be the result of genetic and environmental factors (Rook 1961a):

1. Environmental differences between cows may occur as a result of: different diets; errors incurred through sampling techniques (Thomas and Rook 1983).

2. The genetic differences involve heritability of reproductive traits and other physiological traits.

The genetic influence on milk production has been studied by Donald and Watson (1961) and reported by Mulholland (1984). Donald and Watson found that variance in half sisters and unrelated pairs was three times greater for milk yield at 70 days than in monozygotic twins. Mulholland reported on the way in which genetic improvement accumulates in dairy cattle using high-production sires of reliable proofs, and concluded that yield was the dominant factor and any breeding programme should therefore place maximum emphasis on production. Despite the fact that environmental effects can confound the influence of genetic make-up on milk secretion, ranges of variation accounted for by genetic parameters for milk yield and constituent content have been suggested (Robertson et al 1956; Von Krosigk et al 1960; Tong et al 1977; Chalmers 1979; Thomas and Rook 1983), table 1.5:

Table 1.5: Proportion of genetic variation

Milk	Range of variation accounted for by genetic parameters
Yield	0.25 - 0.90
Fat content	0.32 - 0.75
Protein content	0.45 - 0.75
Lactose content	0.36 - 0.70

As a result of the high heritability coefficients, it should be possible to improve milk composition by selective breeding. However, environmental factors tend to mask genetic potential although work by Dommerholt et al (1977) has attempted to eliminate these errors by describing a 'herd standard cow' in which corrections are applied for age, stage of lactation, breed, calving season and herd production level.

Aschaffenburg and Drewry (1957) identified a genetic relationship in which concentrations of certain proteins were linked with the gene concerned with B- lactoglobulin formation. Animals homozygous for the allele termed Lg^A at a specific locus on the gene were found to produce twice as much B- lactoglobulin than those homozygous for the corresponding Lg^B allele. Interestingly, heterozygotes recorded intermediate values.

Although much work still needs to be carried out in this field of milk production, identification of true genetic potential for productive traits in animals could lead to an improved quality milk.

5. Age of cow

A difficulty when examining the effect of age on animal production is the fact that low yielding cows which are 5th lactation or more are usually culled. As a consequence, data is biased and unbalanced towards young animals, and, unless there is an unusual combination of older cows milking at an early stage of lactation at the same time, age effects on composition are unlikely to have a marked effect. Schutz et al (1990) found that a first parity Holstein cow between 25-27 months at calving produced higher yields and improved composition, whereas an age nearer 30 months was optimum for Jersey and Guernsey breeds. This trend was reflected throughout the subsequent lactations.

Research by Waite et al (1956) has showed that changes in older animals in the solids-not-fat content were mainly a result of lactose changes and not protein, although slight variations were apparent in milk casein. Subsequent work by Rook (1961a) concluded that a progressive decline in milk fat and solids-not-fat was evident with successive lactations and further suggested that changes in milk constituents may be explained by:

1. selective culling of low yielding animals

2. udder deterioration through mastitis or physical damage.

A specific effect on milk production relating to age was investigated by O'Donovan et al (1960) who found that in infection free cows a drop of 1g/kg in the solids-not-fat content of the milk was shown with consecutive lactations.

6. Mastitis history

Mastitis refers to the inflammation of the mammary gland through the interaction of host (cow), pathogen and environment. Mastitis itself covers a complex of diseases which are due to a number of different causes, however, a similar overriding factor among the various forms is that all are of economic importance to the dairy farmer.

There are two forms of mastitis:

1. subclinical - caused by minor pathogens

2. clinical (or chronic mastitis if persistent or recurring), caused by major pathogens such as Staphylococcus aureus; Streptococcs dysgalactiae; Escherichia coli.

Subclinical mastitis is a mild inflammation of the mammary tissue with no visible signs of change in milk or udder. In general though, milk yield and composition of the infected quarter is reduced. Clinical mastitis on the other hand shows visible changes: in the udder due to swelling; in the milk due to the presence of white clots of protein coagulation, and in the physiological state of the animal due to a temperature rise. Acute clinical mastitis shows gross changes in the milk and health of the cow, through reduced appetite, increased body temperature and low milk yield in all quarters. Peracute clinical mastitis is the most severe form with a sudden onset and debilitating effect which renders the animal unable to rise and ultimate death if untreated.

Due to the economic nature of mastitis on a dairy herd, much work has been carried out on the infection in an attempt to control its spread either by selective breeding or management awareness (Bodoh et al 1976; Moxley et al 1978; Griffin et al 1983; Fox and Hancock 1989). In addition, researchers have also examined dietary effects, particulary high concentrate diets on the incidence of mastitis (Emery et al 1969; Holter et al 1982; Keys et al 1984).

The effect of mastitis on milk yield and composition occurs as a result of the inflammation impairing secretory activity (Thomas and Rook 1983), and altering the udder tissue permeability (Rook 1961a). Mastitis invariably reduces the amount of milk from the infected quarter which in subclinical mastitis can be as much as 25% if the quarter is affected for the total lactation. An estimation of the severity of mastitis is calculated by somatic cell counts. A cow at peak lactation will give milk with a cell count of <100,000 cells/ml but this increases with age thereby raising the number with each subsequent lactation. A normal cell count is therefore regarded as around 300,000 cells/ml, whereas subclinical mastitis in one quarter can increase this to >1,000,000 cells/ml, table 1.6 (Dairy Facts and Figures 1987-1989):

Table 1.6: Association of somatic cell count with milk yield loss

Cell count range (cells/ml)	Mastitis Problem	Estimate of milk yield loss (litres per cow per year)
Below 250,000	Low	-
250,000-499,000	Medium	190
500,000-749,000	Medium/high	330
750,000-1,000,000	High	760
>1,000,000	Very high	890

As a result of this effect on milk yield, milk constituent contents are also affected. Milk lactose, potassium and casein contents have been found to decrease, making the milk look watery. In response, sodium, chlorine and protein globulin contents increase with additional slight increases also being found in serum albumin and proteose-peptones (Thomas and Rook 1983). However, the total protein effect tends to vary according to the level of infection. Milk fat changes are generally irregular.

Research investigating the control of mastitis tends to favour preventative measures rather than possible selection for natural resistance and stimulation of immune systems (Bramley et al 1981; Dodd 1983; Hutton et al 1990). Smith (1983) found that most success in mastitis control was due to the utilisation of stringent management factors that reduce pathogen transmission such as pre and post milking teat hygiene.

7. Milking

Additional factors which have been found to affect milk yield and composition are those related to the actual milking of the cow, such as the milk ejection reflex, incomplete milking and the frequency of milking.

During milking, the actual composition varies with time (Rook 1961a). Milk fat is found at levels of only 10g/kg in the initial milk whereas levels of milk lactose and protein are high. Thomas and Rook (1983) explained this phenomenon as the result of the large fat globules being retained within the fine ducts of the udder thereby retarding the passage of fat but allowing the smaller secretions of solids-not-fat to pass through. Towards the completion of the milking the residual milk or 'strippings' is released which is high in fat (100g/kg - 30 to 50% of total fat content), and contains slightly less amounts of lactose and protein. In general, only 90% of milk is removed from the udder, the remaining 10% being retained in the secretory tissue by capillary forces. Work by Linzell and Peaker (1971) on the goat has shown that this retained milk can be removed by using oxytocin, however, milk production can be affected.

The milk ejection reflex is a conditioned reflex where the cow has learned to associate patterns in the parlour (entry, concentrates, teat washing etc.) with milking. If the pattern remains constant the animal's reflex is enforced and milking will be quicker with a higher yield. Variations in the pattern may therefore lower yield, and it is likely (bearing compositional changes during milking in mind) that if yield is inhibited then changes in milk composition will occur. Milk

yield is additionally depressed by local inhibitory effects if milking is incomplete (Wheelock et al 1965).

Another factor affecting milk production is the frequency of milking or milking interval. Once a day milking by Roberts (1987) revealed a drop in milk yield of approximately 30%, which was only increased to near normal lactational levels when twice a day milking was reintroduced. Early work by Wheelock et al (1966) found that the amount of constituents present in milk was curvilinearly related to the milking interval. A linear relationship was evident up to 12 hours between milkings, whereas a curvilinear relationship was apparent by an 18 hour interval. At long intervals between milkings Wheelock et al found that the amount of solids-not-fat decreased whereas the contents of sodium, chlorine, fats and non-casein nitrogen all increased.

AIMS AND OBJECTIVES

The literature reviewed has highlighted a number of relationships between diet and milk composition. There is evidence to suggest that dietary protein effects on milk protein are the result of alterations in the minor protein conmponents. Stage of lactation undoubtedly plays an important part in milk secretion and early suggestions that lactation patterns are fixed have been discounted by later works showing that diet can alter these expected curves. The availability and effects of concentrate source have been reviewed, but research into the differences between starch and sugar carbohydrate sources on milk production are limited.

The aims of the experiments presented in this thesis are

twofold. Firstly, to ascertain if precalving feeding has any effect on milk production postcalving, and whether normal lactational curves can be altered. Secondly, to investigate the effects on animal production of varying the crude protein of diets and the effects of different energy sources.

CHAPTER 2

EFFECT OF PERIPARTUM ENERGY CONCENTRATIONS ON PRODUCTION PERFORMANCE

INTRODUCTION

The practice and management of dairy cow nutrition has substantially changed over the last two decades. The genetic improvement and selection for high yielding dairy cows, and the generalised feeding of high energy rations has led to increased milk production. With this increase in milk quantity and the restrictions of the milk quota regime, much work has been carried out on energy nutrition during the prepartum and postpartum periods, in an attempt to produce a good quality milk (Gleeson 1973; Lodge et al 1974; Johnson and Otterby 1981; Eastridge et al 1988; Flipot et al 1988; Butler and Smith 1989). To find the total economic value of producing a good quality milk, a number of factors have to be considered:

1. silage quality, and optimum supplementation of silage
2. energy requirements for maintenance and milk production
3. the effect (and cost) of peripartum diets on milk composition
4. body condition at calving
5. animal health, and calving to conception interval

From previous work, prepartum feeding appears to have little effect on milk production but obviously significant effect on body condition (Garnsworthy and Topps 1982; Ducker et al 1985; Boisclair et al 1986). Broster (1971) found that cows in poor condition (1.5-2) prepartum gave more milk in early lactation directly from their food and not their body reserves, thus making them biologically more

efficient through a positive energy balance. Lodge et al (1974) additionally found milk from poor condition cows to be of a higher protein content. Gleeson (1973) concluded that cows offered a precalving diet of silage ad libitum had sufficient nutrients for increased milk production and was also economical. Therefore, to examine the effect of the prepartum diet on production postpartum in the following experiment, two diets were compared: silage based and low energy.

It is generally agreed (Berglund and Danell 1987; Flipot et al 1988; Butler and Smith 1989) that after calving cows are unable to consume sufficient nutrients to meet requirements in early lactation. However, as the plane of nutrition increases more of the feed goes to milk production (particularly in high yielding cows) and body depletion eventually reverses to tissue accumulation. The following experiment was therefore also designed to investigate the effect of postpartum high and low energy diets on milk production and body condition.

MATERIALS AND METHODS

[The three experiments described in this thesis all used a similar design - individual feeding through Calan Broadbent gates of silage and concentrates with feed intakes, milk yield and composition, and weights being recorded. Consequently, the materials and methods section described below, in general applies to all three experiments. However, any deviations from the standard materials and methods described are outlined in the relevant chapter.]

1. Experimental

1.1 Animals and their management

The dairy cattle used throughout this work were British Friesian/Holsteins. Average cow liveweight in this experiment was 676kg (SED=32.8) prepartum and 576kg (SED=38.4) postpartum. The lactation number varied from second lactation to ninth lactation cows, and mean milk yield for the previous lactation based on a 305 day estimate was 5694kg (SED=660.7).

All animals were housed in a cubicle house containing two water troughs, and were fed individually through Calan Broadbent gates. Each cubicle measured 2.20m (length) x 1.15m (width), and was covered with sawdust which was raked twice daily and renewed weekly. Slurry from the feed and cubicle passages was scraped twice a day during milking, and the Calan gates and transponders were examined regularly so that any replacements or alterations could be made as necessary. The animals were offered the feeds in 0.61m x 0.61m silage bins weighed out on Weighmaster scales (25kg x 200g divisions). All animals were given sufficient feed to ensure a 5-10% refusal.

The cows were milked twice a day in an 8 x 16 herring-bone parlour. No concentrates were offered during milking, except when the cows in this experiment were put out to grass. Standard milking procedures were used, as recommended in commercial practice. Animals were always weighed after a milking, in a Macklow-Smith Ltd. (IOSB/G 2/81) weighing crate and condition scores recorded were based on the tail-head system (Mulvany 1977).

Cows due to calve were removed from the main group and placed in a large straw bedded pen. The animals were offered silage ad libitum, and once calved remained with the calf for 24 hours. Any calving difficulties were recorded and the cow was returned to the main group for postpartum feeding as soon as possible. Blood analysis was carried out in weeks -1, 3, 7 and 11 of this experiment and samples were taken mainly from the base of the tail while the cows were restrained in the weighing crush.

1.2. Experimental design and diets

Twenty multiparous dairy cows were used in a continuous design experiment. The 20 Feburary/March calving cows were individually fed for 4 weeks prepartum and 8 weeks postpartum. The animals were then put out to grass for a further 4 weeks. Each animal was assigned to a group according to lactation number, weight and calving date in order that each treatment had a cross-section of the animals in the experiment. There were 3 formulated treatment diets:

Table 2.1: Formulated treatments offered

Contents (g/kg DM)	Treatments Silage base	Low ME	High ME
Silage	844	655	373
Soya bean meal	136	142	144
Barley	-	45	211
Sugar beet pulp	-	119	233
Minerals	17	17	15
Megalac	-	23	25
ME (MJ/kg)	11.2	11.8	12.4
CP (g/kg)	176	174	175

The group treatments are outlined in table 2.2:

Table 2.2: Group treatments

Group	Prepartum	Postpartum
1	Silage base	Low ME
2	Silage base	High ME
3	Low ME	Low ME
4	Low ME	High ME

Dry matter intakes were recorded on 4 consecutive days each week during the housed period. Milk samples were taken three days per week with additional samples being taken on the 3rd, 7th and 11th week of lactation for casein analysis. Weights and condition scores were recorded two days per week and blood samples were taken once prepartum, twice postpartum and once while the cows were out to grass. Once out to grass the animals were offered 8kg of concentrates in the parlour and any refusals were recorded. Grass heights in the field were measured weekly, but no herbage intake work was carried out.

2. Diet components

2.1 Silage

2.1.1 Preparation of silage

All the silage used in the experiments was first cut perennial ryegrass (*Lolium perenne*) with the exception of the first experiment in which second cut silage was used for the postcalving period. All silages were cut from the leys prior to the emergence of the seed heads, using a drum mower. The crop was wilted for an average 24 hours, depending upon weather conditions. The forage was then picked up and chopped to an average length of 20mm using a forage harvester. A

fermentation inhibitor (Add-F, BP Nutrition UK Ltd., an acidifier containing formic acid 85%) was applied at a rate of 2.5 litres per tonne as it was chopped, in the treatment of all silages with the exception of experiment 3 which used sulphuric acid (45%) applied at a rate of 3.5 litres per tonne. The grass was then ensiled in a clamp silo and sealed with weighted polythene sheets to exclude air.

2.1.2 Silage sampling and analysis

Grab samples of silage were taken from the materials to be fed each day, and approximately 1 to 1.2kg was dried to a constant weight (in practice, 24hrs was sufficient) in a forced draught oven (Unitherm, 12kW fan heater) at 100^{o}C to determine the dry matter content of the feed samples. Additional samples were bulked monthly over a 4 day period for <u>in vitro</u> analysis for dry matter, crude protein, energy, pH, ammonia and minerals. Feed refusals were also grab sampled and oven dried for a refusal dry matter. The <u>in vitro</u> silage analysis is shown below for experiment 1 (techniques used are outlined in section 3.3.1):

Table 2.3: Analyses of the silages used prepartum and postpartum

Analysis	Prepartum 1st cut	Postpartum 2nd cut
Dry matter (g/kg)	186	177
Dry matter analysis:		
Crude protein (g/kg)	163	122
ME (MJ/kg)	10.85	10.70
D Val (g/kg)	682	670
pH	3.85	3.82
Ammonia (g/kg N)	111.8	109.8
Calcium (g/kg)	5.18	5.64
Phosphorus (g/kg)	3.40	2.74
Magnesium (g/kg)	2.08	2.08

2.2 Concentrates

Animals in the first experiment were offered a complete mixed diet of silage and concentrates. The feedstuffs were mixed in a Cormall mixer, and weighed out into the silage bins. All the dietary concentrates i.e. barley, sugar beet pulp, soya and Megalac were purchased in the form normally found commercially and stored in easy access 1000kg Polybins. Samples of the concentrates were bulked over a 4 day period and analysed monthly using the Neutral Cellulase Digestibility (NCD) method (techniques used are outlined in section 3.3.2):

Table 2.4: Analysis of the concentrates fed pre and postpartum

Analysis	Barley	Sugar Beet Pulp	Soya	Megalac
Dry matter (g/kg)	831	862	879	900
Dry matter analysis:				
Crude protein (g/kg)	176	105	466	40
OM (g/kg)	971	870	931	-
D Value (g/kg)	805	779	843	-
ME (MJ/kg)*	12.8	12.4	13.5	33.2
Calcium (g/kg)	1.0	10.7	3.3	-
Phosphorus (g/kg)	4.6	0.9	6.9	-
Magnesium (g/kg)	1.7	2.9	3.0	-

* ME was calculated using the following equation:

$$\text{Concentrate ME} = (0.14 \times \text{NCD}) + (0.25 \times \text{AHEE})$$

where: NCD = neutral cellulase detergent
AHEE = acid hydrolysed ether extracts

All diets were supplemented with a proprietery mineral/vitamin mix, in a powder form, the composition of which is shown below:

Table 2.5 Composition of Downland GP feed mineral/vitamin mix

Minerals	%	Vitamins	iu/kg	Minerals	mg/kg
Phosphorus	7.0	Vitamin A	320,000	Iodine	700
Calcium	14.5	Vitamin D3	64,000	Cobalt	120
Magnesium	4.5	Vitamin E	250	Copper	1000
Sodium	11.5			Manganese	4000
				Zinc	1600
				Selenium	12

3. Laboratory analysis of samples

3.1 Milk analysis

After agitation, approximately 10ml of milk sample were taken from the parlour jar of each cow and stored in an airtight plastic bottle containing preservative (potassium dichromate $K_2Cr_2O_7$). The bottles were inverted to dissolve the tablet and then stored at $4^{o}C$. Larger 100ml samples were collected at specific intervals for milk casein analysis and stored in preservative-free bottles. All samples were analysed in the Food Technology Department at the Scottish Agricultural College, Auchincruive.

3.1.1 Milkoscan analysis

The 10ml samples were analysed for protein, fat and lactose using a Foss Electric Milkoscan machine (Model 203). The analyser is designed for the fully automatic determination of fat, protein and lactose which is displayed simultaneously by digital readout and automatic printer. The principle of the Milkoscan is similar to that of an infra-red spectrometer in that certain basic components are used:

1. source of radiation
2. optical system for focussing energy

3. radiation detector

4. amplifier and readout display

The relative amount of fat, protein and lactose are detected by the absorption of the infra-red light by a specific chemical bond:

Table 2.6 Chemical bonds relative to milk constituents

Constituent	Chemical bond	Structure	Absorption
Fat	Carbonyl group	R-C=O	5.75nm
Protein	Amino group	R-N-H \H	6.46nm
Lactose	Hydroxyl group	R-OH	9.60nm

The Milkoscan also determines total solids, and solids-not-fat which are printed out with the milk composition.

3.1.2 Casein analysis - Kjeldahl analysis

The 100ml samples of milk were used in the analysis for casein, non-protein nitrogen (NPN) and protein. Rowland's (1938) method was used which determines the nitrogen distribution in milk. The detailed laboratory methods by which total nitrogen, non-casein nitrogen, NPN, proteose-peptone plus NPN, and globulin nitrogen are determined are available in Davis and McDonald (1953). The basis of the analysis for the three components required, i.e. total protein, casein and NPN, is that initially the total protein content was determined using a Kjeldahl analysis. Second, the samples were then treated with acetic acid/sodium acetate buffer to precipitate the casein, and the supernatant was analysed by Kjeldahl analysis for non-casein nitrogen. Thirdly, the milk was treated with trichloroacetic acid to precipitate

total protein and the supernatant was used to determine NPN. Thus, using these figures it is possible to calculate casein nitrogen by difference. Milk NPN is therefore calculated as nitrogen x 6.38 (i.e. protein equivalent).

3.2 Blood analysis

Samples of blood were withdrawn by a veterinary surgeon initially from the jugular vein, but because of the stress the halter imposed on the cow, samples were then taken from the base of the tail. Animals were restrained in a cattle-crush during sampling and the blood was collected in evacuated glass tubes using small bore needles ("Vacutainer", Becton-Dickinson (UK) Ltd., Wembley, Middlesex). Blood serum was obtained by allowing a clot to form and then centrifuging at 1300g for 10 minutes to separate the serum. The serum samples were then sent to the Aberdeen Veterinary Investigation Centre where they were analysed using the Random Access Analyser, (Technicon) for:

1. Glutamic oxaloacetic transaminase (GOT)
2. Glutamate pyruvate transaminase (GPT)
3. Gamma GT
4. Haptoglobin
5. Urea
6. Calcium, Phosphorus, Magnesium
7. Beta Hydroxybutyrate (BHBA)
8. Non-esterified fatty acids (NEFA)

Standard chemical reagents were used, with the exception of the beta hydroxybutyrate which used the Randox reagent, Ireland.

