Short and Long Term Effects of Diet on the Concentration of Protein in the Milk of Dairy Cows

by

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Abstract

The effect of diet on the milk protein concentration from dairy cows was investigated in a series of experiments aimed at both short and long term changes. To study the effect of dietary energy source and protein level, four concentrates were offered at a constant forage:concentrate ratio to 12 animals in mid-lactation on a grass-silage based diet. These provided two sources of energy (barley and sugar beet pulp) and two levels of digestible undegradable protein. Milk protein concentrations from the four diets were 36.3, 35.2, 35.1 and 34.9 g/kg (SED 0.40) for barley high and low protein and sugar beet pulp high and low protein respectively. There was little difference in microbial protein yield between diets, based on estimates of urinary purine derivative (PD) excretion. In a second experiment, three concentrates were offered, which differed in metabolisable energy (ME) density and fat content, to 12 mid-lactation dairy cows on a grass-silage based diet. Mean milk protein concentrations were 31.4, 32.5 and 31.0 g/kg (SED 0.45) for low ME high fat, low ME low fat and high ME high fat concentrates respectively. Milk protein yields did not differ significantly, although milk yields were significantly increased by the inclusion of fat in the diet due to increased lactose synthesis. PD excretion was correlated to fermentable ME intake. To investigate longer term effects of diet on milk protein concentration, 22 dairy cows were paired and offered a control diet of silage or restricted silage, *ad libitum* straw and 0.5 kg protein supplement (prairie meal) during the dry period. All animals were offered the same lactation diet and mean milk yields were the same from both groups. The mean milk protein concentration was significantly affected by dry period diet, with 29.4 and 32.0 g/kg (SED 0.40) for control and supplemented animals over the first 31 weeks of lactation. The experiment was repeated with 36 animals (18 pairs) using similar dry period diets. The over the first 18 weeks of lactation mean milk yields were significantly increased by the supplemented dry period diet, and mean milk protein concentration was only slightly increased (32.7 and 36.3 kg milk/d, SED 0.62; 30.7 and 31.5 g protein/kg, SED 0.28) with no significant difference in dry matter intakes with a proportion of animals. Two experimental lactation concentrates were fed to 6 pairs of mid-lactation animals which had also been offered the dry period diets to study the interaction effects. Mean milk protein concentrations were significantly increased by 2 g/kg with a 'milk protein enhancing' concentrate compared to a 'milk protein depressing' concentrate; milk yields were similar for both diets. No interaction effects were seen. It is concluded that the metabolisable protein supply to the mammary tissue is the most important factor in determining milk protein concentration. This may come directly from the diet, in which case maximising microbial protein efficiency by balancing the rumen degradable protein supply with fermentable ME is important. Avoiding a high acetate fermentation may also be important to ensure a low utilisation of amino acids for gluconeogenesis. Finally, the status of the animal's protein reserves during early lactation is more important than formerly appreciated.

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JM Moorby

Abbreviations

A/C	Allantoin to Creatinine Ratio
ADF	Acid Detergent Fibre
ADS	Acid Detergent Solution
AFRC	Agricultural and Food Research Council (formerly ARC)
AHEE	Acid Hydrolysis Ether Extract
AHEEI	Acid Hydrolysis Ether Extract Intake
Analar	Analytical Reagent
ARC	Agricultural Research Council
ASU	Analytical Services Unit
ATP	Adenosine Triphosphate
AU/C	Allantoin + Uric Acid to Creatinine Ratio
B	Barley (Chapter 3)
BH	Barley High Protein (Chapter 3)
BL	Barley Low Protein (Chapter 3)
BOHB	ß-Hydroxybutyrate
bST	Bovine Somatotrophin
CP	Crude Protein
CPI	Crude Protein Intake
CS	Condition Score
CTAB	Cetyl Trimethyl Ammonium Bromide
d	Day
DAPA	2,6-Diaminopimelic Acid
DM	Dry Matter
DMI	Dry Matter Intake
DNA	Deoxyribonucleic Acid
DOMD	Degradable Organic Matter Digestibility
DUDP	Digestible Rumen Undegradable Protein
DV	Diurnal Variation
EAA	Essential Amino Acids
EDTA	Ethylenediaminetetraacetic Acid
EE	Ether Extract

ER	Endoplasmic Reticulum
ERDP	Effective Rumen Degradable Protein
F:C	Forage to Concentrate Ratio
FIL	Feedback Inhibitor of Lactation
FME	Fermentable Metabolisable Energy
FMEI	Fermentable Metabolisable Energy Intake
g	Gram(s)
g	Acceleration of gravity (9.81 m^{-2})
GF/A	Glass Fibre filter
GPR	General Purpose Reagent
h	Hours
H	High Protein (Chapter 3)
HA	Hippuric Acid
HEHF	High Energy High Fat (Chapter 4)
HPLC	High-Performance Liquid-Chromatography
HPLF	High Protein Low Fat (Chapter 6)
IADF	Indigestible Acid Detergent Fibre
ICP	Inductively Coupled Plasma
IVOMD%	*In vitro* Organic Matter Digestibility
kg	Kilogram(s)
l	Litre(s)
L	Low Protein (Chapter 3)
LEHF	Low Energy High Fat (Chapter 4)
LELF	Low Energy Low Fat (Chapter 4)
LPHF	Low Protein High Fat (Chapter 6)
LW	Live Weight
MAFF	Ministry of Agriculture, Fisheries and Food
ME	Metabolisable Energy
MEI	Metabolisable Energy Intake
MJ	Megajoules
ml	Millilitres
mM	Millimolar
MMB	Milk Marketing Board for England and Wales

mRNA	Messenger RNA
NA	Nucleic Acid
NA-N	Nucleic Acid Nitrogen
NADPH	Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form
NCGD%	Neutral Cellulase Gammanase Digestibility
NDF	Neutral Detergent Fibre
NDS	Neutral Detergent Solution
NEFA	Non-Esterified Fatty Acid
nm	Nanometres
NPN	Non-Protein Nitrogen
NUNPN	Non-Urea Non-Protein Nitrogen
OM	Organic Matter
P	Probability (of Differences Being Due to Random Variation)
PD	Purine Derivative
RDP	Rumen Degradable Protein
REML	Residual (or Restricted) Maximum Likelihood
RER	Rough Endoplasmic Reticulum
S	Sugar Beet Pulp (Chapter 3)
SAC	The Scottish Agricultural College
SD	Standard Deviation
SE	Standard Error
SED	Standard Error of the Differences of the Means
SMRA	Scottish Milk Recording Association
TCORN	(AFRC) Technical Committee on Responses to Nutrients
TDN	Total Digestible Nutrients
tRNA	Transfer RNA
UDP	Rumen Undegradable Protein
UV	Ultra Violet
VFA	Volatile Fatty Acid

Chapter 1

Milk Protein: Its Importance, and Factors Influencing its Concentration in Milk of Dairy Cows

1.1 THE IMPORTANCE OF MILK AND MILK PROTEIN

From the moment the young mammal is born until it is weaned, milk is essential for its survival. The use of milk as a human food extends into adulthood through the exploitation of certain ruminant species. Worldwide, a number of these are used for milk production, including cattle, buffalo, sheep, goats and various camelids. On a large scale, and in the 'developed' countries in particular, dairy cattle are the most important producers of milk.

Until relatively recently, dairy cow breeding programmes have had the major goals of increasing milk yield and increasing milk fat concentration. In recent years, however, there has been a trend towards a reduction in the fat content of many Western diets due to health concerns about fats containing saturated fatty acids, and animal fats in particular (Committee on Medical Aspects of Food Policy, 1984) despite scant evidence (Blaxter and Webster, 1991). Rightly or wrongly, one of the consequences of this is that there has been a general trend towards an increase in the consumption of semi-skimmed and skimmed milk, and a corresponding decrease in the consumption of whole milk (Milk Marketing Board, 1984, 1986 and 1990).

At the same time as a decrease in consumption of whole fat milk, in Britain at least, an increase in disposable income has led to an increase in the consumption of 'luxury' dairy products - butter, cheese, yoghurt, fromage frais etc (MMB, 1984, 1986, 1990). Many such dairy products - with the exception of butter - are based on the precipitation of certain milk proteins by enzymatic (cheese) or fermentation (yoghurt) processes. Cheese producers, for instance, are therefore dependent on the quantity of casein present in milk for their products. Because of this, there is an incentive to produce milk with a high concentration of protein, and this is partly reflected in the present pricing structure for milk from the Milk Marketing Board for England and Wales - e.g. milk protein is more valuable to the producer than milk fat. An increase in milk protein concentration, even at the expense of fat, would increase the value of the milk to the processor. In Europe, with the restrictions of the quota system of milk production, an increase in milk protein concentration at the expense of milk yield may be acceptable in some circumstances.

With the breaking of the monopolies held by the British milk marketing boards this year (1993), there may be interest in altering milk quality to suit individual processors - the ranges within which individual milk constituents can lie may be narrowed by the buyers

so that a more consistent raw material is made available. This will put increased pressure on the milk producer to feed dairy cows in a more controlled manner. One way in which this may be brought about is through the use of supplements designed to have specific effects on milk quality.

Until the late 1970s and early 1980s, relatively little emphasis was placed on milk protein. Most research effort was directed towards increasing milk yields and milk fat concentration. Milk protein was often not considered as it was thought that its concentration could not be changed to any great extent through nutrition. However, milk protein concentration can be influenced quite significantly through nutrition of the animal, albeit perhaps not to the same extent as milk fat.

1.2 MILK PROTEIN

The collective term 'milk protein' covers a wide range of nitrogenous compounds which are found in milk. Crude milk protein, often quoted in the literature, also includes various non-protein nitrogen (NPN) compounds. True milk proteins have been categorised into two broad groups based on their solubility at pH 4.6 and 20°C: the caseins and the whey proteins (Eigel, Butler, Ernstrom, Farrell, Harwalker, Jenness and Whitney, 1984). In addition to these two groups, a further group of proteins which account for approximately 1% of proteins found in whole milk are associated with the fat globule membrane.

1.2.1 Caseins

The caseins are a group of milk proteins that precipitate out of solution when the pH of milk is lowered to 4.6 or below, at a standard 20°C (Eigel *et al.*, 1984). They are additionally characterised by ester-bound phosphate, as having a high proline content and few or no cysteine residues (Jenness, 1985). It is this group of proteins which predominates in cheeses, and so these are the targets for any factors which may increase milk protein. The major caseins currently identified in bovine milk are α_{s1}-casein, α_{s2}-casein, ß-casein, κ-casein and γ-caseins. γ-Caseins have been shown to consist of fragments of ß-casein (Jenness, 1985).

Caseins are made exclusively in the mammary gland and are therefore specific to milk. They are one of the few animal proteins which are produced primarily as a foodstuff, and collectively account for some 70-80% of the proteins found in milk. Casein micelles, as

well as being a source of protein for the young mammal, are also important for carrying the milk minerals such as calcium, magnesium and zinc.

1.2.2 Whey Proteins

These are the proteins that remain soluble in the milk 'serum' (whey) after the caseins have been precipitated at pH 4.6 at 20°C. This definition includes some γ-caseins which remain soluble after acid precipitation of 'the casein proteins'. Major whey proteins include ß-lactoglobulin, α-lactalbumin, blood serum albumin, the iron-binding proteins transferrin and lactoferrin, immunoglobulins and various enzymes. ß-lactoglobulin and α-lactalbumin are produced in the mammary gland. Other whey proteins are formed elsewhere in the body and are transferred preformed into milk.

ß-Lactoglobulin is the whey protein found in the greatest abundance in milk, although its biological activity is unknown. α-Lactalbumin is a 'specifier' to the enzyme galactosyltransferase (see Kuhn, Carrick and Wilde, 1980) which, in tissues other than the mammary gland, is essential for the transfer of galactose from UDP-galactose to *N*-acetylglucosamine to form *N*-acetyllactosamine. In the mammary gland secretory tissue α-lactalbumin complexes with galactosyltransferase to form lactose synthetase, which catalyses the transfer of galactose to glucose, producing lactose.

1.2.3 Non-Protein Nitrogen

NPN compounds which are found in milk include urea, creatine, creatinine, uric acid, orotic acid, hippuric acid and ammonia. Various small peptides and amino acids are also secreted into milk. Together, these amount to some 1 g/l in bovine milk, or some 6% of total milk nitrogen. Most of this is of little nutritive value to humans, and an increase in milk nitrogen as NPN rather than protein amounts to an inefficient use of the nitrogen available to the animal.

1.3 Milk Protein Synthesis

Milk protein concentration is a function of the rate of secretion of milk proteins into the lumina of the alveoli of the mammary gland, and the rate of diffusion of water from blood to the same lumina. The key to understanding the factors controlling milk protein concentration is therefore the understanding of the factors which control both protein production and water diffusion.

Milk proteins secreted into milk are derived from two main sources - mammary gland secretory tissue and other parts of the body (e.g. the liver). In both cases, the proteins are made in the same way as almost all other eukaryotic proteins.

Information coded in the genome of the animal, held in the form of deoxyribonucleic acid (DNA), is expressed starting with the process of transcription. The genes coding the proteins are copied onto ribonucleic acid (RNA), which is processed (e.g. splicing out of introns, addition of 3' poly adenine tail) as it passes out of the nucleus to the site of protein synthesis. In the mammary secretory cells, this occurs at the endoplasmic reticulum (ER), to which the ribosomes attach. Ribosomes are the structures which act to hold the mature messenger-RNA (mRNA) in such a way that the process of protein synthesis, i.e. the polymerisation of amino acids, can occur.

The process of translation, in which amino acids are strung together in the order specified by the mRNA molecule, begins with the priming of transfer-RNA (tRNA) molecules with amino acids specific to semi-unique triplets of RNA bases at a certain position on the tRNA molecule. As the ribosome moves along the mRNA molecule three bases at a time, new codons are presented for the attachment of an aminoacyl-tRNA complex with the complementary anti-codon. The protein grows by the repeated attachment of amino acids, and in the case of milk proteins, a short primary sequence of amino acids causes it to cross through the ER membrane to the lumen (Mercier and Gaye, 1982). Here, the finished proteins are produced following various post-translational modifications (e.g. the removal of signal sequences, cleavage of certain bonds).

The expression of milk protein genes in mammary epithelial cells is under the control of a complex set of peptide and steroid hormones (Rosen, Rodgers, Couch, Bisbee, David-Inouye, Campbell and Yu-Lee, 1986; Vonderhaar and Ziska, 1989). Whilst hormonal control of casein gene expression is primarily at the post-transcriptional level in mouse mammary epithelial cell culture (Eisenstein and Rosen, 1988), there are a number of areas of control before that which may be influenced by nutrition, particularly in terms of the partitioning of nutrients towards the mammary gland. For example, plasma concentrations of insulin and somatotrophin are affected by nutrition, and the degree to which these and their target tissues respond direct nutrients towards or away from their incorporation into milk. However, the identity of these control systems and how they may be modified to

produce the desired response is not clear at present.