GOT relates mainly to liver function and levels of the enzyme can

indicate if acute damage is taking place due to its hypersensitivity. GPT also relates to the liver indicating acute damage, but it is also an indication of heart function. Gamma GT is a liver function enzyme relating to bile clearance and levels show chronic damage. Haptoglobin is similar to GOT in function and is synonymous with the fatty liver syndrome. Urea denotes kidney and some liver function while the minerals calcium, phosphorus and magnesium affect homeostasis in the animal. BHBA represents ketone bodies in the blood and therefore metabolism disorders. NEFA levels indicate fat metabolism and problems associated with utilising fat from the back due to inadequate energy intake.

3.3 Feed analysis

3.3.1 Silage

The *in vitro* analysis of the silage samples was carried out by the Analytical Services Department (SAC). The silage was oven dried at a temperature of 100^{o}C to obtain a dry matter value. The ME of the sample was calculated by the method outlined in the WSAC Research Bulletin No. 42 (1969) using the equation: (IVD x 0.907 + 6.03) x 0.16, where IVD is the *in vitro* digestible organic matter in the dry matter. The crude protein content of the forage was estimated using the procedure described by Spillane (1966). The crude protein and minerals were determined using a Kjeldahl type digestion, the CP being analysed by the indophenol-blue calorimetric technique, while the minerals were analysed using an inductively coupled plasma (pers. comm. J Dunsmuir, SAC). The pH was determined from a macerated solution of the silage and the ammonia was calculated from the volatile nitrogen present (see table 2.3).

3.3.2 Concentrates

The neutral cellulase digestibility analysis of the concentrates was also carried out by the Analytical Services Department SAC. Dry matter, minerals and crude protein contents were calculated by the same techniques outlined for the silage analysis. The ME, NCD and AHEE components were estimated using the methods described in the MAFF booklet (1989) - NCD (Appendix I), and AHEE (Appendix II, procedure B). The organic matter of the concentrates was determined by oven drying the samples for 16 hours at 520^{o}C (see table 2.4).

4. Statistical analysis

The results were analysed using Minitab and Genstat IV computer programmes. The statistical significance of the effect of treatments, as determined by F-test in the analysis of variance, is indicated by asterisks accompanying the SED values in the following tables (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$). Any other statistical analyses or tests used are referred to in the text.

RESULTS

The health of the animals was, in general, satisfactory throughout the experiment. However, there were two separate incidences of lameness and mastitis (Streptococcus dysglactiae); two cows were taken off the experiment as a result of postcalving difficulties, and a further cow refused to operate the electronic gates properly which resulted in her being removed from the experiment. Consequently, 3 cows remained in the SL group, 4 cows in the SH group and 5 cows each in the LL and LH groups. The diets are referred to in the tables and text as follows and

in this order where applicable (e.g. results for each group with SED value and significance): SL = Silage to low ME diet/Group 1; SH = Silage to high ME diet/Group 2; LL = Low ME to low ME diet/Group 3 and LH = low ME to high ME diet/Group 4.

An analysis of variance was carried out on calf weight for the two diets prepartum and was found to be not significantly different between the groups (49.1kg silage base: 47kg low ME; SED = 4.40, NS).

All diets were formulated in advance to a minimum of 174g/kg DM of CP on the basis of analysed feed samples prior to the start of the experiment. However, the feed analysis of samples taken throughout the experiment revealed that the concentrate mixes and silage had slightly more variable CP contents and ME values than predicted. Mean analysis however, showed the CP composition to be similar across diets, and the ME differences between treatments to be within the range expected.

As a result of the higher concentrate contents of the two higher ME diets pre and post partum, total dry matter intake (TDMI) and CP intake were significantly higher in animals offered these diets, as shown in table 2.7:

Table 2.7: Feed intake results

	Prepartum			Postpartum				
Daily DM intake	Silage base	Low ME	SED	SL	SH	LL	LH	SED
Concentrates (kg/d)	1.39	4.42	0.17***	4.02	10.49	4.57	8.64	1.12***
Silage (kg/d)	8.45	7.92	0.51 NS	7.40	6.34	8.21	6.48	0.63NS
TDMI (kg/d)	9.84	12.34	0.66**	11.42	16.83	12.78	15.13	1.27**
CP (kg)	2.08	2.48	0.16*	1.99	2.84	2.21	2.85	0.19***
ME (MJ)	109.9	145.3	7.48***	129.6	206.6	149.9	196.6	13.23***

Liveweight changes were estimated using two approaches (table 2.8). Firstly, by regression on cow weight over the two periods; and secondly by an energy balance calculation using the equations published in MAFF (1975) - for comparison, energy balance calculations were made for the groups using ARC (1980). Both approaches indicate that cows were in negative energy balance on all diets, with the regression calculations showing significant differences in weight loss between treatments.

Table 2.8: Liveweight and energy balance results

	Prepartum			Postpartum				
Analysis:	Silage base	Low ME	SED	SL	SH	LL	LH	SED
Liveweight (kg)	670	686	27.00 NS	497	611	549	647	38.40**
Lwt change by regression on cow weight:								
(kg/d)	+1.0	+1.4	0.35 NS	-2.3	-1.8	-2.9	-1.8	0.27 NS
Condition score	3.00	3.25	0.14 NS	1.75	2.75	2.25	2.75	0.44 NS
TDMI as % of lwt	1.47	1.81	0.10**	1.69	2.86	2.39	2.39	0.37*
Lwt change (kg/d): MAFF (1975)				-2.23	-0.75	-1.43	-0.62	0.53*
Lwt change (kg/d): ARC (1980)				-2.23	0.28	-1.86	-0.13	

Animals in the four groups lost 87.3, 75.2, 90.6 and 73.1kg (SED=20.66;NS) respectively, in liveweight due to calving. Cows in the LH group showed a slower rate of liveweight loss than the other three groups, although the SL group were the only group in positive energy balance when the cows went 'out to grass'. No work was carried out on

liveweight change during the out to grass phase because the cows were weighed only once a week and in the afternoon as compared with three times a week in the morning during the pre and post partum phases, thus making comparisons unreliable.

During the 'at grass' period, milk composition, milk yield, concentrate refusals in the parlour and grass heights were recorded. A high energy, high CP diet was offered to all animals and consisted of fresh herbage and 8kg of parlour concentrate, the analyses of which are shown in table 2.9:

Table 2.9: Fresh herbage and concentrates analysis (weeks 9-12 of lactation)

Analysis	Herbage	Concentrates
Dry matter (g/kg)	162	840
Dry matter analysis:		
CP (g/kg)	207	203
OM (g/kg)	887	912
D val (g/kg)	716	750
ME (MJ/kg)	11.30	13.20
Ca (g/kg)	4.62	7.90
P (g/kg)	4.66	7.90
Mg (g/kg)	2.34	8.80

The results obtained for milk yield and composition are detailed in table 2.10:

Table 2.10: Milk analysis results

	Postpartum					At grass				
Analysis	SL	SH	LL	LH	SED	SL	SH	LL	LH	SED
Milk yield (kg/d)	25.71	26.73	26.99	27.39	2.57 NS	27.46	25.89	29.37	26.83	2.17 NS
Milk protein content (g/kg)	30.72	32.44	29.79	31.96	0.96 NS	31.59	31.66	30.87	32.02	1.22 NS
Milk protein yield (kg/d)	0.79	0.87	0.80	0.87	0.07 NS	0.87	0.82	0.91	0.86	0.08 NS
Milk fat content (g/kg)	41.80	45.00	41.80	42.10	3.45 NS	30.67	38.92	33.10	34.58	2.75 NS
Milk fat yield (kg/d)	1.07	1.20	1.14	1.15	0.14 NS	0.85	1.01	0.98	0.93	0.12 NS
Milk lactose content (g/kg)	47.57	49.65	48.46	48.13	0.71 NS	47.69	48.55	47.71	48.08	0.56 NS
Milk lactose yield (kg/d)	1.22	1.33	1.31	1.32	0.13 NS	1.31	1.26	1.40	1.29	0.11 NS
Milk casein (g/kg)	22.20	23.82	22.24	22.54	0.11 NS	25.20	23.48	23.56	23.75	0.13 NS

No statistically significant differences were apparent in either milk yield, constituent yield or constituent content between any of the four diets during either the postpartum phase or when out to grass (table 2.10). Nonetheless, from the above table it can be seen that there are differences between means: milk protein is higher in the high ME fed cows although this does disappear when the cows were put out to grass. The milk casein results also showed higher contents in the high ME cows although this reversed when at grass (SL diet: 22.20g/kg postcalving cf 25.20 g/kg at grass). Postpartum milk lactose and at grass milk fat results showed differences between the SH and SL groups.

No significant differences were found between the groups in milk casein during any recording week.

Recorded blood parameters, were analysed to give an indication of the nutritional state of a cow. This method relies on recordings being outside the "normal bovine range" to be classified abnormal. The blood analysis results are detailed in table 2.11:

Table 2.11: Blood analysis

Analysis	Normal range	Prepartum			Postpartum				
		Silage base	Low ME	SED	SL	SH	LL	LH	SED
GPT (u/l)	4-15	12.7	15.6	0.057*	14.34	15.30	12.00	17.75	2.103 NS
Gamma GT (u/l)	7.5-21.9	21.0	20.3	0.081 NS	22.49	20.30	24.10	19.63	1.915 NS
B-OH butyrate (mmol/l)	<0.75	0.398	0.271	0.057*	0.686^{ab}	0.383^{a}	0.995^{b}	0.419^{a}	0.206*
Urea (mmol/l)	2.0-6.6	4.98	4.95	0.589 NS	5.18^{a}	3.56^{b}	4.70^{a}	3.66^{b}	0.370**
NEFA (meq/l)	0.1-0.7	0.311	0.49	0.157 NS	0.718	0.82	1.01	0.862	0.212 NS
Lipids (g/l)	1.5-5.0	2.35	3.59	0.444*	6.38	6.02	5.66	6.35	0.714 NS
Magnesium (mmol/l)	0.8-1.1	0.76	0.83	0.040 NS	0.645	0.807	0.767	0.846	0.074 NS

Analysis	At grass				
	SL	SH	LL	LH	SED
GPT (u/l)	16.7	30.4	23.6	27.0	6.65 NS
Gamma GT (u/l)	22.34	21.80	23.20	23.00	2.248 NS
B-OH butyrate (mmol/l)	0.237^{a}	0.462^{b}	0.304^{a}	0.277^{a}	0.068*
Urea (mmol/l)	6.67	6.24	7.80	6.48	0.834 NS
NEFA (meq/l)	0.300	0.420	0.340	0.475	0.100 NS
Lipids (g/l)	7.29	6.93	6.46	6.30	1.182 NS
Magnesium (mmol/l)	0.692	0.833	0.835	0.844	0.066 NS

Note: means with the superscript in the same line do not differ significantly from each other ($P<0.05$)

Results for calcium, phosphorus, serum GOT and haptoglobin were all within the normal bovine range and were not significantly different between the groups over the experimental period.

The results of the blood analysis showed a similarity between groups on the same diet postcalving, regardless of precalving treatment. Blood urea was significantly higher (SED=0.37, $P<0.01$) in the SL and LL groups than in the SH and LH groups, with a t-test analysis showing a significant difference between the SL and SH groups ($t=4.38$, $P<0.001$). These differences disappeared when the cows went out to grass suggesting a dietary effect. Serum GPT and gamma GT were also similarly affected, showing differences between the low ME and high ME fed groups postpartum, but no differences when out to grass.

Beta hydroxybutyrate is a ketone body which when present in high concentrations in the blood, indicates that the animal is energy deficient. It is evident that the silage base cows had a significantly higher level of beta hydroxybutyrate in the blood. Animals in the LL group showed a significantly higher postpartum beta hydroxybutyrate value (0.995 mmol/l; SED=0.206,*) suggesting that the low energy diet was not sufficient to maintain and improve body condition after calving.

Although significant differences were found between means in the GPT, gamma GT, urea, beta hydroxybutyrate and magnesium, during the pre and post partum periods, with the exception of the last two parameters these significant differences disappeared once the cows went out to grass.

The fertility results of the animals in terms of calving to

conception interval were not suitable for analysis. Five cows were served later than usual so that they could replace culled cows on summer calving experiments, and another four were not served but culled at the end of the lactation. In the remaining cows intervals of 79, 88, 72 and 96 days (SED=43, NS) were recorded.

When the cows were 'at grass' the concentrate intake and mean grass height were found not to be significantly different between groups (6.67, 6.89, 7.15, 6.33 kg/d freshweight; SED=0.748, NS for the concentrates and 12.91, 11.53, 11.43, 13.07 cm; SED=1.370, NS for the grass heights), a correlation was found between the two parameters and expressed by the equation $y=10.74-0.326x$ ($r=-0.618$, $P<0.01$), where y=concentrate intake in kg freshweight per day and x=grass height in cms. Thus, as the amount of fresh herbage available decreased, parlour concentrate intake increased to adjust the balance of required ME intake for maintenance, liveweight change and milk production.

Figure 2.1: Grass height and concentrate intake correlation

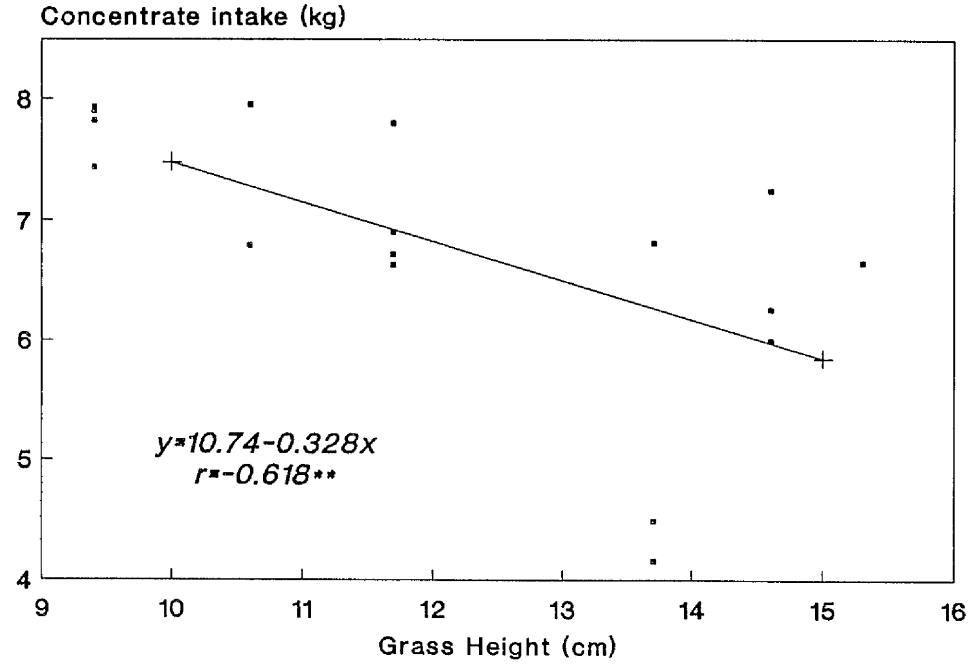
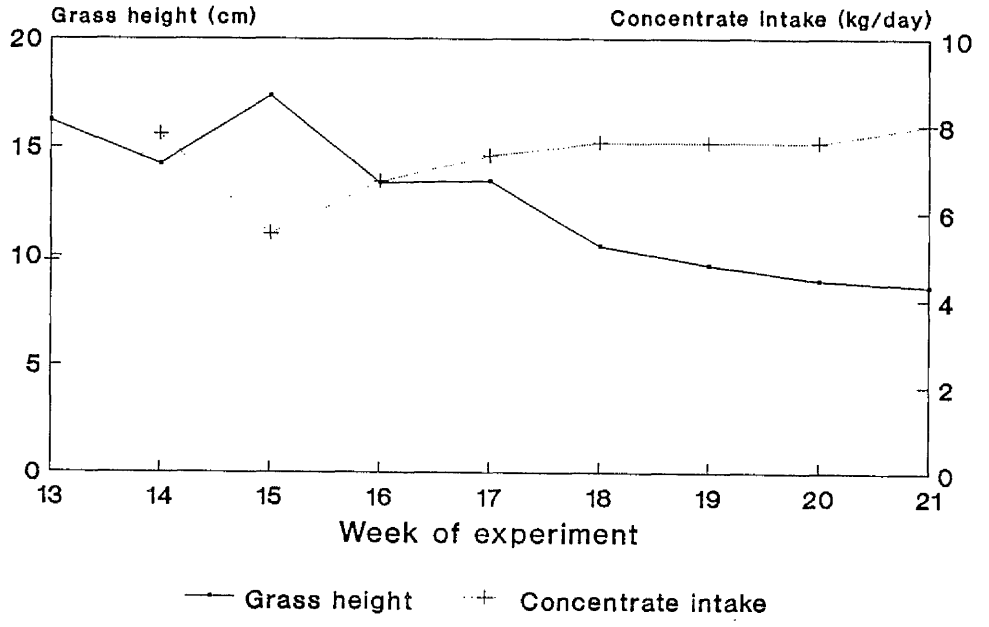


Figure 2.2: Graph of grass height and concentrate intake (at grass) against time



DISCUSSION

The pattern of response in milk composition and dietary intake to the diets offered was in broad agreement with other findings, especially in relation to energy balance in early lactation (Davenport and Rakes 1969; Gardner 1969; Braund and Steele 1972; Lodge et al 1974).

From the five factors mentioned in the introduction, thought to be of importance for producing a good quality milk, the silage used throughout was of good quality despite the CP content being significantly lower in the postpartum silage (122g/kg cf 163g/kg prepartum; $t=2.99, P<0.05$). Supplementation of the forage with concentrates is detailed in table 2.12:

Table 2.12: Composition of complete diets (% on DM basis)

Diet	Forage %	Concentrates %	Minerals %
Silage base	84	14	2
Low ME	65	33	2
High ME	37	61	2

Total dry matter intakes were statistically different between treatments. This was due in part to the effect of the diet, for example, Total dry matter intakes in the low ME fed cows was 12.34kg prepartum and 12.78kg postpartum, with an increase of 2.79kg to 15.13kg occurring in cows offered the high ME diet postcalving. Similar results were also found in the SH group with a jump of 6.99kg, from 9.84kg to 16.83kg being recorded. The decrease in the SL group, from 9.84kg to 9.06kg postcalving is thought to be a cow effect since only 3 cows were in the group after calving all of which consumed small amounts of feed. This group had only 4 cows at the start of the trial (one cow refused to operate the Calan gate) and at calving another animal had a Caesarian which resulted in her leaving the trial through infection. A third cow was in poor condition after calving and a fourth animal was lame during the majority of the postpartum period. Therefore, the dry matter intake results reflect the problems associated with this group and the fact that cows in this group were significantly lighter in weight after calving than the other treatment groups (SED=38.4,***), particularly the high ME fed cows.

Total dry matter intake among treatments for the experimental period paralleled the increase in energy concentration of the three

diets. Coppock et al (1972,1974) recorded mean dry matter consumptions, as a percentage of liveweight, of 3.32% and 3.50% respectively. This was substantially above the values calculated in this work, which tend to correspond more closely with that of Braund and Steele (1972) who claimed a value of 2.84% in older cows. The prepartum percentages were low suggesting that the cows were in adequate condition (3 to 3.25) for calving, thereby only consuming essential energy for maintenance and calf growth. Postpartum the results were improved at 1.69, 2.86, 2.39, 2.39% for SL, SH, LL, LH respectively.

During early lactation the rate of increase in milk production exceeded that of feed intake. Energy balance is therefore an important factor to consider and is defined as the relationship between dietary energy intake and energy utilization i.e. daily energy balance = energy consumed - energy required. Calculation of the energy balance is consequently a more accurate measurement of the metabolic status of the cow than milk yield alone.

The results in table 2.8 are mean group values and these mask a large individual variation between cows in weight changes and energy deficit. Liveweight change (MAFF 1975) towards the end of the postpartum period was similar in all groups (week 12: 0.43, -0.54, -1.57, -0.33 lwt change kg/d; SED=0.77,NS). It is very probable that the animals went into positive energy balance and consequently showed a liveweight gain not long after they went out to grass. This interval to positive energy balance from calving (approximately 9-10 weeks in this experiment) corresponds to that in other work. Coppock et al (1974) stated that the time needed for cows to attain an energy balance decreased from 14 weeks in low supplementation fed cows to 6 weeks in

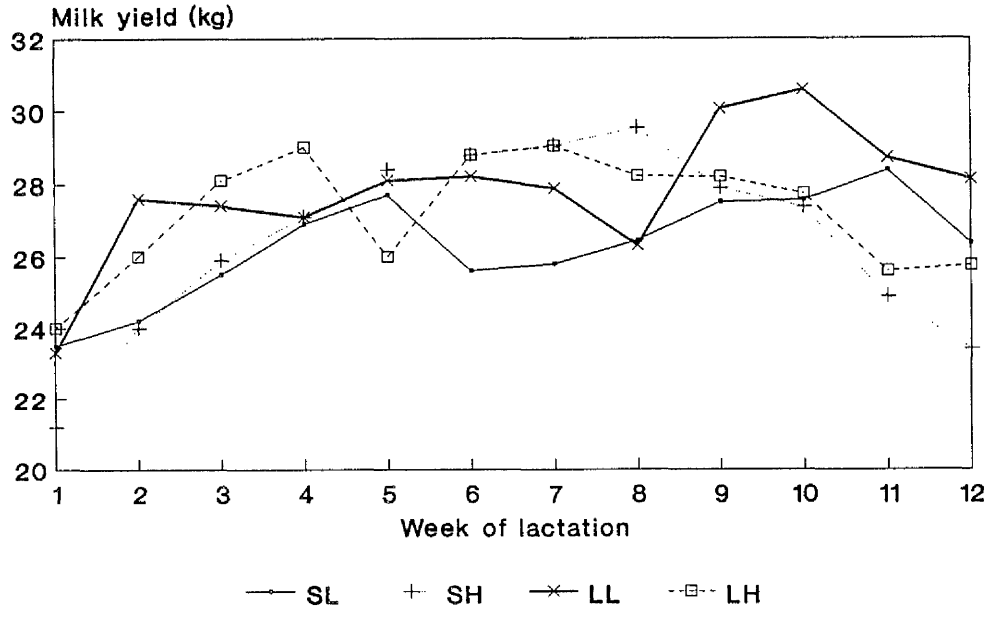
high supplemented cows.