1.4 MILK PROTEIN SECRETION

Milk proteins are secreted into milk by the mammary epithelial cells by the process of exocytosis. Mercier and Gaye (1982) reviewed the early processes in the pathways of secretion of the main milk proteins: the deposition of milk proteins into the lumen of the rough ER (RER) and subsequently into the Golgi apparatus. Linzell and Peaker (1971) discussed a number of hypotheses by which milk proteins may be transported to the apical membrane of the secretory cell and released into the lumen of the alveolus. Vesicles are budded off the Golgi apparatus, and move to the apical membrane. Here, they fuse with the membrane and deposit their contents outside the cell. It is still unclear the way in which the movement of the vesicles is controlled, or even what mechanisms are involved. Wilde and Burgoyne (1992) report the partial involvement of Ca^{2+} ions in goat mammary epithelium, which are utilised in the regulation of many intracellular movement processes, although this is thought not to involve the cortical actin network (Turner, Rennison, Handel, Wilde and Burgoyne, 1992). Their hypothesis allows for the intracellular storage of protein-packed vesicles, with calcium ions promoting their exocytosis, and the autocrine inhibitor found in bovine milk suppressing it.

1.5 NON-NUTRITIONAL FACTORS INFLUENCING MILK PROTEIN CONCENTRATION

The main aim of this work was to consider the effect of nutrition on milk protein concentration, with the ultimate goal being that of determining factors which would bring about an increase. There are, however, several factors other than nutrition which can affect milk protein content that I shall briefly outline below: breed, parity, stage of lactation, disease, season, and environmental temperature.

1.5.1 Breeding

This encompasses two separate but overlapping issues - animal breed and genetic merit. The milk of Channel Island breeds of cattle is renowned for its fat content, but it is also richer in protein and lactose. Jersey cow milk has an average protein concentration of approximately 38.0 g/kg, compared to an average of 31.7 g/kg for Holstein milk (Nix, 1989). However, the amount of protein per unit fat in the milk of different dairy cow breeds differs, with typical protein/fat ratios of 0.72 for Jerseys and 0.84 for Friesians (Nix, 1989).

Breeding (i.e. increasing the genetic merit) for milk protein may well be the way in which sustained increases are best achieved, particularly since artificial insemination has become the norm in Britain and North America. However, it is only in the last two to three years that bulls offering potential increases in milk protein have become widely available in artificial insemination programmes. Despite this, few popular bulls have a high milk protein genetic potential.

1.5.2 Parity

The protein content of milk from individual animals generally increases with each lactation until their third (Ng-Kwai-Hang, Hayes, Moxley and Monardes, 1985). From then on there is a gradual decline in the casein content of milk, although this is compensated for to some extent by an increase in whey protein concentration (Waite, White and Robertson, 1956; Ng-Kwai-Hang *et al.*, 1985). Stanton, Jones, Everett and Kachman (1992) found that protein yields increased to the third lactation, but protein concentration was relatively constant due to a concomitant increase in milk yield.

1.5.3 Stage of Lactation

Milk protein concentration follows an approximate inverse curve to that of milk yield; starting high and declining after parturition, reaching its lowest levels at about peak lactation, before steadily increasing to the end of lactation (Wood, 1969, 1976). Since milk yield is highest when protein concentration is lowest, and vice versa, the daily yield of protein remains relatively constant throughout lactation, although decreasing towards the end of a normal 305 day lactation (Stanton *et al.*, 1992).

1.5.4 Environmental and Seasonal Effects

These are often confounded with nutrition (see later), since, in Britain at least, cows are fed different diets at different times of the year. Heat stress - both cold and hot - affects the animal's maintenance nutrient requirement, which will affect the availability of those nutrients for milk production. In the United States, protein concentration tends to be highest during the colder months of the year, and lowest during the hotter months (Collier, 1985), although in Britain the opposite tends to be seen because of the lower quality diet offered to animals over the winter period.

1.6 NUTRITIONAL FACTORS INFLUENCING MILK PROTEIN CONCENTRATION

There have been a number of excellent reviews in the recent literature which extensively discuss the effects of nutritional factors on milk protein (Emery, 1978; Thomas PC, 1983 and 1984; Spörndly, 1989; Sutton, 1989; Sutton and Morant, 1989; Coulon and Rémond, 1991; DePeters and Cant, 1992).

Emery (1978), Spörndly (1989), and Coulon and Rémond (1991) used published data to calculate regression equations for the effects of various dietary factors on milk protein content from dairy cows. Whilst such analyses are useful to gauge the likely effect of altering a dairy cow diet, they must be treated with some caution. As Emery (1978) notes, the data used in his analyses were not collected with the specific aim of investigating effects on milk protein. Up to that point, some 15 years ago, relatively few papers specifically cited milk protein concentrations; most authors measured milk fat and solids-not-fat. Since then, the interest in milk protein has increased to a stage where a fuller analysis is generally given in the literature. Even now, however, milk protein figures are often derived from analyses related to total nitrogen, and may not take into account the NPN fraction; such figures, therefore, overestimate the true protein content of milk and can be misleading if the NPN is not considered.

There are two major ways in which nutrition of the dairy cow can influence milk composition: directly, with an immediate response to feeding, and indirectly, through the animal's body reserves. A great deal of research effort has been directed at the exploration of the former in recent years, and relatively little at the latter, although the importance of the animal's reserves is something which should not be underestimated.

1.6.1 Direct Nutritional Responses

The nutrition of the dairy cow has been extensively investigated, yet is still far from fully understood. There are many conflicting reports in the literature about the impact of nutrition on milk protein concentration, although a number of general trends have emerged. These can be classified somewhat loosely into responses due to dietary energy and due to dietary protein (Aston and Sutton, 1993). **More detailed discussions of these factors can be found in the introductions to Chapters 3 and 4.**

1.6.1.1 Dietary Energy

Several aspects of dietary energy may influence milk protein content. The source of energy is important, as is the quantity. Energy is not a nutrient in its own right, and references to it here are to the energy-yielding compounds in the diet; the way in which the gross energy of a feedstuff is utilised depends on a number of factors such as rumen fermentation, products of digestion and interactions with other compounds in the feed.

The modern high-yielding dairy cow is normally in negative energy balance in early lactation as the increase in energy intake lags behind early increases in milk production. There is, therefore, much interest in increasing energy supply to the animal by feeding high-energy concentrates, particularly by feeding fat, for example using feedstuffs such as rape seed (canola), whole cotton seed, and full fat soya beans, which can have an important effect on the composition of the milk.

1.6.1.1.1 Energy Level

Energy intake can be increased either by feeding more energy-yielding feeds, by increasing the energy density of the feed, or both. The conclusions of Coulon and Rémond (1991) are relatively simple: an increase in energy supply over a wide range of experimental designs and animal characteristics generally leads to an increase in milk protein production (i.e. yield) and an increase in milk yield. This is in agreement with other authors (Emery, 1978; Thomas PC, 1984; Spörndly, 1989) when the energy is from carbohydrate sources (see below). At moderate to high levels of energy feeding, an increase in energy supply has relatively little effect on milk protein concentration - one reason why milk protein concentration was until recently regarded as being constant. At higher levels, the response of milk yield follows the law of diminishing returns before that of milk protein yield, and hence an increase in milk protein concentration can be seen.

1.6.1.1.2 Energy Quality

The term energy quality refers to the form in which dietary energy is presented to the animal (i.e. the class of feedstuff), and it has important effects on the response of the animal in terms of milk protein concentration.

One of the cheapest methods of increasing the energy supply to the dairy cow is through the use of supplemented unprotected fats (e.g tallow). However, an increase in the

concentration of fat in the diet is often associated with a decrease in milk protein concentration. This has been attributed to both changes in the fermentation of dietary fibres due to the effects of high levels of fat on rumen microbes (Devendra and Lewis, 1974), and changes in the animal's physiology such as change in plasma hormone concentrations and changes in the extraction of amino acids from the blood by the mammary gland (Casper and Schingoethe, 1989). However, a depression in milk protein concentration often occurs when milk yields are increased by feeding additional fat, and protein yields remain constant, resulting in a dilution of the milk protein. Sometimes, the increase in milk yield is concomitant with a decrease in protein yield, resulting in even larger effects. The reason for these effects still remains largely unclear.

Feeding additional energy in the form of a carbohydrate tends to increase milk protein concentration. There are, however, differences in the responses to different carbohydrates: starchy, or non-structural carbohydrates, and fibrous, or structural carbohydrates. There is general agreement in the literature that increasing the energy intake of a dairy cow by supplementing her diet with a starchy concentrate will increase the concentration of the protein in the milk she produces. There are several aspects to this. Supplementing a basal diet of say, grass silage, with a concentrate will tend to decrease the intake of the silage (the substitution effect) and the increase in energy intake will not be in direct relation to the increase in energy content of the concentrate. Furthermore, the concentrate energy is supplied in the form of starch, whilst that of the silage is mostly from fibre. Increased energy, supplied in the form of fibrous or structural carbohydrates has been shown to increase milk protein concentration, but the response is not as great as that elicited by starchy concentrates.

Many of the experiments performed to assess the effects of changing the forage:concentrate ratio of the dairy cow diet have done so by feeding additional concentrates. The effects obtained are therefore often confounded by an effect of increased plane of nutrition. However, when isoenergetic substitution of forage with concentrate occurs, the effect of decreasing the F:C ratio tends to increase milk protein concentration.

Some of the effects produced by differences in carbohydrate sources, i.e. starch versus fibre, are thought to be brought about by differences in rumen fermentation patterns. The

microbial fermentation of cereal starch generally leads to a low acetate:propionate ratio, whilst the fermentation of fibrous carbohydrates provokes the opposite (Lees, Oldham, Haresign and Garnsworthy, 1990); the consequence of this is that the animal which is supplied with more gluconeogenic precursors (i.e. propionate) from starchy diets can benefit in terms of availability of energy for lactation, initially increasing both milk and protein yields and later protein concentration as an increase in glucose precursors can reduce the utilisation of amino acids for gluconeogenesis. Some of these changes may be mediated through the influence that such the products of rumen fermentation have on the concentrations of blood hormones such as insulin. Similar responses from ruminal infusions of volatile fatty acids (VFAs) have been seen (see Thomas, PC, 1984). Furthermore, the capture of rumen degradable protein depends on an adequate fermentable energy source, and much of the crude protein in, for example silage, is lost unless a fermentable supplement is provided, which may be starchy or fibrous.

1.6.1.2 **Dietary Protein**

Like dietary energy, both the quantity and quality of dietary protein can affect milk protein concentration. It was originally thought that dietary protein supply had little effect on milk protein concentration, and then only when severely restricted. Current descriptions of dietary protein requirements of ruminants, such as the Metabolisable Protein system (AFRC Technical Committee on Responses to Nutrients (TCORN), 1992) take into account the rumen degradability of dietary protein and also the protein provided by the growth and digestion of rumen microbes. The effective rumen degradable (ERDP) fraction of dietary crude protein is that which is utilised by the rumen population for growth. This must be balanced with rumen requirements for energy for efficient utilisation. Dietary protein that remains undegraded in the rumen is digested by the animal in the normal manner in the small intestine, and that which is not digested is lost in the faeces.

Dietary protein was found by Emery (1978) to be correlated with an increase in milk protein concentration, but this is in contrast to the findings of Spörndly (1989) who found no correlation with milk protein concentration, but a positive correlation with milk protein yield. An increase in dietary protein has, like an increase in dietary energy, been found to produce increases in milk yield, and this may be one reason for the discrepancies. Another reason is the complexity of the interrelationships between protein and energy in the animal (Oldham, 1984) and the need to not only provide sufficient protein to the

rumen, but also to the animal (i.e. above the requirements of the rumen) and to balance this with the energy requirements of the two. This balancing act is complicated by synergistic or antagonist effects which exist between the protein and energy supplies to the animal depending on the ways in which these resources are delivered, and their effects on the physiology of the animal.

The rumen degradability of the dietary protein source can have important consequences on milk protein concentration. Highly degradable protein sources provided in excess of rumen requirements are effectively wasted. Degradable protein sources are broken down to ammonia in the rumen, from which new amino acids are synthesised by the microbes for incorporation into microbial protein. Much of the ammonia-N in excess of microbial requirements is absorbed by the gut, converted to urea by the animal, and excreted. Feeding high levels of degradable protein to the dairy cow can increase the crude protein content of milk, but this is due to an increase in the NPN fraction of the milk protein, which is of no nutritional value to humans. Urea apparently diffuses into milk in relation to blood urea levels, and is highly correlated to rumen ammonia levels (Oltner and Wiktorsson, 1983; Roseler, Ferguson, Sniffen and Herrema, 1993). It can, therefore, be a useful marker of rumen function in the dairy cow.

Digestible undegradable protein (DUDP) and microbial proteins are broken down in the small intestine to individual amino acids and oligopeptides. These are absorbed by the intestine epithelial cells by Na^+-amino acid cotransporters. In the epithelial cells, oligopeptides are cleaved into the constituent amino acids and these are moved out of the cell, into the interstitial fluid, and eventually into the blood system (Alpers, 1986). Here, amino acids can either be transported directly to the mammary gland, or arrive at the mammary gland after having been through other body proteins. Recent rather controversial evidence has indicated a greater transport and utilisation of amino acids as peptides (Webb, Dirienzo and Matthews, 1993).

1.6.2 Indirect Nutritional Responses

This refers to the utilisation of the animal's body reserves to support lactation when 'direct' nutrition is unable to supply sufficient quantities of nutrients. The high-yielding dairy cow is in negative energy balance during early lactation since her dietary intake is too low to meet the demands of lactation. At this time fat reserves are broken down to

provide energy for lactation. Much research has been carried out since the early 1970s to determine the most efficient ways of managing fat reserves. However, it has become apparent that fat cows are not only prone to more health and reproductive problems than thinner contemporaries (e.g. greater calving difficulties, increased risk of ketosis, longer interval to first oestrous), fat cows are unable to consume as much food due to physical and physiological constraints, confounding the problem further. **The introduction to Chapter 5 gives more detail on this subject, but major points are raised below.**

Contrary to earlier work, milk yields have been found to increase when cows are calved at a relatively low condition score (CS) - although not when emaciated (Garnsworthy, 1988). This is sometimes due to increased energy intake as they are able to eat more than animals which calve at a higher (i.e. fatter) CS. Benefits in early lactation in terms of increased milk protein concentration have also been found with calving at a lower CS.

The use of dairy cow protein reserves as a potentially valuable resource is an area which has not been examined extensively. Protein which is not used immediately by the animal is not stored in the same way that 'spare' energy is. However, body protein can be mobilised in support of processes that need amino acids when the level of crude protein being supplied to the animal is below requirements, such as during foetal growth in late pregnancy, and early lactation. Recent work with rats has shown that labile protein reserves can be mobilised to support about six days of lactation when lactating animals are fed a protein-free diet (Pine, Jessop and Oldham, 1992). Botts, Hemken and Bull (1979) showed that up to 27% of total body protein in dairy cows may be mobilised and that this is therefore potentially available for use by the animal during early lactation in an analogous fashion to fat reserves, and other work has shown that the body protein content follows similar, but less extensive, changes as fat reserves over the course of lactation (Belyea, Frost, Martz, Clark and Forkner, 1978; Gibb, Ivings, Dhanoa and Sutton, 1992).