It is evident from the results that body reserves were being mobilised to support milk production, particulary in the LL group which had three high yielding cows. The body condition at calving reflected the cumulative results of previous feeding (3 & 3.25 for base and low ME respectively), and the effect of maintenance feeding prepartum was minimised because of the capacity of the cows to mobilise body tissue, especially in the SH group. Rook (1976) advocated that giving additional food in late pregnancy facilitated the establishment of peak milk yield - the so called "steaming up" concept. The peak milk yield however, which is controlled by nutrition and hormonal interaction, sets a limit to the yield that may be achieved later in the lactation. Therefore, altering one of the secretion factors, i.e. nutrition, by underfeeding during lactation may lead to irrevocable loss of yield. With respect to this the "steaming up" concept suggests that a bulky food fed in late pregnancy leads to improvement in body condition which enables the animal to cope with the demands of milk production through increased ability to mobilise body tissue, thus minimising the effects of underfeeding. Gardner and Park (1971) however, found that cows on low energy prepartum produced as well or slightly better than liberally fed cows, a finding confirmed by Broster (1971) and Gleeson (1973). This suggests that the animals fed low energy prepartum achieved energy balance quicker than the liberally fed cows because they lost less bodyweight after calving. These cows gained more weight when at grass than the better fed cows. Broster (1971) concluded that a cow in poor condition before calving responded to additional feed prepartum by increased milk yield, whereas cows in moderate and good condition

responded less well or not at all respectively.

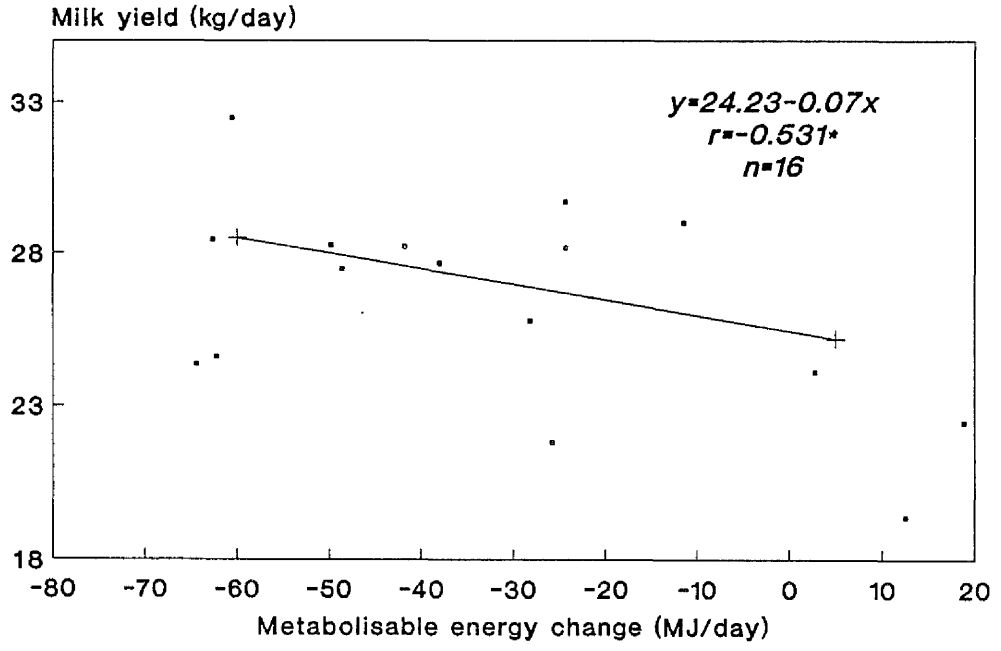
The milk analysis results in this experiment tend to conform in general with the observations of Gardner and Park (1971). The animals in the SH and SL treatments were given a silage base diet prepartum and results in table 2.10 show that in terms of milk yield and constituents, the animals were not significantly different from the low energy treated cows. When the animals went out to grass those that had been on the low energy diet postpartum showed an improvement in milk yield (especially the LL cows) thereby challenging Rook's (1976) claim that the lactation pattern is established on the peak yield shown in weeks 3 or 4 of lactation. Peak lactation was reached in weeks 11, 8, 10 and 4 respectively:

Figure 2.3: Milk yields for weeks 1 - 12 of lactation



During early lactation the deficiency in dietary energy relative to the energy required for milk production resulted in a negative energy balance which was greatest in week 2 of the lactation. The rectilinear relationship between milk yield and energy balance shows that the two factors were negatively related with a correlation coefficient of -0.531, which was found to be significant at the 5% level. This confirmed the conclusion that the cows were milking "off their backs" in the postpartum period.

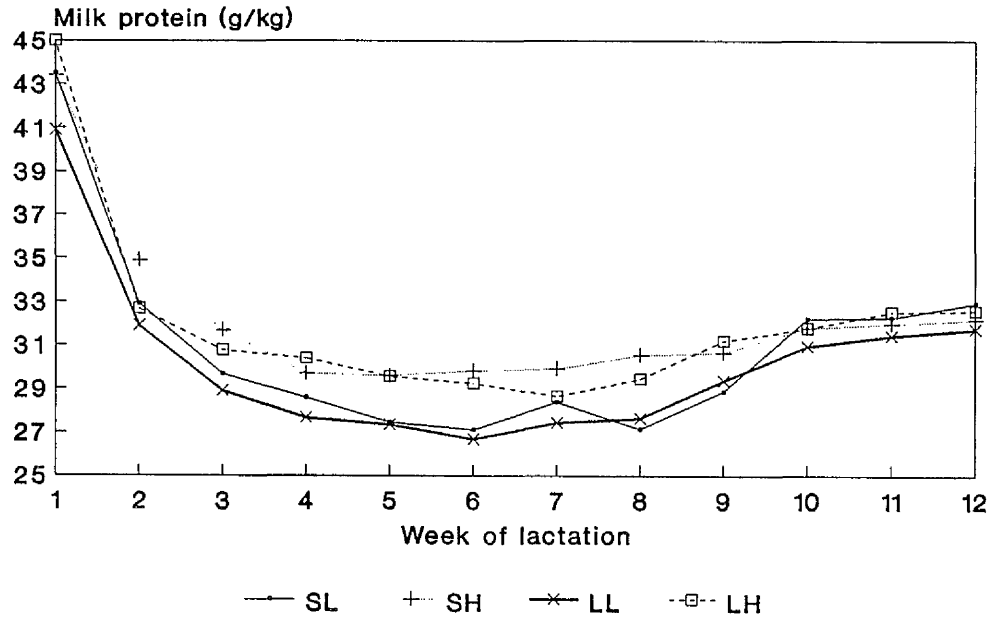
Figure 2.4: Relationship between mean milk yield and average energy balance for the postpartum period



Milk yield was found to be not significantly different between treatments; however, the trend towards higher yields in the higher energy fed cows precalving indicates that a diet of this type fed postpartum leads to maintenance of production as opposed to an increase in production. Additionally, continuation of this feed up to 8 weeks of lactation produces an increased milk yield when the animals were put onto a high energy diet (table 2.10 and figure 2.3).

Milk protein yields were found to be not significantly different over the 12 weeks of lactation. Milk protein contents however, were found to be higher in the postpartum high energy fed cows.

Figure 2.5: Milk protein results for weeks 1 - 12 of lactation



From table 2.10, milk casein content was higher in the high energy fed cows. Milk casein is normally 78% of milk protein and the treatments showed results of 72, 73, 70 and 69% for the groups respectively. These depressions in milk casein percentage are unlikely to be a real effect and could be a result of the analytical technique used in its determination. No measurements were made of NPN (which is mainly urea) using the Kjeldahl analysis, however, blood urea is an indicator of excess protein in the diet which is voided in the urine. If a high crude protein diet is fed with a lower ME than requirement, the efficiency with which the rumen degradable protein (RDP) is utilised by ruminal micro-organisms decreases. Subsequently, this causes decreased protein synthesis and the excess ammonia is absorbed into the blood. In the liver the excess ammonia is converted into urea.

Blood urea (table 2.11) was found to be significantly different between the two postcalving treatments. Prepartum and at grass samples showed no significant differences, although levels in the LL group at grass were 1.2 mmmol/l above the normal range. The reason for this elevated blood urea was unclear, although a possible explanation is that all excess protein in the diet (particularly from the parlour concentrates) was removed from the body as urea in urine. All groups had urea levels near the top of the normal bovine range when at grass, eventhough milk casein percentage approached the normal in all groups. An alternative explanation is that an increase in NPN may be being experienced at the expense of the whey proteins. Work in this area of milk production is obviously required. In general, milk protein was not significantly affected by the treatments, but improved in cows offered high energy diets postpartum and when at grass.

Beta hydroxybutyrate (BHBA) is a major precursor for milk fat and when present in high concentration in the blood plasma, indicates negative energy balance. With the increase in energy demand in early lactation, the beta oxidation of dietary fatty acids results in overproduction of acetyl CoA which stimulates ketone production and finally the onset of ketosis. If assuming that the top of the normal bovine range for BHBA (0.75 mmol/l) is the borderline for ketosis, then the value of 0.995 mmol/l for the LL cows postcalving suggests that the peripartum treatments significantly affected blood ketone levels, causing ketosis in this group. Three out of the five cows in this group (two of which were in the top 5% in terms of milk yield) had BHBA levels significantly above the normal range during the postpartum period. From the results therefore, it appears that the SL and LL treatments were more conducive to abnormal ketone levels than the other treatments, thereby confirming that the high energy diet allowed cows to approach energy balance quicker than the low energy diet.

When comparing blood BHBA results with those of the milk fat content it is clear that the high levels of the blood parameter depressed milk fat content in the low ME group. A linear regression of BHBA and milk fat showed no significance either postpartum or when out to grass ($r=-0.93$ and $r=1.87$ respectively), although the at grass result was approaching significance. The milk fat contents showed no significant differences between treatments during the 12 weeks of lactation. The elevated milk fat content recorded for the SH group in table 2.10 is possibly explained by the blood lipid results. These indicate that there was an increase in the long chain fatty acid (LCFA) absorption from the gut. As a result of this, and in addition to the

mobilised body fat and the synthesis of the milk fatty acids from the acetate and BHBA present in the mammary glands (predominantly the short chain fatty acids - SCFA), milk fat synthesis was increased in the SH group. The low milk fat recorded in the SL group however, despite the high blood lipid level, is perhaps the result of the animals going onto a high energy diet when at grass which is known to cause fat depression.

In terms of economical milk production, the costs of the high energy feeds appear to outweigh the small increases found in milk production. The initial hypothesis that milk might be synthesized more efficiently when feeding maintenance level diets precalving and high energy diets during lactation were not confirmed. The inference from this work is therefore that neither advantage nor disadvantage accrues from feeding low energy diets prepartum and high energy diets postpartum. It is also apparent that prepartum feeding from 4 weeks before calving has little effect on milk production.

Two factors of interest have become apparent from this work which are considered in the next experiment:

1. the changes within the milk protein constituents when cows are offered high crude protein diets - is it the NPN or the whey proteins that substantially increase?

2. and the fact that milk yield may be elevated if cows are put onto high energy and possibly high CP diets later in lactation - at what lactation stage does this have the most significant effect?

Problems associated with this experiment were:

1. not enough cows were available for experimentation.
2. the precalving period was not long enough to establish differences between treatments.
3. more information is required on body condition, and in particular backfat, through the use of ultrasound, and body protein, although methods of measuring this have yet to be perfected.

CHAPTER 3

LACTATIONAL RESPONSES OF DAIRY COWS TO CHANGES IN DIETARY PROTEIN FED AT DIFFERENT STAGES OF LACTATION

INTRODUCTION

With todays' trend towards healthy eating, the consumer is demanding more dairy products that are low in fat and high in protein. Yields of these products (i.e. cheeses, skimmed milks, low-fat spreads) depend to a considerable extent on the protein content of the milk. The quality of these dairy products could therefore be influenced by the composition of the milk protein produced and the relative amounts of the serum proteins, caseins etc. synthesized (Davies and Law 1980). The materials required for milk synthesis are ultimately found in the diet and changes in quantity or quality must obviously affect milk yield and composition. Evidence from a range of experiments points to an increase in milk production and an improvement in milk protein production when a low degradability 'animal' protein source is used in the diet. Feeding this supplementary protein to lactating dairy cows is, however, expensive but nonetheless it is an important aspect of dairy nutrition in the early lactation cow (Casper and Schingoethe 1989).

Feeding high protein concentrates to cows generally has a positive effect on milk yield and milk protein content which can be attributed to an increased supply of energy to the mammary gland (Emery 1978). However, it is generally thought that dietary protein usually affects milk production much more than the protein content of the milk itself. The responses to diet are complex: affecting silage intake and digestibility, and the improvement of rumen microbial protein synthesis

increases the amino acid supply to the small intestine thus maximising the efficiency of utilising ruminal undegradable protein (Casper and Schingoethe 1989).

With the above in mind and the results from experiment 1, this experiment was designed to evaluate the response of cows to changes in dietary protein at different stages of lactation. A simple concentrate feeding system (flat rate feeding) with an adequate supply of forage (silage ad libitum) was utilised to provide an efficient winter management programme for cows.

MATERIALS AND METHODS

[For detailed explanations on materials and methods used, see chapter 2.]

1. Experimental

1.1 Animals and their management

Twenty-four British Friesian dairy cattle were used, consisting of 20 cows (second parity or more) and 4 heifers (first parity). The animals were Autumn calvers, calving between September and November 1988 (SD=12.03 days). Mean cow weight during the experimental period was 576kg (SED=33.25;NS) and mean milk yield based on a 305 day estimate for this lactation was 6100kg (SED=281).

The cows were offered silage ad libitum, and 4kg (freshweight) of concentrates twice daily before each milking. The animals began their experimental period 14 days postpartum and remained in their group for the remainder of the trial. Each cow stayed on the trial for 26 weeks postcalving.

1.2 Experimental design and diets

A continuous design experiment, the trial lasted 36 weeks in total from the first cow calving in mid-September 1988, to the final cow leaving the pen in early May 1989. Each cow was initially fed a standard diet for the two weeks after calving, followed by 3 x 8 week periods on the trial (table 3.1). Using parity number and the milk yields and weights recorded during the first 2 weeks of lactation, cows were allocated to balanced groups which included one heifer and one second lactation cow.

Table 3.1: Treatments offered

Diet	Silage	Concentrates offered (DM)	
		Low	High
Standard	ad lib.	3.44kg +	3.48kg
Low protein	ad lib.	6.88kg	
High protein	ad lib.		6.96kg

The group treatments are outlined in table 3.2:

Table 3.2: Group treatments

	Weeks of lactation			
	Covariance period	Experimental period		
Group	1-2	3-10	11-18	19-26
1	Standard diet	High protein	High protein	High protein
2	Standard diet	Low protein	High protein	High protein
3	Standard diet	Low protein	Low protein	High protein
4	Standard diet	Low protein	Low protein	Low protein

Silage dry matter intakes were recorded on 4 consecutive days each week with fresh silage feeds being adjusted to allow a 5-10% refusal daily. Concentrates were fed twice daily in separate trays (0.6m x 0.35m), and any refusals were weighed and discarded. Milk samples were also taken on 4 consecutive days each week with additional 100ml samples being taken every fortnight from week 4 of lactation for non-protein nitrogen (NPN) and casein analysis. Weights and condition scores were recorded every Wednesday afternoon after milking.

2. Diet components

2.1 Silage

The silages used were first cut perennial ryegrass with ADD-F (BP Nutrition UK Ltd.) additive added at 3 litres per tonne. Grab samples of fresh silage and refusals for oven dry matter analysis were taken daily and bulked samples were taken monthly for *in vitro* analysis.

Table 3.3: *In vitro* analyses of silages used

	Silages	
Analysis	Sep 88-Feb 89	Feb 89-Apr 89
Dry matter (g/kg)	184	167
Dry matter analysis:		
Crude protein (g/kg)	147	156
OM (g/kg)	916	921
D Val (g/kg)	679	682
ME (MJ/kg)	10.9	10.9
pH	3.9	3.8
Ammonia (N g/kg)	109	121
Calcium (g/kg)	6.0	5.6
Phosphorus (g/kg)	3.2	3.3
Magnesium (g/kg)	2.1	1.9

2.2 Concentrates

Animals in this trial were offered industrially formulated pellets, the major composition of which is shown below:

Table 3.4: Composition and analytical target of the low and high protein concentrate

Formulation (g/kg)	Low	High	Analytical target (g/kg)	Low	High
Wheat	139.2	150.0	Protein	140.0	285.0
Wheatfeed	225.0	-	RDP	107.5	107.5
Molassed SBP	225.0	25.0	DUDP	22.2	148.5
Fishmeal	-	75.0	Fibre	85.7	86.7
Molaferm	99.0	101.0	ME (MJ/kg)	11.5	11.5
Palm fat	32.5	23.9	Sugar	120.0	70.0
Soya products	25.4	73.2	Starch	160.0	150.0
Rape extract	117.7	41.3			
Cotton extract	-	150.0			
Maize gluten meal	-	170.5			

Analysis of the monthly concentrate samples, using the NCD method, showed that the concentrates varied from the analytical targets:

Table 3.5: Mean monthly analyses of concentrates

Analysis	Low protein	High protein
Dry matter (g/kg)	860	871
Dry matter analysis:		
Crude protein (g/kg)	171	315
OM (g/kg)	904	903
ME (MJ/kg)	13.05	13.08
AHEE (g/kg)	70.30	79.50
NCD (g/kg)	799.70	812.90
Calcium (g/kg)	11.18	16.21
Phosphorus (g/kg)	7.20	10.86
Magnesium (g/kg)	5.49	7.36

3. Laboratory analysis of samples

[The detailed laboratory analysis of samples described in chapter 2:3 apply to the milk and feed samples taken from this experiment.]

4. Statistical analysis

The majority of the analysis of the data recorded on this trial was analysed using Genstat V by the Scottish Agricultural Statistical Services, based at Edinburgh. Detailed discussions took place on how best to analyse the data to obtain the results required from the experiment. Less detailed work was also carried out using Genstat IV and, to a lesser extent, Minitab. Statistical significance is shown as described in chapter 2:4.

RESULTS

The health of the animals was satisfactory throughout the experiment. Three incidences of lameness were recorded, two were due to injuries and one to a solear ulcer formation. Three cows developed mastitis: bacteriology/sensitivity tests showed the presence of Escherichia coli in two of the cows, and Staphylococcus epidermidis and Streptococcus dysglactiae in the third animal. Examination of milk samples from a fourth cow which was showing a decline in milk yield, revealed the presence of Staphylococcus epidermidis. All incidences of mastitis, with the exception of the fourth cow, occurred shortly after calving, and no further occurrence was found throughout the remainder of the trial. The milk samples were cultured on Todd-Hewitt broth and examined by the SAC Veterinary Investigation Service.

The groups are referred to in general as HHH (Group 1), LHH (Group 2), LLH (Group 3) and LLL (Group 4). However, this trial was mainly an investigation of slow and immediate responses of dairy cows to increased dietary protein. Therefore, to enable comparisons to be made between low and high protein diets, the data was analysed according to concentrates offered i.e. in period 1 there were 2 groups of cows for comparison - those offered high protein concentrates (H) and those offered low protein concentrates (L). In period 2 there were 3 groups of animals - animals remaining on the high protein (HH) and low protein (LL) concentrates, and those changing from low to high protein (LH). In period 3, all 4 groups are represented - HHH, LHH, LLH and LLL. To establish at what stage of lactation an increase in dietary protein had greatest effect, analysis of variance was also carried out on periods 1+2 i.e. first 16 weeks of experiment, and on the total experimental period, 1,2+3 i.e. the 24 weeks of experiment. [Note: in period 2 and period 1+2 there were three unbalanced groups in the cow ratio of 6:6:12, corresponding to the groups HH:LH:LL. As a result, SED was calculated for min:rep (HH:LH) and max:min (LL:HH/LH). In the following tables therefore, the top SED in these periods refers to the max:min value, and the lower SED to the min:rep value.]

No significant differences in concentrate DMI was found between groups (SED=0.018;NS) over the experimental period, but silage DMI was significantly higher in the high protein fed cows (table 3.6).

Liveweight change (lwt), and condition score change (CS) were found to be not significantly different between groups over the experimental period (table 3.7).

Table 3.6: Dry matter intakes for each period

	Period 1			Period 2				Period 1+2			
DM intake	H	L	SED	HH	LH	LL	SED	HH	LH	LL	SED
Silage (kg/d)	10.19	9.05	0.52*	10.26^{a}	10.12^{a}	8.87^{b}	0.50*	10.22^{a}	9.71^{ab}	8.89^{b}	0.50*
							0.58				0.58
TDMI (kg/d)	16.96	15.88	0.55	17.22^{a}	17.02^{a}	15.75^{b}	0.51*	17.09	16.61	15.73	0.52
							0.59				0.60
CP (kg/d)	3.63	2.50		3.70	3.66	2.48		3.67	3.10	2.48	
ME (MJ/d)	199.6	187.6		202.8	200.6	186.5		201.3	196.1	186.2	

	Period 3					Period 1,2+3				
DM intake	HHH	LHH	LLH	LLL	SED	HHH	LHH	LLH	LLL	SED
Silage (kg/d)	9.08^{a}	8.76^{a}	8.34^{a}	7.23^{b}	0.52**	9.84^{a}	9.40^{ab}	8.67^{bc}	8.38^{c}	0.87*
TDMI (kg/d)	16.05^{a}	15.72^{a}	15.30^{a}	14.05^{b}	0.49**	16.74^{a}	16.32^{a}	15.52^{ab}	15.24^{b}	0.48*
TDMI as % lwt	-	-	-	-		2.75	2.79	2.76	2.52	0.15
CP (kg/d)	3.61	3.56	3.50	2.29		3.65	3.24	2.83	2.42	
ME (MJ/d)	191.1	187.4	182.7	168.5		197.8	191.9	185.6	180.9	

Notes 1: In table 3.6 and subsequent tables of results, means with the same superscript in the same line and period do not differ significantly from each other ($P<0.05$).