1.7 CONCLUSION

Several general points about the influence of nutrition on milk protein concentration can be concluded. Dietary energy supply is very important; not only the level of energy, but the form in which it is supplied - carbohydrate (structural and nonstructural) or fat. Linked to this, by rumen function and to a lesser extent by gluconeogenesis, is protein supply. Amino acids are the building blocks of milk protein, and in general, an increase

in these will lead to an increase in milk protein. Microbial protein synthesis from protein and non-protein nitrogen sources and protein that bypasses rumen metabolism are both sources of amino acids. Finally, body protein is an important potential source of amino acids for milk protein production that has received relatively little attention in the past.

Therefore, the main aim of this work was to study three major areas of dairy cow nutrition: i) short term protein supply, and microbial protein production, ii) short term energy supply, and its influence on milk protein production, and iii) protein supply in late pregnancy with the aim of influencing body protein reserves. These topics are reviewed in greater detail in the introductions to Chapters 3, 4 and 5.

Chapter 2

Materials and Methods

2.1 INTRODUCTION

Many of the methods used during the experiments presented in subsequent chapters were common to more than one, and these are presented here. These include analytical methods for the determination of feed composition, milk constituent quantification, and urinary purine derivative analysis.

2.2 FEED ANALYSIS

Each sample was analyzed in duplicate; if the two results differed greatly (by more than 1-5%) then the analysis was repeated. The mean of the two duplicates was taken as the value for the sample.

2.2.1 Sample Preparation

Feed samples were either analyzed immediately, or were frozen and stored at -20°C until processed (see individual descriptions). Most of the analyses were carried out on dried samples, and for this fresh samples of 100 g were oven dried at 60°C and/or 100°C (S & T (Unitherm) Ltd, Birmingham, UK) in aluminium food trays for 24 hours. After cooling, they were weighed to determine the dry matter content. All samples were milled (modified hammer mill, Christy-Norris Ltd, UK) to pass through a 0.6 mm sieve before analysis. The material was then stored at room temperature until analyzed.

Feed dry matter contents (including silage) presented in the experimental chapters of this thesis, unless otherwise stated, were all determined by drying in an oven at 100°C.

Before drying, silage samples were minced (Crypto-Peerless Ltd, London, UK) through a 10 mm plate to ensure an even sample was taken for drying and subsequent analysis.

All reagents used in the analyses, unless stated otherwise, were obtained from BDH (Poole, Dorset, UK) and were of general purpose reagent (GPR) grade, again, unless otherwise stated.

2.2.2 Determination of Acid Detergent Fibre (ADF)

ADF consists of cellulose, lignin, and cutin (Van Soest and Wine, 1967).

Reagent:

Acid Detergent Solution (ADS):

Sulphuric acid, 95% (Analar),	275 ml
Cetyl trimethyl ammonium bromide (CTAB),	200 g

The sulphuric acid was added to about 2 litres of distilled water, and mixed thoroughly. The CTAB was added to this, and the solution was made up to about 10 litres with water, allowing the foam to subside between each addition of water.

Procedure:

1. 1 g (Sample Weight) of 100°C dried material in excess (100 ml) ADS was heated to boiling in a 500 ml conical flask. It was refluxed slowly for 1 hour on electric mantles (Electrothermal Engineering Ltd, London, UK).

2. Whilst still hot, the sample was filtered onto a Whatman glass fibre (GF/A) filter (Whatman International Ltd, Maidstone, UK) supported on a Whatman 541 cellulose filter. The residue was washed 3 times with hot tap water, with a final rinse of cold acetone.

3. The residue, GF/A filter, and muffle basin were dried overnight at 100°C, cooled in a desiccator, and weighed (Weight A).

4. The residue (on its filter and basin), was ashed in a muffle oven (Lenton Thermal Designs Ltd, Worthing, UK) at 500°C overnight, cooled in a desiccator, and reweighed (Weight B).

5. $$\text{ADF g/kg DM} = \frac{(\text{Weight A} - \text{Weight B})}{\text{Sample Weight}} \times 1000.$$

2.2.3 Determination of Indigestible Acid Detergent Fibre (IADF)

IADF is determined as ADF, followed by incubation with cellulase to remove the cellulose component of the ADF residue. IADF was used as an internal marker for whole tract organic matter digestibility (Penning and Johnson, 1983), calculated as 1 minus the ratio of feed to faecal IADF concentration.

Reagents:

Acid Detergent Solution (ADS): as for ADF determination.

pH 4.6 Buffer:

Anhydrous citric acid (Analar),	10.33 g
Anhydrous disodium hydrogen orthophosphate (Analar),	13.28 g

The citric acid was dissolved in 532.5 ml of water (to make a solution of 19.4 g/l). This was mixed with the disodium hydrogen orthophosphate dissolved in 467.5 ml water (to make a solution of 28.4 g/l) to make the final buffer.

Enzyme Solution:

Trichoderma viride cellulase	6.25 g
(Fluka Biochemicals Limited, Gillingham, Dorset, UK)	

The cellulase was dissolved in 1 litre of pH 4.6 buffer (made up fresh daily).

Procedure:

1. 2 g (Sample Weight) of 60°C dried material in excess (200 ml) ADS was heated to boiling in a 500 ml conical flask and refluxed slowly for 1 hour.

2. 1 g (Weight A) of 60°C dried material was dried at 100°C overnight, allowed to cool in a desiccator to room temperature, and re-weighed (Weight B).

3. Whilst still hot, the sample was filtered onto a Whatman glass fibre (GF/A) filter supported on a Whatman 451 cellulose filter. The residue was washed 3 times with hot distilled water, with a final rinse of cold acetone.

3. The residue and GF/A filter were carefully transferred to a screw-top plastic jar. 50 ml cellulase solution was added, and the jar was vigorously shaken to break up the residue disk.

4. The residue was incubated for 10 days at 40°C, with daily vigourous shaking.

5. The residue was refiltered onto a GF/A filter, washed with tap water, dried overnight at 100°C, allowed to cool in a desiccator, and was then weighed (Weight C).

6. The residue was ashed at 450°C overnight, allowed to cool, and then re-weighed (Weight D).

7. $$\text{IADF g/kg DM} = \frac{(\text{Weight C} - \text{Weight D})}{\text{Sample Weight}} \times \frac{\text{Weight B}}{\text{Weight A}} \times 1000$$

8. Whole tract apparent organic matter digestibility = 1 - (feed IADF / faecal IADF)

2.2.4 Determination of Neutral Detergent Fibre (NDF)

NDF consists of hemicellulose, cellulose, lignin, cutin and some cell wall proteins (Van Soest, 1963; Van Soest, Robertson and Lewis, 1991).

Reagents:

Neutral Detergent Solution (NDS):

Sodium dodecyl sulphate,	150 g
Disodium EDTA (Analar),	93 g
Sodium tetraborate,	34 g
Disodium hydrogen orthophosphate, anhydrous,	22.8 g
2-Ethoxyethanol,	50 ml

The EDTA and sodium tetraborate were dissolved in distilled water with gentle heating and the sodium dodecyl sulphate and 2-ethoxyethanol were added. In a separate beaker, the disodium hydrogen orthophosphate was dissolved in distilled water with gentle heating. The two solutions were added together and diluted to 5 l. The pH was checked to be between 6.9 to 7.1.

Amylase solution 1:

Bacterial α-amylase (*Bacillus subtilis*, Fluka Chemicals Ltd, Gillingham, Dorset, UK)	0.8 g
Neutral detergent solution,	200 ml

The amylase was dissolved in the NDS with stirring.

Amylase solution 2:

Bacterial α-amylase (*Bacillus subtilis*, Fluka)	2 g
2-Ethoxyethanol,	10 ml
Distilled water,	90 ml

The amylase is dissolved in the water with stirred, and the 2-ethoxyethanol was added.

Procedure:

1. 0.5 g (Sample Weight) of 100°C dried material was covered with 25 ml amylase solution 1 in a 100 ml beaker, covered with a watch glass, and incubated overnight at 40°C.

2. The sample was transferred with an NDS washbottle to a 500 ml conical flask. 2 ml of amylase solution 2 and excess (100ml) NDS was added. The sample was heated to boiling and refluxed slowly for 1 hour on electric mantles.

3. Whilst still hot, the sample was filtered through a Whatman GF/A filter supported on a Whatman 541 cellulose filter. The residue was washed with plenty of hot tap water and given a final rinse of acetone.

4. The residue, GF/A filter and muffle basin were dried overnight at 100°C, cooled in a desiccator, and weighed (Weight A).

5. The residue (on its filter and basin), was ashed in a muffle oven (Lenton Thermal Designs Ltd, Worthing, UK) at 500°C overnight, cooled in a desiccator, and reweighed (Weight B).

5. $$\text{NDF g/kg DM} = \frac{(\text{Weight A} - \text{Weight B})}{\text{Sample Weight}} \times 1000$$

2.2.5 Determination of *In Vitro* Digestibility

This procedure, a modified version of the Tilley and Terry (1963) technique (Alexander, 1969) was carried out by the technicians of the Analytical Services Unit at Auchincruive.

Briefly, fresh (i.e. undried) material was incubated in filtered ovine rumen liquor anaerobically at 38.5°C for 24 hours, with an additional 24 hours after adjustment with HCl to pH 6.9, and a final 48 hours digestion at pH 1.2 with the addition of aqueous pepsin solution. The residue was filtered, dried at 100°C, weighed, ashed at 480°C, and weighed again. Control analyses allowed the determination of residual organic matter (from the rumen liquor) and hence the organic matter of the sample.

2.2.6 Prediction of Metabolisable Energy

For silages and very simple concentrates (as used in the experiment described in Chapter 3), the metabolisable energy (ME) content was predicted using the digestible organic matter in the dry matter (DOMD) calculated from the *in vitro* organic matter digestibility (IVOMD%), described in Section 2.2.5. The ME content of the silage was estimated as 0.16 x DOMD (see Thomas and Chamberlain, 1982).

For concentrates that were made from a number of raw materials, particularly those with a high fat content, ME was predicted using the E3 equation (Thomas, Robertson, Chamberlain, Livingstone, Garthwaite, Dewey, Smart and Whyte, 1988):

$$ME\ (E3) = NCGD\% \times 0.14 + AHEE\% \times 0.25$$

where NCGD% is the neutral cellulase gamanase digestibility expressed in g NCGD/100g and AHEE% is the acid hydrolysis ether extract content of the dry matter expressed in g AHEE/100 g (Section 2.2.11). NCGD (MAFF, 1992) determination was carried out by the staff of the Analytical Services Unit at Auchincruive, and is a modification of the NDF determination (Section 2.2.4), where the incubation of the material in amylase solution is replaced by an incubation with cellulase and gamanase (both supplied by Enzymatix, Cambridge, UK).

2.2.7 Determination of Starch

Soluble sugars are extracted from the sample with 40% ethanol. The starch is gelatinised and incubated with amyloglucosidase to convert it to glucose, which is subsequently determined spectrophotometrically (Feedingstuffs Evaluation Unit, 1981).

Reagents:

Ethanol Solution, 40% V/V.

Sodium acetate-acetic acid solution :

Anhydrous sodium acetate, pH 4.0,	164 g
Glacial acetic acid,	120 ml

The sodium acetate was dissolved in water, the acetic acid was added, and the solution was diluted to 1 litre in distilled water.

Amyloglucosidase suspension (Sigma, Poole, Dorset, UK), 2% W/V in distilled water.

Glucose standard solution (0.5 mg/ml):

Glucose (Analar),	0.5 g
Distilled water,	1 litre
Toluene,	A few drops

Working solutions of 0, 10, 20, 30, 40 and 50 μg/ml were prepared fresh by diluting the appropriate amounts of standard solution with distilled water.

Tris-phosphate Buffer:

Tris(hydroxymethyl)aminomethane (Analar),	36.3 g
Sodium dihydrogen orthophosphate dihydrate (Analar),	50 g
Distilled water,	1 litre
Phosphoric acid (Analar),	A few drops

The tris(hydroxymethyl)aminomethane and sodium dihydrogen orthophosphate dihydrate were dissolved in about 900 ml of the water. The pH was adjusted to 7.0 with phosphoric acid, and the solution was diluted to 1 litre.

Glucose oxidase-peroxidase solution:

Glucose oxidase (Sigma, Poole, Dorset UK),	0.100 g
Peroxidase (Sigma, Poole, Dorset, UK),	0.003 g
2,2' Azino-di (3-ethyl benzthiazoline sulphonic acid), diammonium salt (Analar),	0.050 g

These were diluted to 100 ml with tris-phosphate buffer; the resulting solution was stored in a dark glass bottle in a refrigerator.

Procedure:

1. 80 ml of 40% ethanol was added to 1 g (Sample Weight) of 100°C dried sample in a 120 ml bottle which was stoppered. The flask was allowed to stand for 1 hour, during which it was shaken vigorously 6 times to ensure thorough mixing of the contents.

2. The suspension was filtered through a Whatman GF/A filter supported on a 541 cellulose filter, the residue being washed with 40% ethanol solution. The residue and GF/A were transferred to a 120 ml conical flask with 20 ml distilled water, and the residue was shaken to ensure dispersion.

3. The residue was boiled gently for 3 minutes, the flask was capped with aluminium foil, and autoclaved in a pressure cooker for ½ hour at 120°C.

4. After allowing to cool, 20 ml water, 2.5 ml sodium acetate-acetic acid buffer and 2 ml amyloglucosidase suspension were added and mixed thoroughly. Two drops of toluene were added, and the suspension was incubated at 55°C for 18 hours overnight.

5. The suspension was filtered through a fluted Whatman 541 paper into a 250 ml volumetric flask, the residue was washed thoroughly with distilled water, and the filtrate diluted to volume.

6. Approximately 2.5 ml of the extract was filtered through a Whatman No. 1 cellulose filter, and 2 ml of this was diluted to 100 ml with distilled water.

7. To prepare the standard graph, 1 ml of each working glucose standard solution was used in place of the diluted extract in step 8.

8. 1 ml of each diluted extract was pipetted into a 10 ml stoppered tube. 2 ml of distilled water and 2 ml of the glucose oxidase-peroxidase reagent were added, and the reaction was allowed to proceed in a dark cupboard for 30 minutes.

9. The absorbance of the standards and each sample extract was measured with a Vitatron spectrophotometer (Fisons Instruments, Crawley, UK) using a 10 mm optical cell at 560 nm.

10. Standard glucose solution absorbances were entered into a computer program, which performed a curve fitting procedure. Sample absorbances were entered into the same program, and sample starch content was presented.