2: CP and ME intakes were significantly higher in the high protein fed cows.

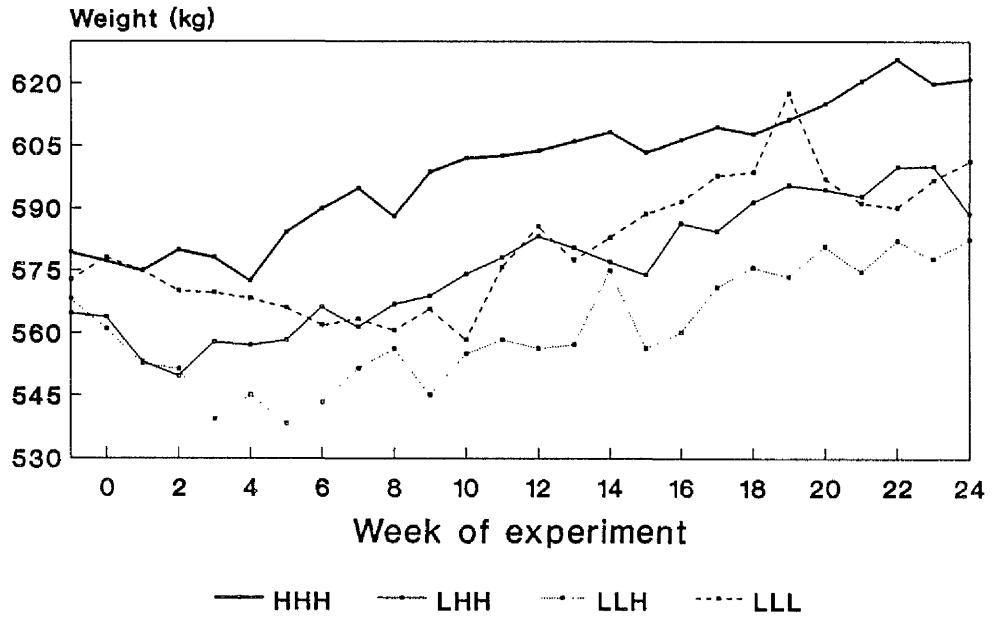
Table 3.7: Liveweight and condition score change

	Period 1			Period 2			
Change	H	L	SED	HH	LH	LL	SED
Lwt (kg)	15.8	-6.9	14.91	5.7	19.3	17.5	13.47 15.56
CS	-0.15	-0.19	0.16	0.00	0.17	0.15	0.15 0.18

	Period 3				
Change	HHH	LHH	LLH	LLL	SED
Lwt (kg)	14.3	2.3	22.3	9.5	8.09
CS	0.17	0.42	0.00	0.17	0.22

From the results obtained on liveweight, it is evident that animals offered the high protein concentrate in period 1 achieved energy balance within the first 10 weeks of lactation. A mean liveweight gain of 15.8kg was recorded in these animals despite a marginal decline in condition score. It also appears from the weight data that the HHH and LLL groups gained more weight during the lactation than the LHH and LLH cows, as shown in figure 3.1:

Figure 3.1: Graph of liveweight against weeks of experiment



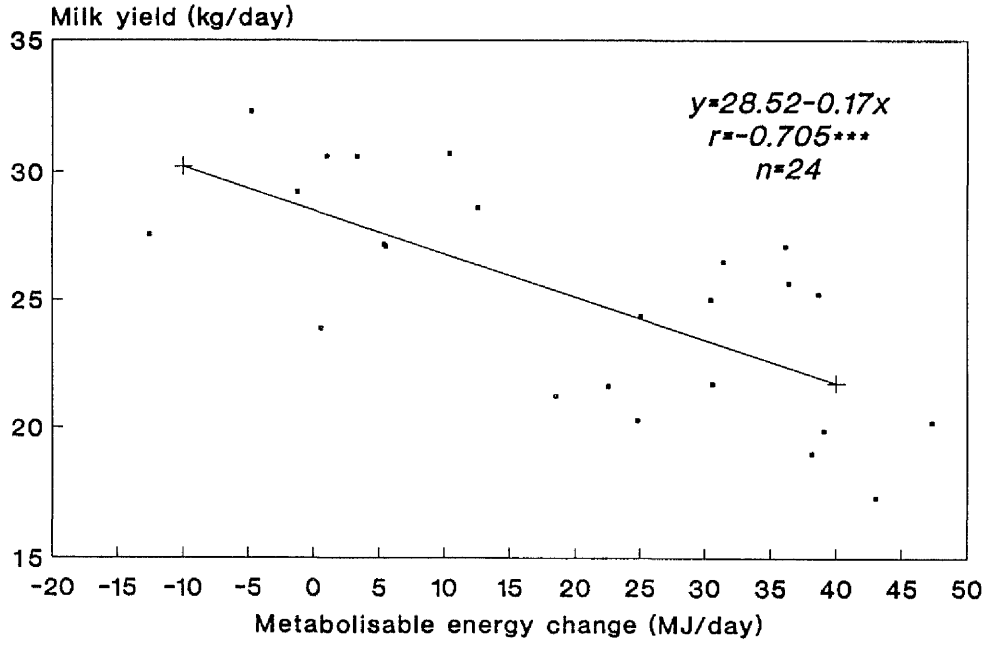
However, liveweight gain results (MAFF 1975) for the total experimental period showed a greater weight increase in the LHH and LLH animals, although not significantly. Energy balance was also found to be not significantly different between the groups over the total 26 weeks:

Table 3.8: Energy balance and liveweight gain

	Periods 1,2+3				
	HHH	LHH	LLH	LLL	SED
Lwt gain (kg/day) (MAFF 1975)	0.44	0.76	0.71	0.55	0.33
Lwt gain (kg/day) (ARC 1980)	0.63	0.61	0.75	0.50	
Energy excess (MJ/day) (MAFF 1975)	14.1	24.9	23.3	17.9	10.42

The rectilinear relationship between milk yield and energy balance showed that the two factors were negatively related with a correlation coefficient of -0.705, significant at the 0.1% level.

Figure 3.2: Relationship between milk yield and energy balance



Mean treatment milk outputs were adjusted by covariance analysis using the yield, protein, fat and lactose contents recorded during the first 2 weeks of lactation as the independent covariates. The results obtained for milk yield and composition are detailed below (table 3.9).

In period 1 milk yield was significantly higher in the high protein fed cows. In period 2 there was a significant difference between the HH and LL group ($t=2.77, P<0.05$), with the LH group having an intermediate value. No significant differences were found in any of the milk composition parameters over the three periods. Nonetheless, milk protein was found to increase in animals changing to the high protein diet later in the lactation (from periods 2 and 3).

Figure 3.3: Milk protein content - LHH

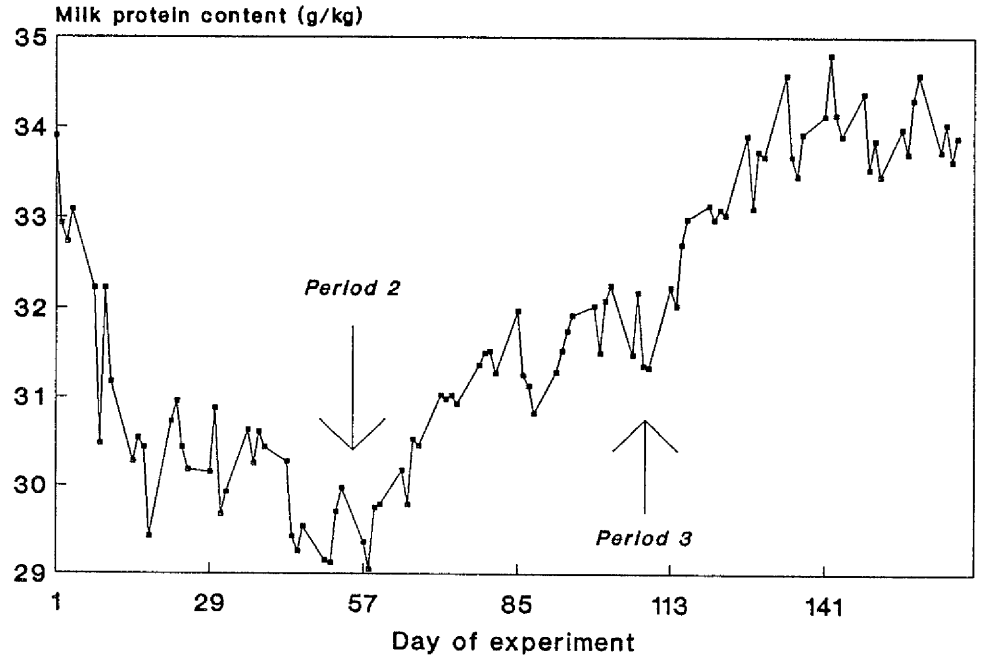
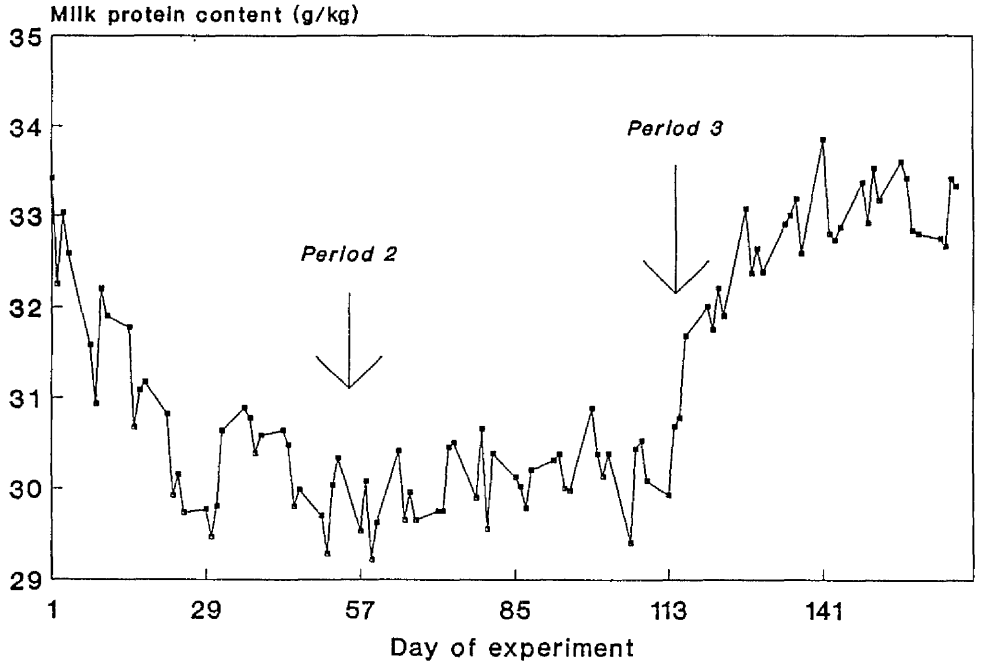


Table 3.9: Milk analysis

	Period 1			Period 2				Period 1+2			
	H	L	SED	HH	LH	LL	SED	HH	LH	LL	SED
Milk yield (kg/d)	27.0	24.6	0.78**	26.5[a]	25.0[ab]	22.5[b]	1.46* 1.68	26.8[a]	25.2[ab]	23.4[b]	1.06* 1.23
Milk protein (g/kg)	31.0	31.2	0.90	30.9	31.4	30.4	1.10 1.23	31.0	31.2	30.8	0.97 1.12
Milk fat (g/kg)	40.2	40.1	1.30	37.8	36.6	36.1	1.70 2.01	39.0	38.6	38.0	1.40 1.61
Milk lactose (g/kg)	48.6	48.5	0.80	49.7	48.9	48.9	0.80 0.94	49.1	48.6	48.8	0.80 0.92

	Period 3					Period 1,2+3				
	HHH	LHH	LLH	LLL	SED	HHH	LHH	LLH	LLL	SED
Milk yield (kg/d)	22.9	22.1	21.6	18.9	1.80	25.5	24.1	22.4	22.2	1.40
Milk protein (g/kg)	32.0	34.1	32.7	31.9	1.30	31.3	32.2	31.3	31.3	1.14
Milk fat (g/kg)	37.9	38.7	35.6	37.8	2.10	38.6	38.6	37.0	38.1	1.66
Milk lactose (g/kg)	47.1	46.8	46.2	47.1	1.00	48.5	48.0	47.6	48.5	0.91

Figure 3.4: Milk protein content - LLH



A gradual improvement in milk protein was evident in all groups over the lactation. Milk protein yield showed a significant difference between groups in period 2 ($t=2.5, P<0.05$) - table 3.10.

Table 3.10: Milk constituent yields

	Period 1			Period 2				Period 1+2			
Yield (kg/d)	H	L	SED	HH	LH	LL	SED	HH	LH	LL	SED
Protein	0.82	0.77	0.03	0.79[a]	0.79[a]	0.69[b]	0.04*	0.80[a]	0.79[ab]	0.73[b]	0.03*
							0.04				0.04
Fat	1.07	0.99	0.06	0.98	0.93	0.81	0.07	1.02	0.99	0.89	0.06
							0.09				0.07
Lactose	1.30	1.19	0.05	1.31	1.22	1.10	0.09	1.30	1.22	1.14	0.07
							0.10				0.08

	Period 3					Period 1,2+3				
Yield (kg/d)	HHH	LHH	LLH	LLL	SED	HHH	LHH	LLH	LLL	SED
Protein	0.70	0.76	0.71	0.61	0.05	0.77	0.79	0.70	0.71	0.04
Fat	0.84	0.87	0.80	0.68	0.07	0.96	0.95	0.86	0.82	0.07
Lactose	1.07	1.03	1.01	0.89	0.10	1.22	1.16	1.08	1.08	0.09

The milk protein, casein and NPN results from the Kjeldahl analysis are given below. The differences in milk protein content from those determined by the Milkoscan can be accounted for by:

1. the samples for the Kjeldahl analysis being taken fortnightly over two milkings whereas samples for the Milkoscan analysis were taken four times per week over eight milkings;

2. the results were not covariance adjusted.

From table 3.11 it can be seen that milk casein was significantly higher in the high protein fed cows in period 1. For the remainder of the experimental period though, no significance was found eventhough a slow increase was observed throughout the lactation in all groups.

The NPN results showed significant responses to dietary protein in all cows offered the high protein concentrate at some stage of the experimental period. From the table, two levels of NPN content were observed: above 2g/kg in high protein fed cows, and around 1.5g/kg in low protein fed cows. Animals in the LHH and LLH groups tended to increase from the low level to the high level when offered the high protein diet. This is clearly seen in figures 3.5-3.8.

Table 3.11: Kjeldahl results for milk protein fractions

Analysis	Period 1			Period 2				Period 1+2			
(g/kg)	H	L	SED	HH	LH	LL	SED	HH	LH	LL	SED
Protein	32.55	30.55	0.99	32.89	32.24	30.77	1.00 1.16	32.72	31.30	30.71	1.00 1.15
Casein	25.35	23.82	0.60*	25.76	25.09	23.97	0.80 0.95	25.55	24.35	23.96	0.70 0.81
NPN	2.03	1.48	0.07***	2.06[a]	1.98[a]	1.57[b]	0.07*** 0.09	2.04[a]	1.68[b]	1.55[b]	0.07*** 0.08

Analysis	Period 3					Period 1,2+3				
(g/kg)	HHH	LHH	LLH	LLL	SED	HHH	LHH	LLH	LLL	SED
Protein	35.17	34.82	34.36	32.06	1.38	33.54	32.48	31.74	31.37	1.19
Casein	27.54	27.25	26.40	24.83	1.18	26.22	25.31	24.60	24.44	0.90
NPN	2.15[a]	2.06[a]	2.05[a]	1.77[b]	0.09**	2.08[a]	1.81[b]	1.68[b]	1.67[b]	0.08***

Figure 3.5: NPN and casein contents - HHH

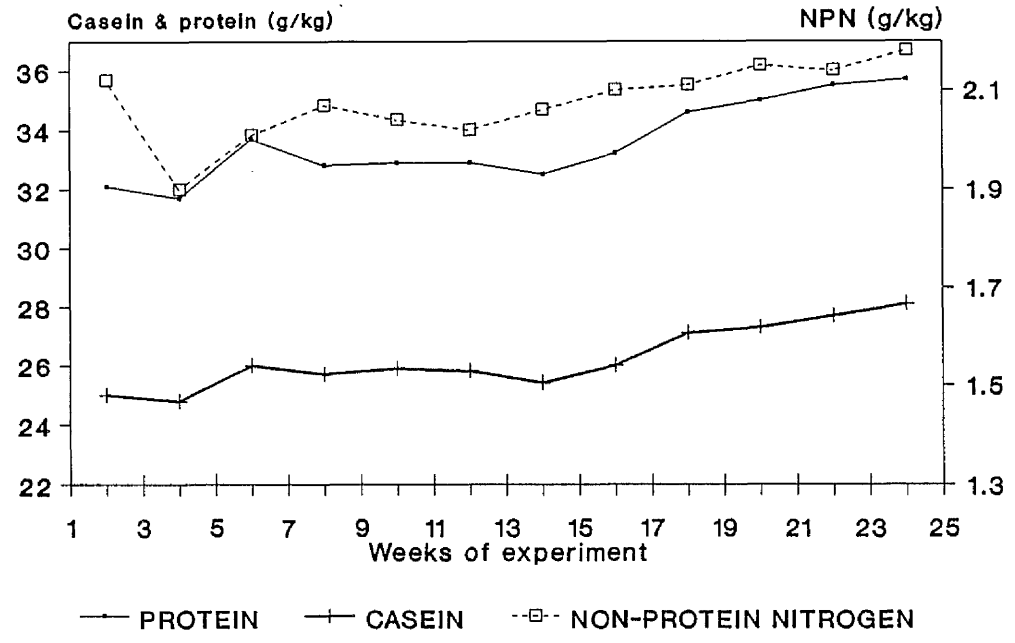


Figure 3.6: NPN and casein contents - LHH

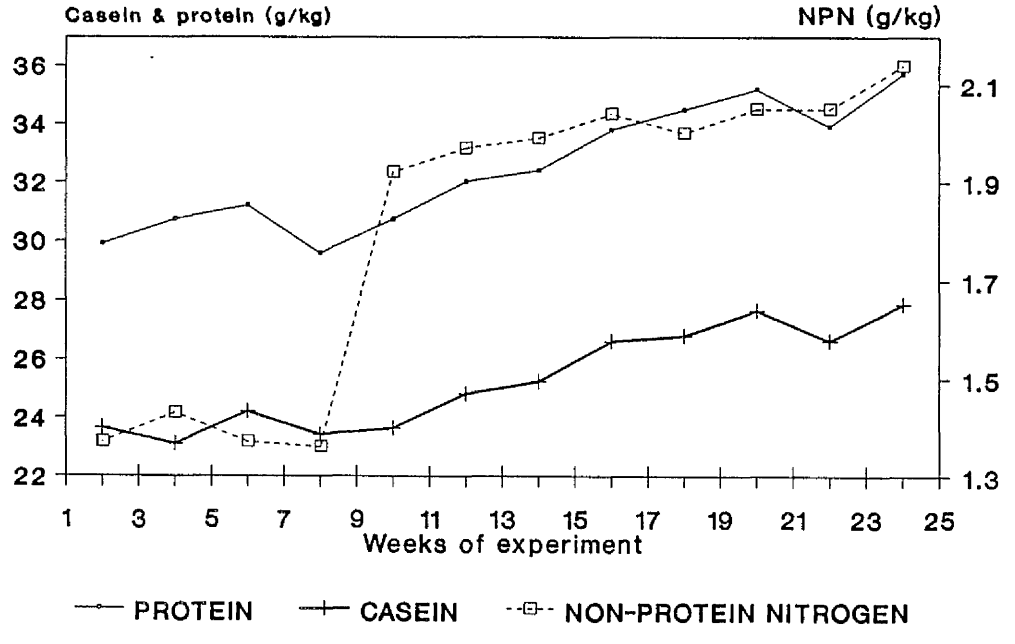


Figure 3.7: NPN and casein contents - LLH

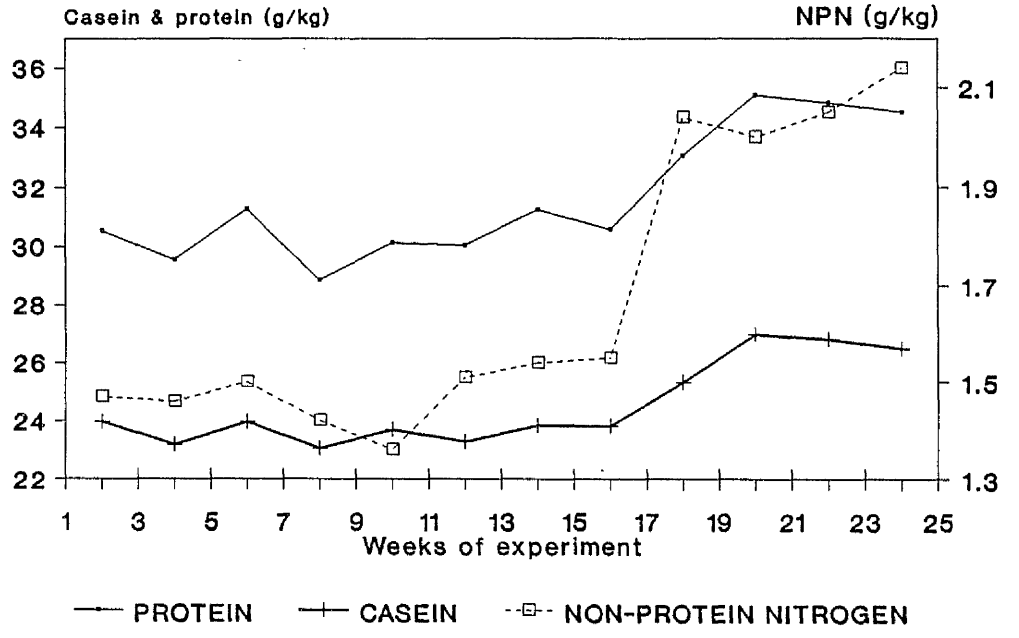
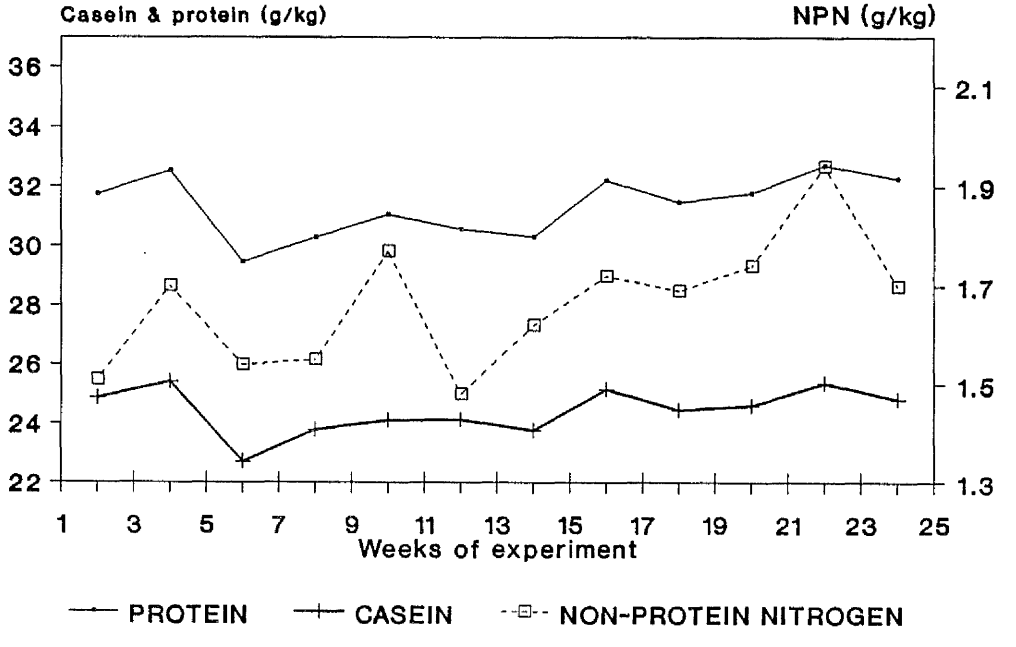


Figure 3.8: NPN and casein contents - LLL



Figures 3.6 and 3.7 show an increase in NPN of 0.6g/kg and 0.5g/kg respectively. The milk protein and casein results indicate slow responses to dietary protein change, whereas the NPN shows an immediate response. Therefore, if milk casein and NPN are increasing it follows that there must be a corresponding decrease in the whey proteins. Milk protein, casein and NPN values are known, so the whey protein % can be calculated by difference. Table 3.12 shows the percentage composition of the milk protein from the Kjeldahl analysis:

Table 3.12: Percentage composition of milk protein

	Period 1		Period 2			Period 3			
Composition	H	L	HH	LH	LL	HHH	LHH	LLH	LLL
Casein	77.9	78.0	78.3	77.8	77.9	78.3	78.3	76.8	77.0
NPN	6.2	4.8	6.3	6.1	5.1	6.1	5.9	6.0	5.5
Whey proteins	15.9	17.2	15.4	16.1	17.0	15.6	15.8	17.2	17.5

From table 3.12 it is evident that NPN in the high protein fed groups has increased at the expense of the whey proteins in all periods, with the exception of the LLH cows in period 3 where milk casein was depressed.

DISCUSSION

The experiment was carried out under commercial, but strictly controlled conditions. The pattern of response in milk production and feed intake to the diets was in close agreement with results from previous work (Thomas 1983; Castle and Watson 1984; Reeve et al 1986; Casper and Schingoethe 1989).

The use of data recorded in weeks 1 and 2 of lactation as independent covariates was justified by previous published work where observed improvements in milk production through nutrition and experimental design did not occur consistently. This may have allowed inherent differences in milk yield and milk composition between animals to be expressed as treatment effects. The covariance adjusted results therefore, take into account any within cow or between cow differences occurring as a result of genetic or previous treatment effects.

Much research has been carried out on silage dry matter intake in relation to supplementation with concentrates, of varying amounts and crude protein contents (Vadiveloo and Holmes 1979; Castle 1982; Thomas 1987). In general, the inclusion of a concentrate supplement in the diet leads to a depression in silage intake, termed the 'substitution rate'. All groups in this trial were flat rate fed 8kg (freshweight) of concentrates per day which eliminated any changes in silage dry matter intake, susceptible to a step rate feeding system. Therefore any differences in dietary intake were attributed to the crude protein content of the concentrates.

Castle (1982) found that the increase in the protein content of a supplement in the diet led to a reduction in substitution rate and

subsequent research by Reeve et al (1986) also indicated a drop in substitution rate when the crude protein content of the concentrate was increased. This effect was apparent in this experiment: the substitution rate, calculated at the simplest level from equation 14.3 (Thomas 1987), where -

$$\text{Substitution rate} = \frac{(1 - \text{concentrate digestibility})}{(1 - \text{forage digestibility})}$$

showed a value of 0.59 in the high protein diet compared with 0.63 in the low protein diet. This suggests that the crude protein in the diet had a marked effect on silage DM intake which is discernable in the feed intake results. Table 3.6 shows that cows offered the high protein concentrates had silage dry matter intakes significantly higher than the low protein fed cows. The mode of action which caused the decline in substitution rate is unclear, however, Beever and Gill (1987) suggested ruminal and metabolic effects associated with the decrease in protein supply from the silage. Results up to 18 weeks (periods 1+2) of lactation and over the total experimental period showed a progressive decline in silage dry matter intake from the HHH group to the LLL group in silage DM intake.

TDMI among treatments corresponded closely with the results obtained for silage DM intake. Nevertheless, no significance was found between the H and L cows in period 1, which is attributed to changes in concentrate intake. The concentrate intake in period 1 was marginally higher in the low protein cows. Large variations in concentrate intake were found in periods 2 and 3 with high intakes

being associated with high CP fed cows. TDMI as a percentage of liveweight was not significantly different between groups and was in close agreement with reviewed work mentioned in chapter 2.

The treatments had small and non-significant effects on liveweight and condition score change. Cows offered the high protein concentrate in period 1 achieved energy balance within the first 10 weeks of lactation although all groups showed a mean liveweight gain over the experimental period (table 3.8). This corresponded to the energy excess in the dietary intake when the ME for milk production and maintenance had been subtracted (MAFF 1975).

The relationship between CP in the diet and energy intake relative to milk production has been well documented (Gordon and Forbes 1970; Tamminga 1982; Castle and Watson 1984; Thysen et al 1988). Lactational responses to the increased dietary protein showed that milk yield was significantly higher in the high protein fed cows up to 18 weeks of lactation. The LH group had intermediate values between the HH and LL groups but was found to increase slightly from the 24.6kg/d in period 1 to 25kg/d in period 2. A precise comparison between milk yield and energy balance (figure 3.2) demonstrated that milk yield had a positive correlation with ME intake. Cows with high milk yields tended to have energy intakes lower than required and three animals with mean yields above 30kg/d were found to be in overall energy deficit during the experimental period suggesting that they were milking 'off their backs'. In relation to the treatment effects on milk yield therefore, feeding a high protein concentrate from week 3 of lactation has significant effect up to week 18 but no effect other than to

marginally increase the yield when fed from weeks 11 or 19 of lactation.

The milk constituents fat and lactose showed no significant effects in either content or yield. Milk fat yield declined progressively during the lactation with the highest values being found in the high protein fed cows. The lactose yield was shown to slightly improve in cows offered the high protein diet from period 2, however, all groups showed a sharp fall in period 3 (table 3.10).

Efficient bacterial protein synthesis is dependent upon an adequate supply of dietary energy and rumen degradable protein (RDP). However, increasing dietary protein results in losses of ammonia from the rumen which puts a burden on the liver through increased detoxification. To prevent this in the treatment diets, a low degradable animal protein was used in the concentrates (table 3.4). The dry matter and nitrogen degradabilities of the formulated pellets are given below:

Table 3.13: DM (g/gDM) and N (g/gN) degradabilities after incubation in the rumen

	DM degradability		N degradability	
Hours	Low protein	High protein	Low protein	High protein
0	0.537	0.542	*	0.419
2	0.525	0.521	0.341	0.391
4	0.626	0.656	0.464	0.553
8	0.713	0.720	0.616	0.625
18	0.814	0.814	0.807	0.773
24	0.876	0.859	0.912	0.795
48	0.898	0.901	0.936	0.901

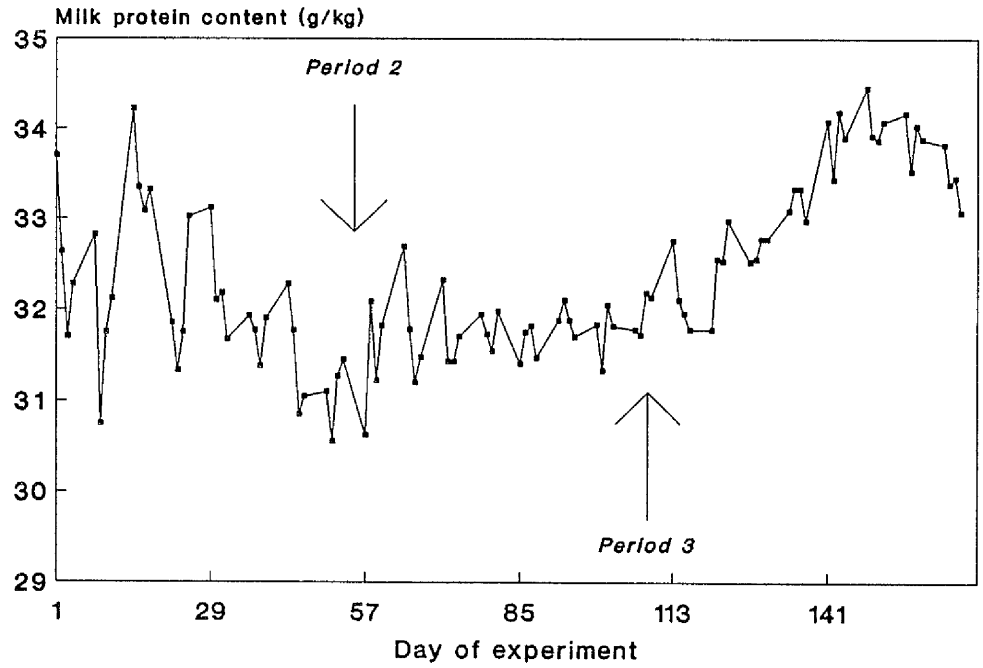
[The nitrogen degradability at 18 hours suggested that a much larger than anticipated proportion of the additional N from the high protein concentrate was in the form of RDP, (pers. comm. RJ Dewhurst).]

The effect of dietary protein on milk protein secretion is attributed to the increased supply of amino acids to the small intestine, which is mediated through the endocrine responses. The significant improvement in milk yield and milk protein yield (tables 3.9 and 3.10) up to 18 weeks of lactation is characteristic of animals that have been infused intra-abomasally with crude protein fractions, particulary casein (Schwab et al 1976; Mepham 1982). The subsequent increases found in milk protein content after infusion have been related mainly to the improved amino acid supply and not to increases in NPN content. The treatment diets had no significant effect on milk protein content although improvements were evident in cows fed high protein concentrates from periods 2 and 3. Over the total experimental period (table 3.9) the HHH, LHH, and LLL groups all showed mean milk protein content of 31.3g/kg. The LHH group however, showed an improvement of approximately 1 g/kg over the other groups during the total experimental period. The greatest effect on milk protein was seen in period 3 when the LHH group showed an increase of 2.7g/kg and the LLH an increase of 2.3g/kg from period 2 values. Nevertheless it should be borne in mind when interpreting period 3 milk protein changes that the standard milk protein curve (Rook and Campling 1965) does begin to steadily improve from a mid-lactation plane, which occurs during this period, and hence results could be confounded by endocrine effects and not dietary ones (although diet may have some influence over hormone secretion - Bassett 1978; Trenkle 1978). Postpartum protein levels are

high in colostrum, but these gradually fall to a minimum in the 6th or 7th week. Figures 3.3 and 3.4 showed deviations from the normal curve in weeks 11 and 18 respectively when cows were offered high protein concentr Figure 3.9 for the HHH group shows that the milk protein curve was maintained at postcalving levels with only a small decrease in milk protein content occurring in early to mid lactation.

In general, results obtained for milk protein yield conform to those of milk protein content, although yield in the high protein fed cows in period 2 was significantly different from the low protein animals. This is reflective of the milk yield results in period 2. The LHH group showed consistently high protein yields eventhough they were statistically non-significant from the other groups over the total experimental period.

Figure 3.9: Milk protein content - HHH

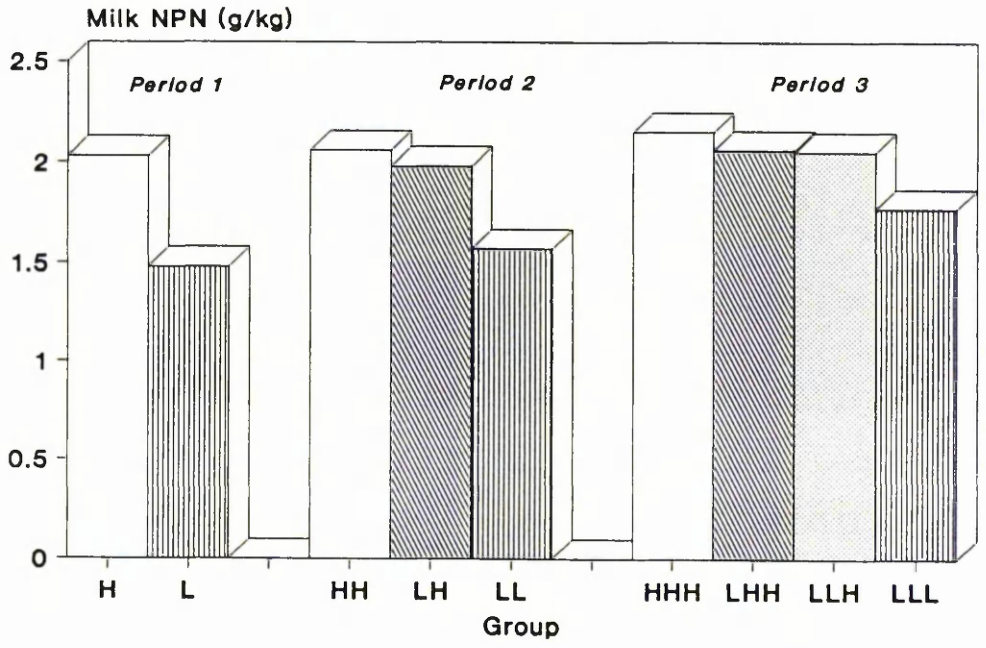


Milk casein is synthesised in the mammary gland and the term 'casein' denotes the group of milk-specific proteins characterised by ester bound phosphates, high proline contents, few cysteine residues and low solubility at pH 4 to 5. Milk casein content improved slowly in all groups during the lactation and was found to be significantly higher in the high protein fed cows in period 1. The improvements in milk casein content are reflective of the changes in milk protein content since milk protein is composed of 78% casein (the remainder being made up of 17% whey proteins and 5% NPN). The percentage composition of the milk protein (table 3.12) shows that milk casein was maintained in all groups at the expected level. The slight drop shown in the LLH group in period 3 is unexpected, although Thomas (1983) stated that diet had a greater effect on mammary synthesised proteins (i.e. casein) than on bovine serum albumin (whey proteins). From table 3.12 however, it is evident that the above effect was not apparent in this work: the whey proteins were consistently depressed in favour of the NPN fractions in response to dietary CP.

The belief that improvements in milk protein content after infusion with CP are related mainly to amino acid supply and not to NPN was not evident in this work. The serum proteins and NPN fraction of milk protein are derived from the blood. Urea diffuses freely across the ducts and tubules of the mammary gland, thus giving a correlation between blood and milk urea (Thomas 1980). The NPN content of the milk is determined by events that influence plasma urea concentrations. Increased dietary protein intake ultimately leads to improved urea production through catabolism of dietary protein to ammonia by deamination of amino acids. Consequently, milk NPN is related to

dietary protein intake:

Figure 3.10: Relationship between dietary protein and milk NPN



The immediate response of the milk NPN to increased CP intake is consistent with previous work. Rook and Line (1962) concluded that additional dietary protein had no effect on milk yield or composition other than to cause an increase in milk NPN. No effect on milk fat, lactose and overall yield was evident and as far as milk protein composition was concerned, evidence from this work indicates that high CP diets mainly affect the minor protein components. Thomas (1983) suggested that most effects on protein content occur when the supplements are of low rumen degradability.

The conclusions of this work correspond with those of Castle and Watson (1984) who stated that with a high digestibility, well preserved

grass silage of satisfactory protein content, the inclusion of a low degradability protein in the supplement has no beneficial effects on milk production.

Experiments 1 and 2 have investigated dietary effects and dietary crude protein effects respectively. However, no specific energy source (i.e. starch or sugar) was used in experiment 1 and no detailed work on protein interaction was carried out in experiment 2. Experiment 3 therefore examines both these aspects and their combined effect on animal performance.

CHAPTER 4

DIETARY INTERACTION OF ENERGY SOURCE AND CRUDE PROTEIN CONTENT: LACTATION AND INTAKE EFFECTS

INTRODUCTION

A result of housing dairy cows over winter periods is that farmers face high feeding costs when supplementing silage with concentrates. The situation is not improved if the silage is of poor quality since this increases refusals and may lead to the deterioration of the animal's health through insufficient energy intake. From previous experiments, a basic maintenance diet of mainly roughage is essential for prepartum cows, which makes the availability of a good quality silage all the more important. Consequently, any supplement which improves palatability* of poor quality roughages will increase silage dry matter intake, and ultimately save on winter costs.

Evidence from trials points to an improvement in lactic acid fermentation in silage when molasses is either applied to the grass or used as an additive in the clamp. However, many factors including weather affect silage fermentation and as a result, in many cases supplementation of expected and unexpected poor quality silage with concentrates is currently the obvious conclusion, eventhough a further option may be to improve silage palatability. Molaferm 20 (United

[Note: palatability* in this work is used in the context of improved flavouring. Animals on the sugar diets were found to quickly consume any silage covered in molaferm, before approaching the remaining forage.]

Molasses) is a blend of cane molasses and condensed molasses solubles. Research into improving the palatability of silage with molaferm 20 as opposed to plain molasses is limited, however, much work has been carried out on sugar beet pulp supplementation (molassed or unmolassed), and starch substitution in the diet (Castle et al 1981; Mayne and Gordon 1984; Thomas et al 1986; Huhtanen 1988). The advantages of feeding molasses as a supplement are well documented (Owen et al 1967; Hemingway et al 1986; Thomas and Rae 1988; Morales et al 1989):

1. it increases the palatability of poor quality roughages
2. it improves microbial efficiency in the rumen
3. it reduces the dustiness of a feed
4. it provides high digestible energy.

Including a starch concentrate in the diet has led to slight improvements in microbial efficiency, eventhough barley supplements tend to increase intraruminal recycling of nitrogen (Chamberlain et al 1985). A sugar supplement however has no such effects on ruminal nitrogen but shows increases in microbial nitrogen utilisation, particulary if the diet includes a mineral buffer (Newbold et al 1987; Thomas and Rae 1988). These conclusions however were not confirmed by Mayne (1989) who found no evidence of improved bacterial protein synthesis in dairy cows offered grass silage-based diets, even when sodium bicarbonate was included in the supplement along with molasses.

In this work Molaferm 20 was used as a true supplement and not as a simple silage replacement in that it was poured onto the silage to improve palatability (eventhough the silage used was of good quality -

table 4.3). Previous work has shown that feeding low levels of molasses (1.6kg DM) has little effect on microbial efficiency (Thomas and Rae 1988), so in this experiment starch diets were compared with sugar diets which contained 2.9kg DM of molaferm. The treatments were formulated to two crude protein levels and the effect of substituting the starch with sugar in the supplement was investigated in terms of subsequent animal performance.

MATERIALS AND METHODS

[For detailed explanations on materials and methods used, see chapter 2.]

1. Experimental

1.1 Animals and their management

18 British Holstein/Friesian dairy cattle were used in this experiment, all of which were second parity or more. The animals were Autumn calvers calving between September and November 1989 with a mean number of days calved of 77 days (SD=30.2). Mean cow weights during the experimental periods were 578 (SED=30.93,*), 589 (SED=32.07,NS) and 589 (SED=32.28,NS) for periods 1, 2 and 3 respectively. Mean milk yield, calculated on a 305 day estimate for the experimental lactation was 6751kg (SED=206).

The animals began their experimental period at least 6 weeks postpartum and remained in the trial for 12 weeks.