11. Alternatively, sample starch (g/kg DM) = μg glucose x 1.125 x 10
where % starch = μg glucose in 1 ml aliquot x $\frac{100 \times 125 \times 100 \times 0.9}{1000 \times 1000 \times \text{Sample Weight}}$

2.2.8 **Determination of Water Soluble Carbohydrates (Sugars) - Concentrates**

The Luff-Schoorl method depends on the extraction of sample sugars in aqueous ethanol. The extracted sugars are inverted and reduced, and the excess reducing agent is titrated. This method is set out in EEC Directive 71/250/EEC:OJ NO.L 155, 12.7.71, p.21.

Reagents:

Ethanol solution:

40% (V/V) neutralised to phenolphthalein.

Carrez solution 1:

Zinc acetate dihydrate (Analar),	21.9 g
Glacial acetic acid (Analar),	3 ml

The zinc acetate dihydrate was dissolved in water, the acetic acid was mixed in, and the volume made up to 100 ml with water.

Carrez solution 2:

Potassium ferrocyanide (Analar),	10.6 g

This was dissolved in water and diluted to 100 ml.

Methyl orange solution (Analar):

0.1 g per 100 ml water.

Hydrochloric acid solution (Analar), 0.1 M and 4 M.

Sodium hydroxide solution (Analar), 0.1 M.

Luff-Schoorl reagent:

Copper sulphate pentahydrate (Analar),	25.000 g
Citric acid monohydrate (Analar),	50.0 g
Sodium carbonate, anhydrous (Analar),	143.8 g

The copper sulphate was dissolved, with heating, in water and made up to 100 ml. The citric acid was dissolved in water and diluted to 100 ml. The sodium carbonate was dissolved in approximately 400 ml warm water, to which, after allowing to cool, the citric acid was carefully added with stirring. The copper sulphate solution was added, and made up to 1000 ml. This was allowed to settle overnight, and was then filtered. The pH of the solution should be about 9.2 and the molarity of the reagent should be Cu 0.1 M : Na_2CO_3 2 M.

Sodium thiosulphate solution (Analar), 0.1 M:

24.818 g dissolved in water and made up to 1000 ml.

Starch indicator solution:

Sodium chloride (May & Baker, Dagenham, UK),	33 g
Soluble starch (Analar),	1 g

The sodium chloride was dissolved with continuous stirring in 100 ml boiling water. After allowing to cool, with stirring, the clear liquid was decanted off. The starch was made into a cream with a small amount of water, the decanted liquid was added and heated to boiling with continuous stirring. The solution was allowed to cool.

Sulphuric acid solution, 6N:

Concentrated sulphuric acid (Analar),	160 ml

The acid was carefully added with stirring and cooling to 840 ml of water.

Potassium iodide solution:

Potassium iodide (Analar),	30.000 g

Made up to 100 ml with water, fresh each day.

Anti-bumping granules, washed in water and dried.

Anti-foaming agent:

Amyl alcohol (3-methylbutan-1-ol or iso-amyl alcohol) (Analar).

Procedure:

Steps 1-7 involve the extraction of sugars from the sample, steps 8-10 the inversion of those sugars, and steps 11-14 their determination by titration.

1. 1.000 g of 100°C dried sample was re-dried at 100°C overnight, allowed to cool, and re-weighed for a dry matter correction factor for use by the calculation software.

2. 2.500 g of each 100°C dried sample was weighed into a diluvial in preparation for analysis. Each sample was added to 200 ml of 40% ethanol in a 1 litre plastic bottle, using some of the ethanol to transfer it, and mixed for 1 hour on a rotary shaker (35-40 turns/minute).

3. 5 ml Carrez solution 1 was added and stirred for 1 minute.

4. 5 ml Carrez solution 2 was added and stirred for a further minute.

5. The residue was transferred quantitatively to a 250 ml graduated flask using 40% ethanol, mixed and diluted to volume with ethanol. It was then transferred to a 250 ml tallform beaker and allowed to settle. Approximately 125 ml of the supernatant was filtered under pressure using a Whatman 451 filter paper.

6. 100 ml of filtrate was evaporated in a fume cupboard to approximately half volume in a 250 ml shortform beaker, with a few anti-bumping granules, in order to eliminate most of the ethanol.

7. The cool residue was transferred quantitatively to a 100 ml graduated flask with water, made up to volume with water, mixed, and if necessary, filtered. This solution is inverted and used to determine the amount of total sugars.

8. 50 ml of the extract was transferred to a 250 ml Quickfit flask. Three drops of methyl orange solution were added. Using a pasteur pipette, 4 M hydrochloric acid was added, with continuous stirring, until the liquid turned pink.

9. 15 ml of 0.1 M hydrochloric acid was added, and the mixture was heated to boiling and refluxed for 30 minutes with a few anti-bumping granules.

10. The mixture was cooled rapidly in a tray of cold water to room temperature, and 15 ml of 0.1 M sodium hydroxide solution was added. It was then transferred to a 100 ml graduated flask, and diluted with water to volume. For feeds with a high sugar content the resulting solution should be diluted further so that 25 ml contains less than 60 mg reducing sugars expressed as glucose.

11. 25.0 ml of the inverted sugar extract was added to 25.0 ml of Luff-Schoorl reagent in a 150 ml Erlenmeyer flask. Two anti-bumping granules and 1 ml amyl-alcohol were added, a reflux condenser was fitted, and the mixture was heated on a hot plate range (model EV 16, Gerhardt UK Ltd, Crewe, UK) which had been regulated to bring the liquid to the boil in 2 minutes ($\pm$ 5 seconds). At boiling, the heat was lowered to allow simmering for exactly 10 minutes. The mixture was then cooled immediately in a tray of cold water, and left for at least 5 minutes before proceeding with the titration.

12. A blank titration was carried out (without boiling) on a mixture of 25.0 ml Luff-Schoorl reagent and 25 ml water, following the steps below.

13. 10 ml of potassium iodide solution was added, immediately followed by 25 ml of 6 M sulphuric acid, carefully added in small increments with constant gentle swirling to avoid excessive foaming.

14. Sodium thiosulphate solution was used to titrate the solution using a digital burette (Bibby, UK). Sodium thiosulphate was added until a dull yellow colour appeared, at which point 1 ml of the starch indicator was added. Further sodium thiosulphate was added to finish the titration; the end-point is milky-white, flesh-coloured, or off-white depending on the colour of the sample.

15. Sample sugar content, expressed as sucrose, was calculated by computer program based on a standard curve.

2.2.9 Determination of Water Soluble Carbohydrates (Sugars) - Silage

This method, carried out by the staff of the Analytical Services Unit at Auchincruive was based on that of McDonald and Henderson (1964) and the arsenomolybdate reagent of Nelson (1944).

The determination is carried out on the water soluble extract from 20 g sample of fresh (i.e. undried) silage. The sucrose is inverted with sulphuric acid, protein is precipitated with zinc sulphate and barium hydroxide. The extract glucose content was determined spectrophotometrically after colour development with Somogyi's reagent (alkaline copper tartrate) and Nelson's arsenomolybdate reagent, and compared with standard solutions of glucose.

2.2.10 **Determination of Ether Extract (EE)**

The EE content of a feed is the fats and oils soluble in petroleum ether. The method employs the Soxtec extraction equipment (Tecator, Bristol, UK).

Reagent:

Petroleum ether, 40-65°C boiling point.

Procedure:

1. 2 g (Sample Weight) of 60°C dried material was weighed into a cellulose extraction thimble, and was covered with a plug of cotton wool. The thimble was fitted with a metal collar.

2. Aluminium extraction beakers were dried at 100°C, allowed to cool in a desiccator, and weighed accurately (Weight A). The extraction unit water heating system was heated to 70°C.