1.2 Experimental design and diets

A 3x4 week changeover design, based on Latin squares, the experiment lasted from January to April 1990. Although no specific covariance period was designated, the animals were fed a standard diet of silage *ad libitum* and parlour concentrates for the 2 weeks prior to the start of the experiment. Cows were allocated to 3 blocks on the basis of current milk yield, liveweight and lactation number. From these they were randomly assigned to 6 treatment groups:

Table 4.1: Group treatments

Group	1	2	3	4	5	6
Feed:	Starch	Sugar	Starch/ Sugar	Starch	Sugar	Starch/ Sugar
Symbol:	St	S	S/S	St	S	S/S
CP content (g/kg) of concentrates offered in:						
Period 1:	160	160	160	240	240	240
Period 2:	240	240	240	160	160	160
Period 3:	160	160	160	240	240	240

The formulated treatment concentrates are outlined in table 4.2:

Table 4.2: Formulated treatment concentrates

Contents (g/kg)	Treatment concentrates St16	S16	S/S16	St24	S24	S/S24
Barley	854	240	597	648	130	389
Molaferm 20	-	477	239	-	475	238
Soya	117	152	135	324	365	345
Minerals	29	31	30	28	31	30
ME (MJ/kg)	12.6	12.8	12.7	12.8	12.5	12.6
CP (g/kg)	161	160	161	240	241	241

All detailed recordings, with the exception of liveweight, were taken during the last week of each experimental period. Silage dry matter intakes were recorded on 4 consecutive days per recording week with fresh silage feeds being adjusted to ensure a 5-10% refusal daily. Concentrates were fed twice daily in concentrate trays, and animals on the sugar and mixed diets received molaferm once a day, poured onto the fresh silage after the morning concentrate feed. Milk and additional 100ml samples were taken on 4 and 2 consecutive days per recording week respectively, with the 100ml samples being bulked over 3 to 4 recordings for Kjeldahl analysis. Liveweights were recorded 3 days each week after the afternoon milking.

2. Diet components

2.1 Silage

The silage used was 1st cut perennial ryegrass with sulphuric acid added at 3.5l per tonne. Grab samples of fresh silage and refusals were taken daily for oven dry matter analysis, and additional samples were bulked during each recording week for *in vitro* analysis:

Table 4.3: *In vitro* analysis of silage

Analysis	Silage
Dry matter (g/kg)	184
Dry matter analysis:	
Crude protein (g/kg)	155
OM (g/kg)	929
D Val (g/kg)	714
ME (MJ/kg)	11.4
pH	3.8
Ammonia (N g/kg)	101
Calcium (g/kg)	5.6
Phosphorus (g/kg)	3.6
Magnesium (g/kg)	2.1

2.2 Concentrates

The animals were offered concentrates that were bought in the form normally found commercially, and mixed into required treatments for storage in 1000kg Polybins.

No separate mixes were formulated for the S/S16 and S/S24 diets: concentrates were used from the 4 formulated mixes (table 4.5) at a 60% substitution of starch with sugar. The 4 concentrate mixes were formulated in advance to 160g/kg DM and 240g/kg DM CP (table 4.2) on the basis of previously analysed feed samples. However, analysis of samples taken during the experiment (table 4.5) revealed that the observed CP contents were more variable than expected. Nevertheless, when the amount of concentrate offered from the different mixes is taken into account, it can be seen that the low protein and high protein diets were comparable:

Table 4.4: Crude protein content of concentrates offered

Diet	DM offered (kg)		CP content (g/kg)		CP Content	
	Mix	Molaferm	Mix	Molaferm	(kg)	(g/kg)
St16	6	-	144	-	0.86	143
S16	3.1	2.9	179	96	0.83	138
St24	6	-	210	-	1.26	210
S24	3.1	2.9	305	96	1.23	205

Samples of the mixes were bulked during the recording weeks and analysed monthly using the NCD method:

Table 4.5: Analysis of concentrate mixes and molaferm 20

	Concentrate mixes				
Analysis	St16	S16	St24	S24	Molaferm
Dry matter (g/kg)	864	861	846	866	771
Dry matter analysis:					
Crude protein (g/kg)	144	179	210	305	96
OM (g/kg)	929	902	926	899	836
ME (MJ/kg)	12.5	12.4	12.7	12.6	11.9
AHEE (g/kg)	27.9	28.5	29.3	32.1	7.0
NCD (g/kg)	866	826	858	848	831
Calcium (g/kg)	9.7	9.1	9.4	12.5	11.1
Phosphorus (g/kg)	7.3	9.5	7.3	8.8	0.5
Magnesium (g/kg)	3.8	5.0	4.0	5.0	5.0

3. Laboratory analysis of samples

[The detailed laboratory analysis of samples described in chapter 2:3 is applicable to samples taken from this experiment.]

3.1 Concentrate analysis: molaferm 20

The dry matter of the molaferm was determined by freeze drying the sample for 7 days at 10^{o}C - all subsequent analyses were corrected to a freeze dried basis. The minerals and crude protein contents of the molaferm samples were calculated using the methods outlined for the silage analysis (chapter 2:3.3.1). ME, NCD, AHEE and OM were estimated by the techniques described for the concentrate analysis (chapter 2:3.3.2).

4. Statistical analysis

The majority of the analysis of the data recorded on this trial was analysed using Genstat V, at the Scottish Agricultural Statistical Services, Edinburgh. Treatment effects were assumed to be constant over

the three periods with no carry over effect occurring from one treatment to another. Detailed work was also carried out on Genstat IV and to a lesser extent Minitab, and statistical significance is shown as described in chapter 2:4.

RESULTS

No incidences of lameness or mastitis were recorded during the experimental period, with the exception of one fourth lactation cow which was lame from the start of the experiment. A decline in milk yield and dry matter intake in another animal resulted in all data recorded for that animal in period 3 being omitted from the analysis. Bovine spongiform encephalopathy (BSE) was diagnosed after culling.

Statistical analysis of the changeover data was carried out on the assumption that there was a constancy during periods in the animals, with no carry over effects occurring from previous treatments. A small portion of the analysis examined 'between' cow effects in relation to feed, i.e. sugar, starch or mixed. The majority of the information obtained however, was specifically for 'within' cow effects after adjustments had been made for the 3 periods. The 'within' cow results examine the protein factor effects (160g/kg CP and 240g/kg CP), and the treatment diet effects (protein factor and feed - St16,S16,St24 etc.). An experiment of this design allows precise work to be carried out on small numbers of cows, and additionally in this experiment enables the interaction between dietary crude protein and energy source to be examined.

Feed intake results are shown in table 4.6:

Table 4.6: Dry matter intake

Daily Intake (kg)	Treatment diets							Feed				Protein factor (g/kg)		
	St16	S16	S/S16	St24	S24	S/S24	SED	Starch	Sugar	Mixed	SED	160	240	SED
Silage	8.39	8.69	8.58	9.05	9.13	8.67	0.841	8.72	8.89	8.63	0.798 0.378	8.54	8.95	0.218
TDMI	14.45	14.81	14.83	14.91	15.34	14.88	0.838	14.68	15.07	14.89	0.795 0.377	14.70	15.04	0.218
ME (MJ)	176.2	170.0	172.4	177.7	177.1	177.3	7.49	177.0	173.6	174.9	5.29	172.9	177.4	4.32
CP	2.24	2.15	2.19	2.64	2.63	2.65	0.102	2.44	2.39	2.42	0.072	2.19	2.64	0.059***
TDMI as % of lwt	2.58	2.43	2.73	2.48	2.46	2.68	0.125	2.53^{ab}	2.44^{a}	2.71^{b}	0.089*	2.58	2.54	0.072

Note: 1. the 2nd SED value in the feed analysis refers to the SED calculated when comparing means of different energy sources at the same crude protein level
2. means with the same superscript in the same line do not differ significantly from each other ($P<0.05$).

The two weeks prior to the start of the experiment were used mainly to retrain animals to specific Calan gates, therefore feed intake covariance data was not available.

The treatment diets had no significant effect on either silage or total dry matter intakes. A correlation was found between silage dry matter intake and crude protein intake with $r=0.749, P<0.01$ indicating that the two factors were positively related.

An inevitable difficulty when analysing liveweight change is the errors that arise during the actual weighing of the cows. In this experiment liveweight change examines minor changes in weight over a 4 week period and, time of weighing or last milking and physiological state of the cow all contribute to weight change.

Animals were weighed 3 times per week after the afternoon milking. However, variations in time of weighing occurred and where possible anomalies have been omitted from the analysis. The weights recorded were analysed by calculating the liveweight regression for each cow for each period, and from this estimating a rate of weight change. Analysis of variance was carried out on the rate of weight change to give mean values for each treatment diet, feed and protein factor.

Liveweight change was also calculated by the equations given in the ME balance calculations (MAFF 1975). These take into account the energy intake from the diet, energy required for milk production in the specific cow, and energy required for basic maintenance.

The results for liveweight change by regression and by ME balance (MAFF 1975) are shown in table 4.7:

Table 4.7: Liveweight change

Liveweight change	Treatment diets							Feed				Protein factor (g/kg)		
	St16	S16	S/S16	St24	S24	S/S24	SED	Starch	Sugar	Mixed	SED	160	240	SED
By regression on cow weight:														
(kg/day)	-0.03	0.63	0.31	0.69	0.84	0.70	0.277	0.33	0.74	0.51	0.217 0.245	0.30	0.74	0.141**
ME balance:														
Energy (MJ/day) (MAFF 1975)	1.00	8.60	11.50	-0.50	5.90	6.80	8.09	0.20	7.30	9.10	5.72	7.0	4.0	4.67
Lwt change (kg/day) (MAFF 1975)	0.04	0.26	0.34	-0.08	0.16	0.19	0.274	-0.02	0.21	0.27	0.194	0.21	0.09	0.158
Lwt change (kg/day) ARC 1980:	0.16	0.67	0.44	0.04	0.41	0.30								

From table 4.7 it is evident that there was no direct relation between liveweight change by regression and by ME balance. The difficulties in weighing cows mentioned above, outweigh the value that can be placed on the results and consequently the ME balance calculated using a number of recorded factors is probably the more accurate result. From these results it can be seen that cows offered the St24 treatment diet were in overall energy deficit during the experiment. Three high yielding animals were offered this diet and were calculated to be still in overall negative energy balance in period 3 of the experiment.

The sugar and mixed feeds showed positive effects on ME balance, and figure 4.1 shows that cows on the two feeds were in energy excess for the majority of the experimental period:

Figure 4.1: ME balance for the three feeds

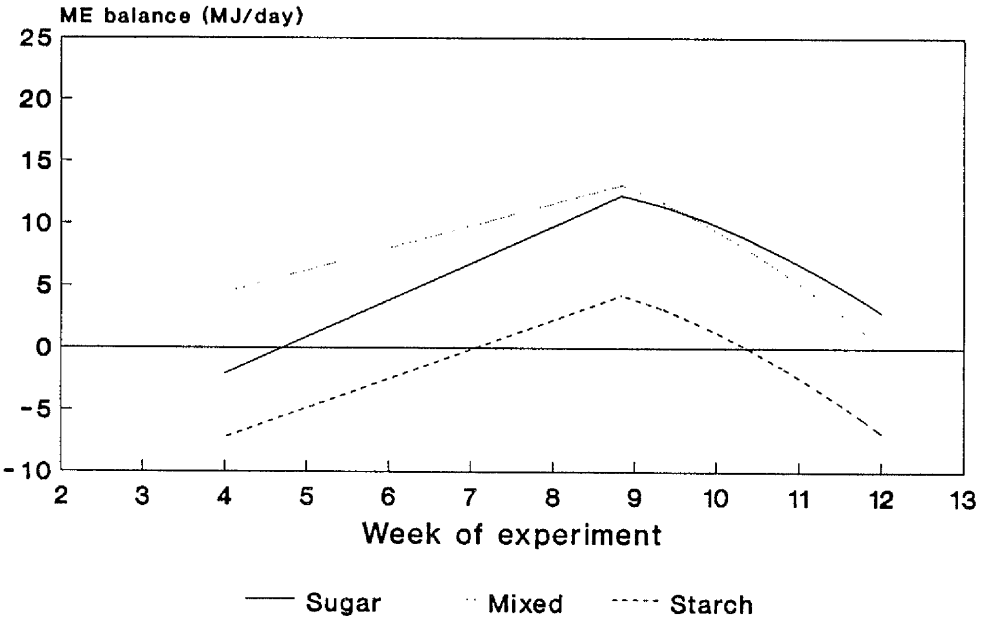


Table 4.8 shows the results obtained for the milk analysis. The milk yields and constituent contents recorded in the two weeks prior to the start of the experiment were used as independent covariates in the adjustment of mean treatment milk outputs by covariance analysis. The covariance coefficient was satisfactory in all treatments with the exception of the milk fat. A value of 0.60 and 0.64 was estimated for milk fat content and milk fat yield respectively. The low value may be atttributed to errors incurred through sampling or analysis techniques.

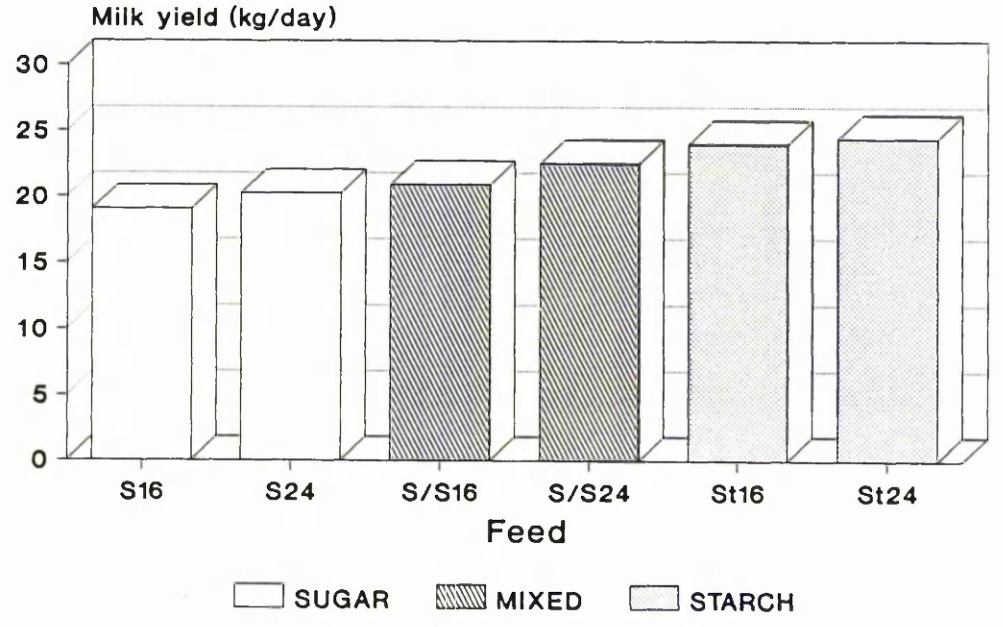
However, discussion of these results is made bearing the low covariance coefficient in mind.

It is apparent from table 4.8 that the treatment diets had no significant effect on milk yield and milk constituents. Nonetheless, the starch diets showed higher values in yield and milk constituents with the exception of the milk fat which was depressed in both starch treatments: figure 4.2.

Table 4.8: Milk analysis

Analysis (kg/d)	Treatment diets							Feed				Protein factor (g/kg)		
	St16	S16	S/S16	St24	S24	S/S24	SED	Starch	Sugar	Mixed	SED	160	240	SED
Milk yield	24.0	19.0	20.9	24.4	21.4	22.5	1.44	24.2	20.2	21.7	1.44 0.60	21.3	22.8	0.33***
Milk composition analysis (g/kg):														
Protein	31.9	30.8	30.7	31.6	30.3	31.3	0.71	31.7	30.5	31.0	0.67 0.35	31.1	31.1	0.20
Fat	38.3	40.4	43.0	39.4	41.5	42.4	1.94	38.9	40.9	42.7	1.82 1.22	40.6	41.1	0.68
Lactose	48.8	47.3	47.4	48.6	47.0	47.1	0.73	48.7	47.2	47.3	0.71 0.33	47.8	47.6	0.19

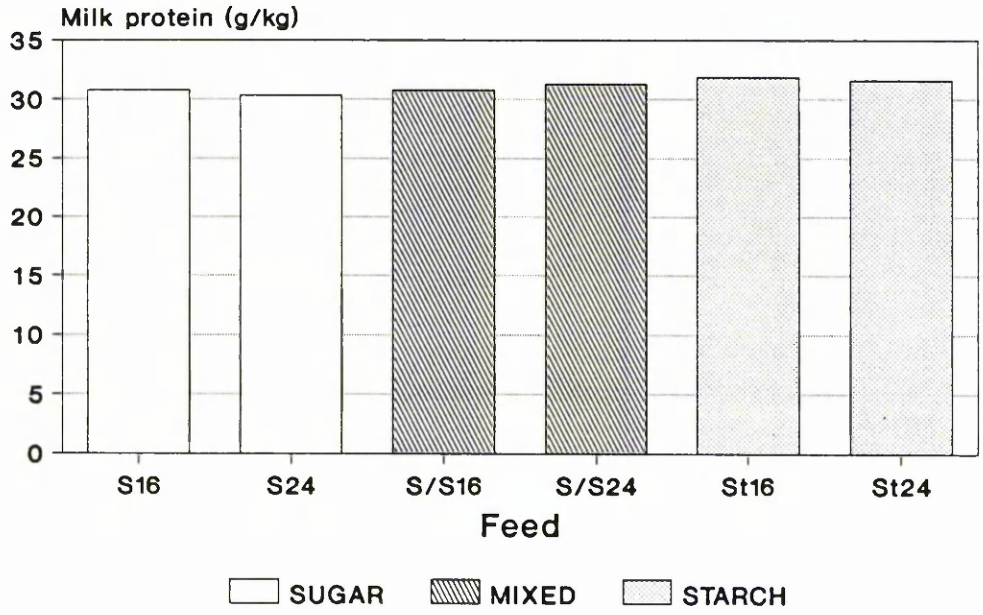
Figure 4.2: Milk yield for the treatment diets



On average, cows given the diets containing 240 g CP/kg recorded milk yields significantly higher than animals offered the lower crude protein diets. Although milk fat and lactose showed no significant effects in the treatment diets and protein factor levels, the calculated feed effect F-ratios calculated for the feed analyses were both approaching significance, indicating that some slight feed effects were occurring.

The sugar fed animals showed lower milk protein contents than the starch fed cows although differences between the contents for each treatment were only marginal:

Figure 4.3: Milk protein contents for the treatment diets



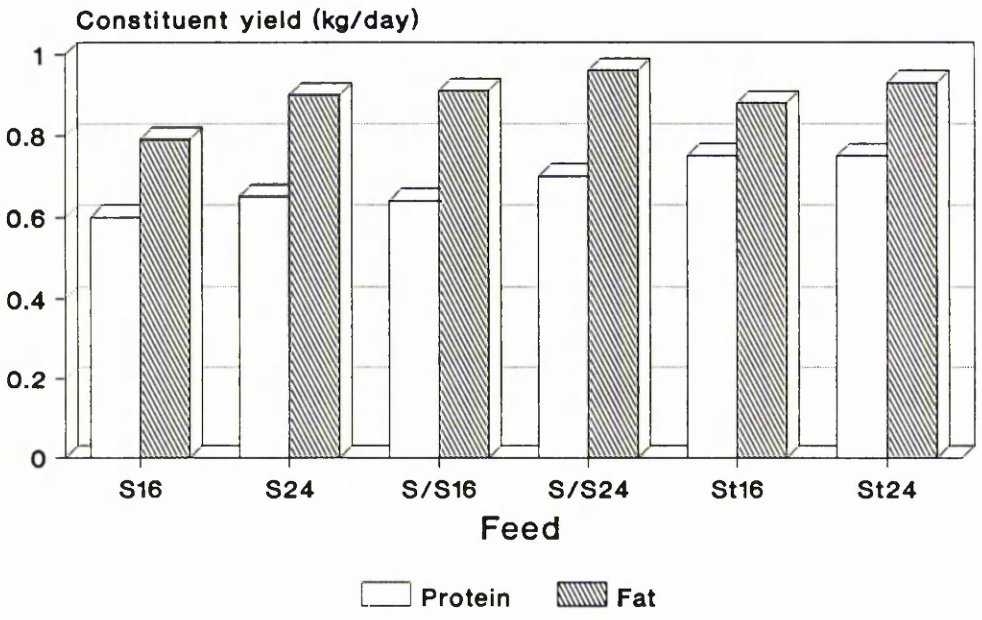
There was no significant effect of the energy source on the yields of milk constituents. However, significant differences were found in the constituent yields between the low protein and high protein diets (table 4.9):

Table 4.9: Milk constituent yields

Analysis (kg/day)	Treatment diets							Feed				Protein factor (g/kg)		
	St16	S16	S/S16	St24	S24	S/S24	SED	Starch	Sugar	Mixed	SED	160	240	SED
Protein	0.75	0.60	0.64	0.75	0.65	0.70	0.047	0.75	0.63	0.67	0.046 0.021	0.66	0.70	0.012**
Fat	0.88	0.79	0.91	0.93	0.90	0.96	0.059	0.90	0.85	0.93	0.055 0.028	0.86	0.93	0.016***
Lactose	1.16	0.91	0.99	1.18	1.01	1.06	0.074	1.17	0.96	1.02	0.073 0.030	1.01	1.08	0.017***

Although not significant, the starch groups showed high milk protein yields while milk fat yield was depressed, as can be seen in figure 4.4:

Figure 4.4: Protein and fat yields for the treatment diets



As a result of no samples being taken for Kjeldahl analysis in the two weeks before the trial commenced, all the results obtained by Kjeldahl analysis are therefore not covariance adjusted. Table 4.10 illustrates the results obtained from the analysis and it can be seen that again milk NPN (in protein equivalent) is significantly higher in high protein fed cows (with the exception of the S24 cows). The S24 treatment fed animals were found to be not significantly different from the low protein fed animals suggesting that the treatment utilised excess nitrogen in the rumen more efficiently than the mixed and starch diets at the same CP level.

Table 4.10: Kjeldahl results for milk protein fractions

Analysis (g/kg)	Treatment diets							Feed				Protein factor (g/kg)		
	St16	S16	S/S16	St24	S24	S/S24	SED	Starch	Sugar	Mixed	SED	160	240	SED
Protein	31.39	29.54	29.89	31.41	30.08	30.33	1.451	31.40	29.81	30.11	1.387 0.604	30.28	30.61	0.349
Casein	24.85	22.81	23.53	24.50	23.21	23.46	1.144	24.67	23.01	23.49	1.084 0.519	23.73	23.72	0.300
NPN (Protein equivalent)	1.43[a]	1.41[a]	1.49[a]	1.69[b]	1.51[a]	1.67[b]	0.057*	1.56	1.46	1.58	0.050 0.040	1.44	1.62	0.023***

Note: results not covariance adjusted

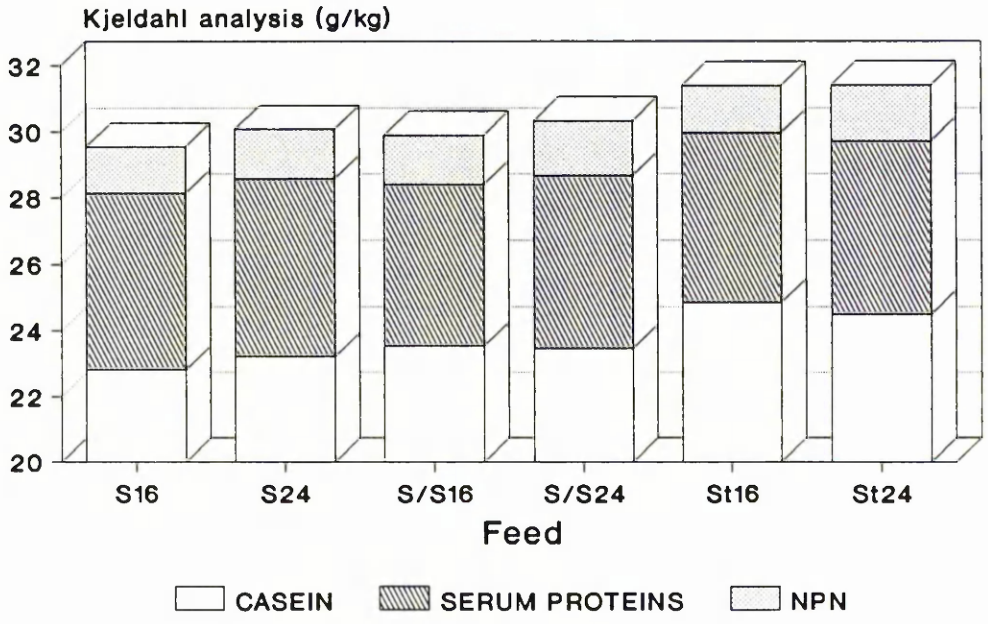
The percentage composition of the milk protein constituents is shown in table 4.11 and illustrated in g/kg in figure 4.5 (the serum or whey proteins have been calculated by difference):

Table 4.11: Percentage composition of milk protein fractions by Kjeldahl analysis

Composition (%)	Treatment diets St16	S16	S/S16	St24	S24	S/S24
Casein	79.0	77.2	78.7	78.0	77.2	77.3
Serum proteins	16.4	18.0	16.3	16.6	17.8	17.2
NPN	4.6	4.8	5.0	5.4	5.0	5.5

	Feeds Sugar	Mixed	Starch	Protein factor (g/kg) 160	240
Casein	77.2	78.0	78.6	78.4	77.5
Serum proteins	17.9	16.8	16.4	16.8	17.2
NPN	4.9	5.2	5.0	4.8	5.3

Figure 4.5: Composition of milk protein fractions (g/kg)



An increase in NPN content as a result of increasing dietary protein was not however observed in the sugar diet (S24). However, milk casein percentage remained at the expected percentage around 78% in all treatments. The effect of the sugar treatment S24 appeared to be a control of urea in the milk by either improved ammonia utilisation in the diet or by repression of NPN by increased whey protein formation.

DISCUSSION

An important aim of an experiment comparing starch and sugar supplements is to ensure that equal amounts of dry matter from the two feeds are consumed so that animal performance is not influenced by unequal intakes of the two energy sources. In this experiment the objective was achieved since concentrate and molaferm refusals were negligible. Consequently, any differences observed in animal production can be attributed either directly or indirectly to feed effects, especially in view of the fact that silage dry matter intake was not significantly different between treatments.

For most results, intermediate values were recorded in the mixed diets.

1. The effect of concentrate energy source

Barley as a concentrate supplement is known to have an adverse effect on silage intake through its influence on the silage 'fibre' digestion (HRI Report 1984). Barley has been found to lower the rate of fibre digestion which slows the passage of digesta through the rumen thereby reducing feed intake through the effect of rumen fill.

Modification of the barley by chemical treatment has shown a slower fermentation rate, and therefore restricted the associated fall in rumen pH. However, further investigations found that this effect was confined to multiparous cows who as a result of being fed the treated barley showed increased silage dry matter intake (HRI Report 1985).

In this experiment there were no effects on feed intake which is contrary to other workers who have shown that concentrate supplements containing sugars have positive effects particularly on dry matter intake. Karalazos and Giouzeljannis (1988) found that costs were lower in their sugar fed cows than their starch supplemented ones because the animals ate more of the forage and thus required less concentrates. The nutritional advantages of feeding a sugar based supplement is that they avoid the build-up of ruminal protozoa and the associated intraruminal recycling of nitrogen that occurs in response to a starchy supplement. In addition they increase microbial fixation of ammonia, provide a source of highly degradable protein and readily available energy from their sucrose and inverted sugar content. The lack of response on feed intake in this experiment could be due to silage quality; the way in which the molaferm was fed; the behaviour of the animals behind the Calan gates.

Total dry matter intake as a percentage of liveweight was significantly higher in the mixed diet compared with the sugar feed. This anomaly is unexpected since mean TDMI and weight change by regression (table 4.7) were both found to be intermediate between the starch and sugar values. The starch fed animals tended to have higher milk yields, and because they had intakes similar to the other diets, were assumed to be utilising body reserves and thus showing a greater

liveweight loss (figure 4.7).

Animals on the sugar diets were found to be in positive energy balance, 7.1MJ/day higher than the starch fed cows, who were additionally showing an overall liveweight loss. This may be due to the greater milk yields found in these animals who were most likely utilising body reserves and thus partitioning metabolisable energy input from the diet into milk production. Although starch fed cows showed yields nearly 4kg higher than the sugar animals surprisingly this difference was not significant, however, examination of the F-value showed it to be approaching significance. Milk yield, milk protein and lactose contents were higher in the starch diets however, a decline was evident in milk fat, as shown in table 4.8. The milk fat depression was surprising since the forage to concentrate ratio in the barley diets was comparable with those of the other diets with a mean of 59% (range 58-61%). This is higher than the 50% value suggested in the literature (reviewed by Sutton 1989) below which milk fat content is depressed. This low fat content was reflected in milk fat yield. From table 4.9 it is evident that although the starch fed cows' yield of 0.90kg/day was higher than the sugar fed cows' fat yield, it was still lower than the mixed diet fed cows' yield, suggesting that the diet depressed yield.

The results from the milk analysis indicate therefore that a decline in milk fat content occurred in the starch based diets, and that no beneficial effects occurred in milk yield or constituents in the sugar based diets. This is in contrast with the work of Broster et al (1970) where sucrose had a small beneficial effect on milk yield,

increased lactose and protein contents, and depressed milk fat. These findings were in agreement with the work of Lofgreen and Otagaki (1960) and Owen et al (1967), where sugars were reported to be used less efficiently for milk production when given as large supplements, and additionally when fed at low levels showed a milk fat depression. Broster et al (1970) suggested that the length and type of experiment was a contributory factor, finding that a milk fat response may occur several weeks after introducing the carbohydrate to the ration, especially in a continuous design experiment of over 10 weeks duration (Bowman and Huber 1967, cited in Broster et al 1970).

The Kjeldahl results showed no significant differences between the energy sources although the starch NPN was found to be the intermediate value and not the expected mixed diet NPN. Sugar supplements in diets containing large amounts of rumen degradable protein, (84% of the CP in the silage used in this work), have shown a reduction in post-prandial concentrations of ammonia in the rumen by providing a readily available energy source to support microbial growth and promote ammonia fixation (HRI Report 1985). The lower than expected NPN value for the starch diet also suggests that the diet suppressed urea formation and that some effect from the high protein starch diet was still occurring on milk NPN formation. [It is interesting to note that the variation found in the previous experiment between protein calculated from the Milkoscan, and that determined by Kjeldahl was not as variable in this work. This was a result of the similar sampling procedure used for the two analyses.]

The barley therefore, despite the associated problems mentioned above, tended to promote higher milk yield and milk protein content and

a depressed milk fat content compared with the other two diets.

2. The response to the two protein levels

The two protein levels in this work were formulated to 160g/kg and 240g/kg CP, the higher concentration being obtained by partial replacement of the energy source with soya bean meal (table 4.2).

The responses to increasing supplementary CP intake in milk yield and silage dry matter intake relative to the energy source and its influence on production are shown in table 4.12:

Table 4.12: Effect of increased CP intake from the supplement on milk yield and silage dry matter intake

Energy Source	Silage DMI increase (kg/day per kg increase in supplementary CP intake)		Milk yield increase	
Barley	+1.84	(+0.17)	+1.20	(+1.87)
Molaferm	+1.01		+5.51	
Mixed	+0.21		+3.87	

[Note: figures in parenthesis from Mayne and Gordon (1984)]

From the above it is evident that the forage intake response to increased supplementary protein was more pronounced in the barley diets than the molaferm ones. Mayne and Gordon (1984) found lower responses in silage intakes than those reported by Castle and Watson (1975, 1976). They attributed this to the high digestibility of the silage used (691g digestible organic matter per kg DM, DOMD), and they further quoted work by Gordon (1980) who observed DM increases which were low

with high digestibility silages and high with low digestibility silages. The findings of this work contrast with those conclusions, in that a high digestibility silage was used (714g DOMD) and high silage dry matter intake increases were recorded. One possible explanation may have been the high concentrate feeding level used in the work of Mayne and Gordon (1984), of 10kg/day while this experiment only used 6kg DM per day of concentrates. Nevertheless the protein levels obviously had considerable effect on silage dry matter intake in the barley based diets.

Gordon and McMurray (1979) investigated the optimum level of protein in the supplement for dairy cows on grass-silage based diets. They defined a relationship between milk yield and protein concentration in the supplement and calculated that a CP level of 250g/kg DM in the concentrate produced maximum profitability. From table 4.12 it is evident that the high protein concentrate had an effect on milk yield in the sugar and mixed diets. An increase of 5.51kg in the molaferm diets was recorded in milk yield per kg increase in supplementary CP intake which is a positive effect from the increased dietary CP intake. This was confirmed to a point in table 4.8 for the milk analysis where the high protein level showed a milk yield of 22.8kg/day compared with 21.3kg/day in the low protein ($P<0.001$). However, these values take into account the starch and mixed diets as well. Nevertheless, this increased response in milk yield to high protein diets is in accordance with the results reported in experiment 2 (chapter 3).

The milk constituent yields (table 4.9) showed significant differences between the two protein levels in all three constituents.

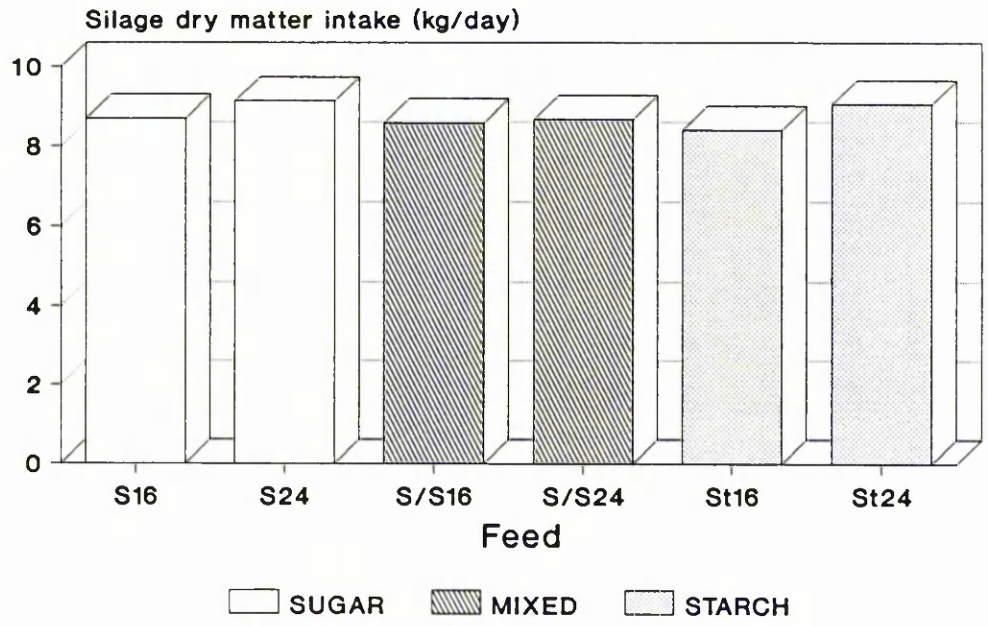
This is relative to the significance found in milk yield although milk protein yield was significant at only the 1% level and not the 0.1% level found in the other two yields. The higher yields were consistently found in the high protein diets.

The Kjeldahl analysis showed identical casein values but NPN was significantly higher in the high protein diets. The level of NPN content was not as high as that found in the 315g CP/kg DM diets used in experiment 2 suggesting that the conclusion of Gordon and McMurray (1979) that 250g CP/kg DM was optimum for maximum profitability was substantially true when payment for true protein in the milk is considered.

3. The response to dietary interaction of energy source and protein level

The effects of energy source and protein level are more clearly seen when examined in the treatment diets results (figure 4.6).

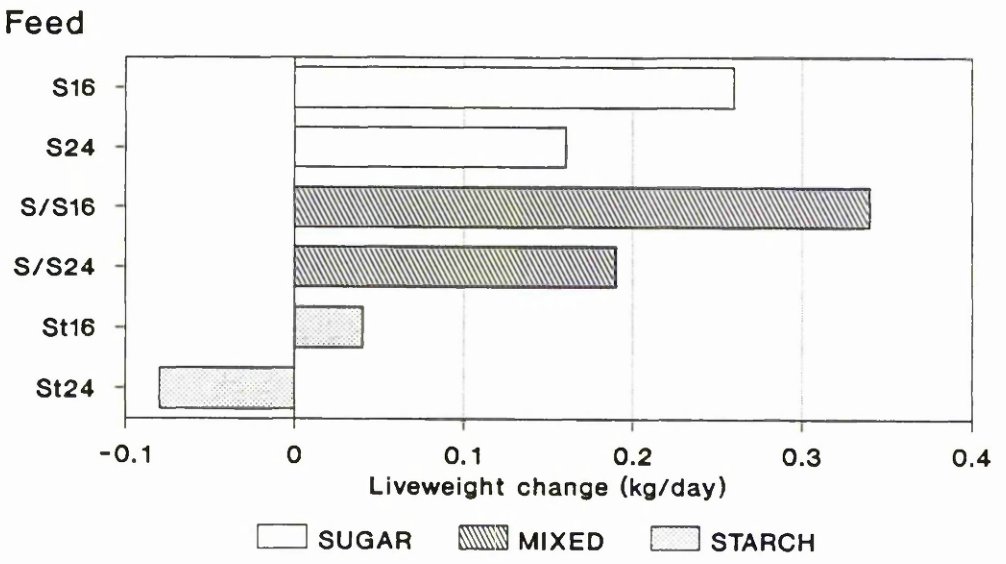
Figure 4.6: Silage dry matter intake for the treatment diets



Intakes were not significantly different between treatments.

From figure 4.7 and table 4.8, it is evident that the starch fed animals (at both protein levels) were partitioning the energy from the diet into milk production, thus producing the higher milk yields while showing an overall liveweight loss.

Figure 4.7: Liveweight change for the treatment diets - (MAFF 1975)



The starch diets showed milk and constituent yields, protein and lactose contents consistently higher than the sugar diets. In addition, within the starch diet, the higher protein treatment yielded 0.5kg of milk per day more than the low protein treatment. However, and in contrast with the findings of experiment 2, the milk protein content was marginally higher in the low protein diet. The interaction between barley energy source and high CP in the diet (St24) effectively depressed milk protein content, which was additionally found in the S24 diet as well. When yield is taken into account (table 4.9) it is obvious that this was compensated for in the starch diets which produced identical yields (0.75kg/day), but not in the sugar diets where the high protein treatment yielded 0.05kg/day more milk protein

than the low protein treatment.

The low milk yield for the S16 diet is unexpected because the effectiveness of the covariate was high and the same cows produced a higher yield while on the S24 diet, thereby eliminating a 'within' cow effect. The low milk fat contents in the St16 and St24 treatments are perhaps a little more difficult to explain. Although previous work has shown that milk fat is depressed in cereal fed cows (Thomas and Rae 1988) a number of factors may have contributed:

1. the effectiveness of the covariate was low - 0.60, possibly due to sampling errors
2. 'within' cow effects, although this is probably unlikely in 6 variable cows.

The related constituent yield results for the St24 treatment (table 4.9) show an improvement in fat yield although this is additionally confounded by its relationship with milk yield. All fat yields were found to be higher in the high protein treatments.

The Kjeldahl results confirm the findings of the Milkoscan in that milk protein content was highest in the starch fed cows. Marginal improvements were evident in the high protein treatments but were not reflected in milk casein results. Percentage composition of the milk protein fractions showed that milk casein was increased from its normal percentage of 78% to 79% in the St16 treatment. Serum proteins were lower in the starch diets than the sugar treatments. Milk NPN was therefore not greatly affected by the high protein concentrate as was anticipated from the results of experiment 2. Nevertheless, the St24 and S/S24 showed NPN results significantly higher than the other

treatments. The NPN value recorded for the S24 which was found to be significantly lower than those for the St24 and S/S24 and may be explained by the improved utilisation of the dietary nitrogen found in sugar diets. One of the factors determining microbial nitrogen production in the rumen is the energy release during fermentation and the rate of ATP generation (Huhtanen 1988). This appears therefore to put non-structural carbohydrates such as molasses in a favourable position because of their fermentation rate (Johnson 1976).

The conclusions of this experiment are that the starch based diets showed a greater reaction in silage dry matter intake to increased dietary crude protein. Liveweight change results calculated by regression on cow weight were unreliable even when measured frequently because of the number of hormonal and nutritional factors affecting weight. However, liveweight change calculations from energy balance equations (MAFF 1975) showed that the starch based diets partitioned dietary energy into milk production through improved milk yields and a liveweight loss. Animals offered a starch based concentrate showed greater performance in milk production. In terms of milk constituents the starch diets in this experiment provided a better quality milk with a high protein level and a low butterfat which suits the demands of the consumer today. However, the St24 diet milk protein contained a significantly higher amount of NPN than the St16 treatment which is of no advantage in industry, particulary if a system of payment for true protein is introduced. The high protein sugar diet showed no such increase in milk NPN thereby making it more acceptable to dairy product manufacturers. Finally, in terms of cost effectiveness, the sugar diets appeared to increase the palatability of the silage from observations

made on feeding behaviour. Therefore, if it can improve a poor quality silage or generally increase silage intake, the amount of concentrates required for feeding in a winter management programme is reduced, making it cost effective for the farmer and adequate for maintenance in the cow.

CHAPTER 5

GENERAL DISCUSSION

In these three experiments, a study has been made of the dietary effects on milk production in the dairy cow. The results of each individual experiment have been discussed in the relevant chapter; in the following discussion an attempt is made to integrate and evaluate the main findings of the experiments with respect to literature information, and to draw some broader conclusions.

MILK NON-PROTEIN NITROGEN

The factor of greatest interest to arise from this work was the effect of diet on the milk protein constituents, in particular non-protein nitrogen.

The presence of NPN in milk protein was established in early work on fractionation of milk protein mainly by the Kjeldahl analysis. However, it was only about thirty years ago that work on the effect of diet on milk NPN was examined in some detail. Rook (1961a) stated that increases in dietary protein content invariably had no effect on milk yield or composition other than to increase NPN when offered above the Woodman standards. Therefore variations in milk NPN content could confound the effect of diet on milk total protein content. Nevertheless, some importance can be attached to milk NPN. Thomas (1980) suggested that although milk NPN represented less than 5% of total crude protein and had no feed value, the importance of the

constituent lay in the fact that its concentration in milk influenced milk protein stability. He further stated that through its relation to plasma urea concentrations, it was affected by events that influence blood urea such as the production of urea from deamination of amino acids in the rumen and liver which increases with dietary protein intake. Thus, Thomas (1980) concluded that dietary protein intake and milk NPN were related (see figure 3.10, chapter 3). In a later paper, Thomas (1983) indicated that with the exception of the influence of diet on milk NPN, changes in nutrition affected all the major protein constituents similarly, and classed them in terms of 'true' protein.

The direct effects of diet on milk NPN have been examined in this work, with the exception of experiment 1 where effects were observed indirectly due to the non-analysis of milk for milk protein constituents other than casein. The detailed work of experiment 2 showed clearly the immediate effect of high levels of dietary crude protein on milk protein constituents with significant increases occurring in the NPN. Little effect, other than normal lactational trends were evident in milk casein. The introduction of different energy sources showed that sugar based diets at high crude protein levels (experiment 3) appeared to suppress milk NPN formation possibly due to efficient ruminal nitrogen utilisation. When comparing the NPN levels of the two experiments at the same stage of lactation (periods 2 and 3 in experiment 2), it can be seen that the lower dietary crude protein level used in experiment 3 was reflected in the lower NPN values. The high crude protein diets in the latter experiment showed NPN contents of around 1.6g/kg whereas in the former experiment these were elevated to over 2g/kg. The low crude protein diets were slightly

more comparable eventhough the higher values were again in experiment 2. Consequently, it is concluded that from the results obtained dietary crude protein is related to milk NPN and furthermore, that feeding simple silage and concentrate diets at high crude protein contents has little effect other than to simply significantly increase milk NPN, unless a sugar based concentrate is offered.

The results of the effect of diet on the NPN content of milk have been discussed and compared with the limited information available in the literature. However, the importance of these findings and its impact on producer and commercial practice is discussed below.

Naturally, each contributor in the dairy products chain looks for an economical transaction in his specific area of the market. The producer (usually the farmer) at the bottom of the vertical chain of the market hopes to produce a good quality milk at reasonable cost, whereas the dairy products manufacturer (near the top of the chain) wishes to purchase a quality milk containing high proportions of desired constituents. Amies (1984) examined changes in the producer payments scheme before the introduction of milk quotas, but nevertheless highlighted compositional changes in terms of payment for producers and trends towards milk high in protein and fat. The alterations in payment for raw milk introduced at that time touched upon the changes consumers were demanding for the low fat, high protein diets they were consuming. Thus in terms of producer practice, the farmer was required to produce a high protein milk to achieve higher payments. In commercial terms, dairy product manufacturers are nowadays even more selective and examine the makeup of the proteins, in particular milk casein content. (Milk casein is of great importance in

milk product formations - see Hayes 1984; Tow 1984). Thus, the use of high protein diets to boost milk protein content may be of value in the long term to the producer, but it is of no benefit to the manufacturer when the increases are mainly in the NPN content of the milk.

Attempts to overcome this problem have been made in the United States and France where payments for 'true' protein, and not 'total' protein were introduced. However, and not surprisingly difficulties have arisen, not least from the fact that introduction in lone states (e.g. New York) has left farmers in that area being paid lower prices for their 5% lower protein milk than those in surrounding states that sell 'total' protein milk. Although this problem is not so pronounced in France where all milk is priced on 'true' protein, the establishment of the Single European Market in 1992 has ironically induced France to consider reverting back to 'total' protein.

Perhaps the root of the problem is the way in which milk protein content is measured. The Kjeldahl analysis of milk determines protein content by measuring total nitrogen content and multiplying it by 6.38 (protein tends to be 15.67% nitrogen). The problem with this established laboratory procedure lies in the fact that not all nitrogen in the milk is found in milk protein. Ironically, despite this, modern infra-red milk analysing machines, although they do not measure nitrogen but estimate protein levels based on chemical bonds, report results according to calibration resulting from the Kjeldahl test for nitrogen. Thus, calibration is probably the main problem and was consequently examined in detail by Barbano and Clark (1989). In general, opinion is that the machines available can be calibrated for

'true' protein, and efforts are underway to perfect methods for casein calibration, if a switch away from 'total' protein is made.

In conclusion therefore, to overcome the problems associated with NPN in the milk, the universal introduction of payment for 'true' protein milk would have to be introduced to satisfy the producer and manufacturer (who would consequently pay for what he required), and, an accurate system of calibration of milk analysis machines would need to be developed (as outlined by Barbano and Clark 1989), so that all industrial machines were compatable.

STAGE OF LACTATION

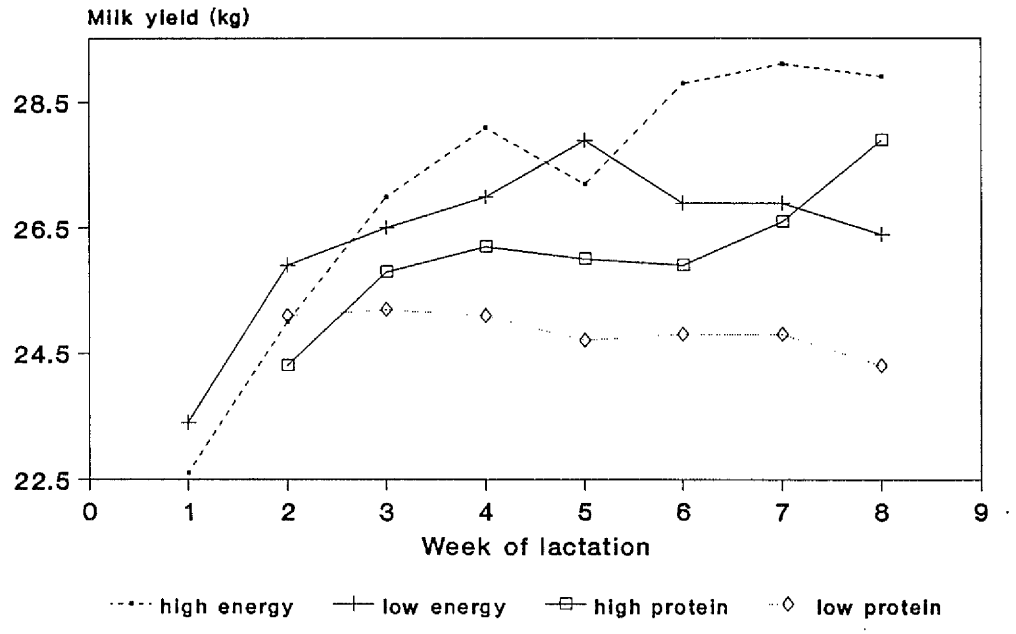
Another factor of interest to arise from this work was the effect of diet on milk production at different stages of lactation.

The effects of stage of lactation on milk composition are well documented, and have been reviewed earlier (Waite, White and Robertson 1956; Rook 1961a; Rook and Campling 1965; Jenness 1974; Thomas and Rook 1983). However, the effects of diet at different stages of lactation are less well reported with researchers opting for short term changeover experiments on dairy cows in mid-lactation when milk yield is relatively constant. Nonetheless, the importance of diet around parturition and in early lactation is widely recognised and recent work is looking more at events around calving and dietary effects at different stages of lactation (Clements et al 1989; Reeve et al 1989; Mayne 1990; Small and Gordon 1990).

The effects of dietary energy and dietary protein on milk production during lactation have been examined in this work. Figure 5.1

illustrates the milk yields of cows on the postpartum low and high energy diets (experiment 1), and the low and high protein diets (experiment 2), for the first 8 weeks of lactation:

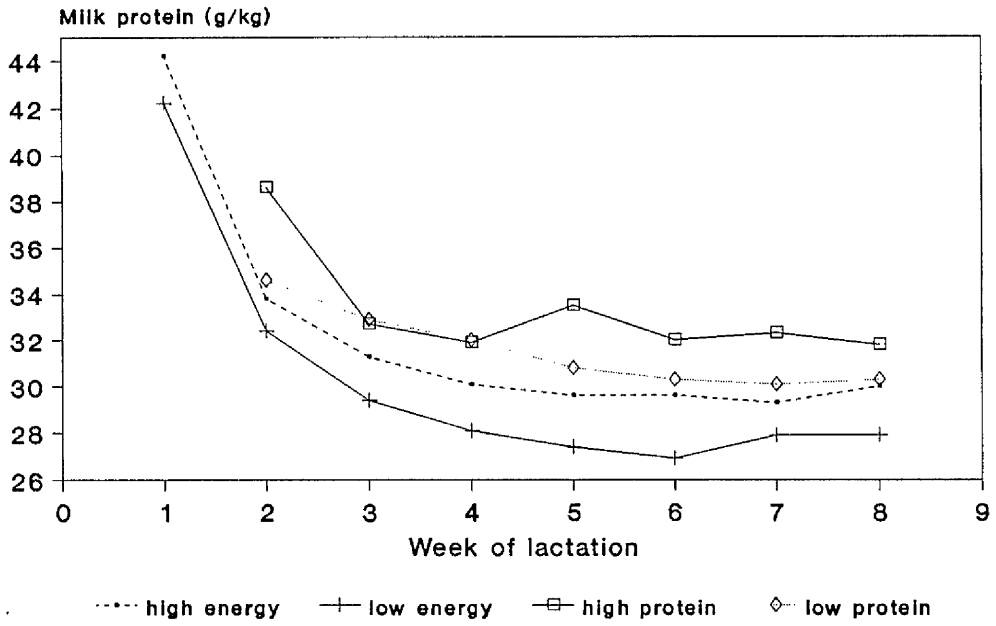
Figure 5.1: Milk yields of cows offered different energy/protein diets



Both energy diets showed sharp increases in milk yield within the initial 5 weeks of lactation, after which the high energy fed cows continued to yield more milk at a higher level, whereas the low energy fed cows showed a decline in yield. The decline in yield from week 5 was also evident in the low protein cows where slight increases in weeks 6 and 7 were followed by a fall in yield. The high protein diet however showed slow responses in yield until week 7, with an increase occurring from 25.5 kg/d (in week 6) to 27.5 kg/d (in week 8). The

opposite effects of the diets were evident in milk protein content:

Figure 5.2: Milk protein contents of cows offered different energy/ protein diets



In this figure it appears that the protein diets showed higher milk 'total' protein contents than the energy diets. A proportion of this can be attributed to increased milk NPN in the high protein fed cows. However, milk NPN contents were not measured in the energy fed cows and it is perhaps unwise to speculate on how much of the improvement is due to NPN, particulary because of the different method employed (Kjeldahl) in measuring NPN. Nevertheless, figure 5.2 illustrates that milk 'total' protein can be increased to a greater extent with protein diets than with energy diets.

In experiment 1, prepartum energy appeared to have little effect on milk production postpartum. It is therefore suggested that perhaps the length of precalving feeding (4 weeks) was not a long enough period to establish any effect and thus future work should examine dietary effects applied many weeks if not months prepartum. This has also been found in previous work: Gardner (1969) offered two energy diets (at digestible energy levels of 115% and 160% of maintenance requirement) to Holstein cows for 6 to 8 weeks prepartum and found no significant effects on milk yield or composition; Flipot et al (1988) offered grain feeds (the grain mixture was offered at 0.25% of body weight for half of the cows and 0.75% for the remaining half) from 2 months prepartum but also found that milk yield was not influenced by the diet; Ducker et al (1985) however, revealed some effects when energy diets were offered from 10 weeks prepartum with significant increases recorded in milk protein yield in the higher energy diets, but again with no effect on milk yield. The above results seem to indicate that dietary effects on postpartum milk production occur if diets are offered from at least 10 weeks before parturition. However, early work by Davenport and Rakes (1969) revealed no significant effects on milk production (with the exception of milk fat which was significantly higher in the higher level fed cows in the early stages of lactation) when different levels of diets were offered for the total dry period. Thus, it appears that future studies involving prepartum effects on milk production need to examine responses to diets offered in late lactation before the onset of the dry period.

A future area of research would be the effects of manipulating cow condition (in terms of both energy and protein status) during the dry

period upon subsequent animal performance. Early work by Garnsworthy and Topps (1982) demonstrated that cows with low condition scores (1.5-2) at calving produced more milk directly from the feed rather than from body fat, and were biologically more efficient over the experimental period than cows with higher condition scores at calving. Subsequent work by Garnsworthy and Jones (1987) revealed that thin cows (condition score 2) consumed more feed and had a higher milk protein content than cows calving in a fat condition (3.5). Neilson et al (1983) examined the changes in backfat that occur during lactation by measuring the fat over the 10th and 13th ribs and the 3rd lumbar vertebra with an ultrasound scanner, from which the backfat area index was calculated. They concluded that, with the exception of the lowest yielding animals, backfat, if present at calving, was generally mobilised during the lactation. However, management of the animals not only affects body fat reserves but body protein as well. The literature provides little evidence of work investigating the separate effects of protein depletion eventhough it may be a factor that enhances the susceptibility of dairy cows during inadequate nutrition to lower milk protein content. Robinson (1986) working with sheep found that performance was influenced by body protein reserves, and it is therefore evident that future work needs to be centred around: interactions between body reserves and milk protein content; possible utilisation of labile fat and protein, and effects on milk production; perfecting a method of accurately measuring protein reserves in the dairy cow.

A positive effect of prepartum feeding from the above cited experiments was that heifers and cows offered high energy diets before

calving had shorter intervals to ovulation and conception respectively.

In experiment 2 the lactational responses of the dairy cows to increased dietary protein were mainly found in the NPN of the milk. However, animals offered the high protein concentrates showed significantly higher milk yields when fed from weeks 3 and 11 of lactation, but this diminished to non-significant levels as the lactation progressed.

In conclusion, in terms of stage of lactation:

1. feeding high levels of RDP (over 170g CP/kg) at different stages of lactation had little beneficial effect on animal performance.

2. prepartum feeds need to be offered from at least 10 weeks before calving in future experiments on dietary effects on milk production

ENERGY SOURCE

Owing to the adverse effects of high levels of starch on silage dry matter intake (Thomas et al 1986; Thomas and Thomas 1989), rumen fermentation (Chamberlain et al 1985; Huhtanen 1988) and milk fat production (Sutton et al 1987), there has been increasing interest in replacing starchy supplements with either fibrous products such as sugar beet pulp (Mayne and Gordon 1984) or soluble carbohydrate products such as molasses (Morales et al 1989; Mayne 1989).

A main limiting effect of using barley in the supplement is the increase in ruminal protozoa thereby increasing intraruminal recycling of nitrogen. Replacing the barley with soluble carbohydrates such as

molasses, or condensed molasses solubles has been shown to lead to more efficient nitrogen utilisation in the rumen because of the improved rate of fermentation associated with these energy sources (Chamberlain et al 1985; Thomas and Rae 1988).

Although limited data is available on animal production trials involving diets of high silage and sugar based supplements, it appears from experiment 3 that the starch based diets depressed milk fat, despite the forage to concentrate ratio being above the level found in other low forage trials to cause milk fat depression. A possible explanation is that milk fat in the sugar and mixed diets was improved through partitioning of excess energy (calculated by ME balance) from milk protein to milk fat. However, it is possible that energy was partitioned into body weight although in view of the short term experiment and unreliability of liveweight regression data, this could not be confidently assumed.

From the milk production results the barley fed cows performed better than the sugar fed animals (although not significantly), with the exception of milk fat. However, a factor of interest to emerge from this work which has implications on both producer and commercial practice is the effects of different energy sources on milk NPN content.

The improvement in silage palatability through the addition of molaferm 20 stimulated animals on the sugar diets to consume the 'coated' silage as quickly as possible after application. Examination of empty forage bins after refusal weighing revealed no presence of molaferm 20 on the container walls or base. This suggested that all

silage covered in molaferm was consumed within the 24 hours between forage feeds. The implications of this for the producer is that poor quality forage could be improved and utilised in dairy cow feeds through the addition of a liquid feed supplement. In this way, expenses incurred through expensive supplementation can be minimised. For the commercial manufacturer of the product, molaferm 20 represents a marketable feed which when offered as part of a silage supplement can produce milk of a quality and quantity not significantly different from that produced with a barley supplement. Thus, future work should examine the effects of sugar supplements on silage intakes both by mixing the sugar with the silage during ensiling, and by the frequency of feeding of the sugar based supplement.

With reference to the earlier section on milk NPN and its impact on producer and commercial practice, results from the final experiment tend to indicate that sugar supplements, offered at high crude protein levels, suppress significant increases in milk NPN. Experiment 2 revealed that the use of an animal protein source in diets resulted in levels of milk NPN content that were 1 to 1.2% above the normal protein constituent percentage of 5% for NPN, whereas a cereal protein source increased the level to only, at maximum, 0.5% above normal percentage. Thus, the cereal source of crude protein (soya bean meal) had less of an effect on milk NPN than the animal source (fishmeal). In addition, the inclusion of a sugar source in the concentrate mix further reduced expected elevated NPN contents to values not significantly different from those recorded for the lower protein diets. In conclusion therefore, use of these diets (starch or sugar) in dairy herds results in both groups producing nearly as well as each other, with similar

milk casein contents (important for dairy products), but with better quality milk protein (in terms of proportions of NPN) being found in the soluble carbohydrate supplemented cows.

PRACTICAL IMPLICATIONS: the manipulation of milk production

The results of the three experiments have highlighted the difficulty of improving milk 'true' protein content in cows offered diets containing high quality grass silage.

Future experimental work should therefore concentrate on the following areas:

1. developing an accurate method of calibrating infra-red milk analysis machines for milk casein
2. investigating nutritional responses of dairy cows during total lactation studies rather than short-term changeover experiments
3. using the above mentioned long term trials to investigate manipulation of cow state prepartum, and in particular, any apparent mobilisation of body protein reserves
4. examining the effect of frequency of feeding of sugar based supplements on forage intake.

SUMMARY

1. The nutritional and non-nutritional factors influencing dairy cow milk production were reviewed. Particular attention was paid to the effects of diet.

2. Three experiments were undertaken to study further the effects of diet on milk production, particulary milk protein.

3. In the initial long term experiment, three complete but different ME diets were formulated and used to investigate the effects of diet prepartum and postpartum on animal performance using blood analysis to aid the interpretation of the effects. The higher ME diets were found to significantly increase TDMI, both pre and post calving, and to significantly increase TDMI as a % of lwt. Animals offered the higher energy diets showed a slower weight loss than the lower energy diets. Peak milk yield was achieved earlier in the higher energy fed cows postcalving, and milk yield was demonstrated to be negatively correlated ($P<0.05$) with energy balance. Cows offered the lower ME diet postpartum were shown to increase milk yield when put out to grass, suggesting that diet could affect milk production at different stages of lactation. No significant effects on milk fat or lactose were evident. Milk protein yield was not significantly different between groups over the total postpartum period but milk protein content was consistently higher in the higher energy fed cows. Milk casein was also found to be higher in these animals although values were depressed from the expected 78% of milk protein composition, reflecting changes in the remaining fractions: serum proteins and NPN. The conclusions from this experiment were that the

lower energy fed cows produced as well as the higher energy cows, and that in terms of economical milk production the costs of the high energy diets appeared to outweigh the small increases found in milk yield and constituents.

4. The two points of interest arising from the first experiment: possible effect of diet on stage of lactation, and changes in the milk protein constituents, were the basis for the design of the second experiment. In a long term continuous design experiment, animals were offered a high protein diet from weeks 3 (period 1), 11 (period 2) and 19 (period 3) of lactation. The substitution rate of silage with concentrates was found to be lower in the high protein fed cows, 0.59 compared with 0.63 (in low protein cows). The high protein animals had a significantly higher silage DMI, with those offered the high protein concentrates from week 3 of lactation achieving energy balance within 10 weeks of lactation. The milk analysis results (excepting the Kjeldahl results) were adjusted by covariance to eliminate any between-cow effects. Milk yield was significantly higher in animals on the high protein diet in periods 1 and 2. Milk protein yield was significantly higher in the high protein cows up to 18 weeks of lactation; milk protein content improved in animals offered the high protein concentrates from week 11 or 19 of lactation. The milk protein constituents showed significant increases in NPN in the high protein diets at the expense of the whey proteins; milk casein was unaffected. The conclusion from this experiment was that with a good quality silage, inclusion of a low degradability animal protein in the supplement had no beneficial effects on milk production.

5. The aim of the final experiment was to examine the effect of energy source (starch:barley; sugar;molaferm 20) at two levels of dietary protein (160, 240 g/kg CP DM) on milk production. The trial was a changeover design based on Latin squares. The starch diets showed a larger response to the increase in dietary protein than the sugar diets (1.84 compared with 1.01 kg/d per kg increase in supplementary CP intake). Milk results, apart from the Kjeldahl results, were adjusted by covariance. The starch diets tended to show higher values for milk yield and constituents (with the exception of milk fat) than the sugar diets with the higher protein level producing 0.5 kg/d more milk than the lower protein level. However, the sugar diets showed a greater response in milk yield to increased dietary protein (5.51 kg/d per kg increase in supplementary CP intake). The high CP diets showed significantly higher milk yields and constituent yields than those for the low protein diets, but milk protein content was usually higher in the low protein diets. Milk casein content appeared to be unaffected by the diets whereas the milk serum proteins were depressed in favour of the NPN, particulary in the high protein diets. Milk NPN content was significantly higher on the high protein starch and mixed diets but not on the sugar one. The conclusions of this experiment were that the starch based diets showed better milk production while the sugar diets improved silage DMI and produced a better quality milk protein.

6. It was concluded that improvements in milk protein content could be achieved through nutritional effects, but that the increases obtained were not necessarily in the constituents desired but in the NPN fraction. Using dietary CP levels of 240 g/kg DM and a vegetable

based protein source appeared to slightly improve milk protein content. Feeding supplementary protein to lactating dairy cows from week 11 of lactation appeared to improve milk production. Manipulation of milk production by dry cow feeding obviously requires more research and at an earlier period than 4 weeks prepartum. Finally, the possible introduction of payment for 'true' protein in the milk requires detailed work on milk protein constituents and their changes in relation to the high protein supplemented diets usually fed.

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APPENDIX

Estimated ME requirements for maintenance, milk production and liveweight change using the following equations (MAFF 1975):

Maintenance $Mm = 7.9 + 0.086W$

Milk production $EV_1 = 0.0623\ BF + 0.0331\ SNF - 0.381$

BF and SNF (g/kg)

$M_1 = EV_1 \times Y$

SNF = Protein + Lactose + Ash (7.5)

Liveweight change

1. Mg = 26.5 MJ/kg loss
2. Mg = 32.3 MJ/kg gain

Mm = ME required for maintenance (MJ/day)

W = Liveweight (kg)

EV_1 = Energy value of milk (MJ/kg)

BF = Butter fat content (g/kg)

SNF = Solids-not-fat content (g/kg)

M_1 = ME required for milk production (MJ/day)

Y = Milk yield (kg/day)

Mg = ME required for body gain or loss (MJ/day)

Equations used to estimate the ME requirements for maintenance, milk production, liveweight change and the efficiency of ME usage for milk production in dairy cows (ARC 1980):

Maintenance $Mm = (0.53 (W/1.08)^{0.67} + 0.0043 W)/km$

Milk production $Mp = Y \times (1.509 + 0.0406 F)/kl$

Liveweight change $Mg = 27.36$ for liveweight gain
$Mg = 21.84$ for liveweight loss

Mm = ME required for maintenance (MJ/d)

W = Cow liveweight (kg)

km = The efficiency of ME usage for maintenance = 0.72

Mp = ME required for milk production (MJ/d)

Y = Milk yield (kg/d)

F = Milk fat content (g/kg)

kl = The efficiency of ME usage for milk production = 0.62

Mg = ME required for liveweight gain or loss (MJ/kg)