3. The thimbles were attached to the magnets on the Soxtec 1040 extraction equipment. Approximately 50 ml of petroleum ether was measured into each extraction beaker, and the beakers were placed on the heating elements with a little water to ensure good heat conduction.

4. The thimble mechanism was jacked down onto the beakers, the top valves were opened, and the thimbles were lowered into the ether (to the "Boiling" position) and left for 20 minutes.

5. After the boiling period, the thimbles were raised to "Rinse" and left for 25 minutes.

6. After rinsing, the top valves were closed, the lever was put to "Evaporate", the air flow was turned on, and the equipment was left for a further 15 minutes.

7. The extraction beakers were dried at 60°C for 1 hour, desiccated for ½ hour, and weighed (Weight B). They were then re-dried for a second time of ½ hour, desiccated for ½ hour and reweighed (Weight C).

8. $$\text{EE g/kg DM} = \frac{(\text{Weight B} + \text{Weight C})}{2 \times \text{Sample Weight}} - \text{Weight A}) \times 1000$$

2.2.11 Determination of Acid Hydrolysis Ether Extract (AHEE)

AHEE is the ether extract of a sample, consisting of the EE plus fats and oils released from the sample after a digestion with acid (MAFF, 1992).

Reagents:

Petroleum ether, 40-65°C boiling point.

Hydrochloric acid, 3M.

Procedure:

1. An ether extraction, described in steps 1-7 of section 2.2.10, is carried out with 2 g (Sample Weight) of 60°C dried material.

2. The ether was allowed to completely evaporate from the residue of the extraction, which was then transferred with a spatula from the thimble to a 500 ml round-bottomed flask.

3. 1 teaspoonful of Supercel filter aid was added to the residue, followed by 100 ml 3M HCl. A reflux condenser was fitted to the flask, the contents were brought rapidly to the boil and refluxed for 1 hour.

4. The flask was allowed to cool *in situ* for at least 40 minutes to ensure no fat was lost during filtration.

5. The digested material was filtered into a sintered crucible (P2 porosity) and was washed with copious water to remove all traces of the acid. It was then dried overnight (in the crucible) at 60°C.

6. The contents of the crucible was transferred with brushing back into the original thimble. A second ether extraction was performed but with prolonged extraction times of 40 minutes boiling (step 4, section 2.2.7) and 45 minutes rinsing (step 5, section 2.2.7).

7. $$\text{AHEE g/kg DM} = \frac{\text{1st extraction fat weight} + \text{2nd extraction fat weight}}{\text{Sample Weight}}$$

2.2.12 **Kjeldahl Digestion**

This technique was carried out by the technicians of the Analytical Services Unit at Auchincruive. It was a standard Kjeldahl digestion in an acid solution of 40 g selenium dioxide dissolved in 2 l of 95% sulphuric acid. 0.25 g of 100°C dried material was digested in 7.5 ml acid digestion solution, on a Tecator 1006 heating block (Tecator Ltd, Bristol, UK). 3 ml of hydrogen peroxide was used as the oxidant.

2.2.13 Determination of Protein Content

This technique was carried out by the technicians of the ASU at Auchincruive. After a Kjeldahl digestion (Section 2.2.12), the indophenol blue method was used to determine the nitrogen content of the sample material (0.25 g starting weight, diluted to 75 ml in distilled water after digestion). Absorbance was measured at 584 nm, and the N content calculated from a linear regression performed on standards. Protein content was calculated as N x 6.25.

2.2.14 **Determination of Minerals**

This procedure was carried out by the Auchincruive ASU technicians, and consisted of a Kjeldahl digestion (Section 2.2.12) of the sample material followed by determination of mineral contents by atomic emission spectrometry (Alexander, Dixon and McGowan, 1985).

Reagent:

Acid reagent:

Nitric acid, 12%,	approx. 1 l
Triton X-100,	1.0 g

The Triton X-100 was dissolved in nitric acid, and made up to 1 l, to make a 0.1% solution.

Procedure:

1. A Kjeldahl digestion was carried out on 0.250 g of sample material, as steps 1-3 section 2.2.12.

2. 1 ml of acidified Triton X-100 was added to 5 ml of diluted digest. Mineral content of this was calculated according to the manufacturers instructions using an Instrumentation Laboratory 100 inductively-coupled plasma (ICP) spectrometer (Thermoelectron Ltd, Warrington, UK), using standards for comparison.

2.3 MILK ANALYSIS

Milk samples were analyzed for a variety of constituents, and the method of preservation depended on the analysis used. Milk destined for determination of fat, protein and lactose using the Milko-Scan analyzer (Foss Electric, Denmark) was preserved with one Lactab (potassium dichromate/sodium chloride tablets; Thompson and Capper Ltd, Runcorn, Cheshire, UK) and stored in a refrigerator; all other milk was either stored in a refrigerator without preservatives and analyzed as soon as possible, or frozen and stored at -20°C.

All milk samples were analyzed by the Department of Dairy Technology at Auchincruive, except for the determination of milk minerals, which was carried out by the Analytical Services Unit at Auchincruive. The analyses used are presented in the following sections for clarity.

2.3.1 Fat, Protein and Lactose

Milk samples were determined for these using the Milko-Scan 203 milk analyzer (Foss Electric, Denmark). This used near-infrared detection of these three constituents calibrated on milk samples provided by the SAC Crichton Royal Farm, Dumfries, for which the constituents had been determined using reference methods. Milk protein was calibrated using a Kjeldahl analysis of total nitrogen (N x 6.38), and the Milko-Scan machine therefore gave an estimate of milk crude protein on the assumption that the ratio of true protein to NPN was constant. Since this near infrared technique does not measure the NPN fraction, any change in the NPN:true protein ratio of milk measured in this way alone could not be seen, and this accounts for some of the differences between milk crude protein concentrations measured by Milko-Scan and Kjeldahl analyses.

2.3.2 Milk Minerals

Unpreserved milk samples for mineral analysis were freeze dried (Virtis Company, Gardiner, NY, USA) at 0°C under a vacuum (to approximately 10 millitor) on aluminium food trays. The freeze dried milk was then analyzed for minerals as described in section 2.2.14, with the exception of chloride which was measured by titration.

2.3.3 Determination of Total Milk Nitrogen

This was done following the procedure as defined by BS 1741 Section 5:2 1990 Modified, which is a Kjeldahl digestion.

2.3.4 Determination of Milk Casein

The casein content of milk was defined as the protein precipitated from milk at pH 4.6 at 20°C. It was measured following the International Dairy Federation method FIL-IDF 29:1964. Total nitrogen content of the milk was determined, and the casein nitrogen content was calculated as the difference between this and the nitrogen content of the filtrate of milk after the casein had been precipitated using an acetic acid-acetate buffer. Casein = (Total N - Non-Casein N) x 6.38.

2.3.5 Determination of Milk Non-Protein Nitrogen

This was determined by Kjeldahl analysis after the true milk protein content had been precipitated using trichloroacetic acid.

2.3.6 Determination of Milk Urea Nitrogen

Milk urea nitrogen was determined using Sigma test kit No. 640, following the procedure for the determination of urea in blood serum. Milk urea concentration was calculated by multiplying by 60/28.

2.3.7 Determination of Milk Organic Acids

This was carried using the high-performance liquid-chromatography (HPLC) technique of Marsili, Ostapenko, Simmons and Green (1981).

A variety of organic acids were quantified in milk using this method, including uric acid. In order to determine whether allantoin could be measured in this way, spiking experiments were undertaken to see where, if at all, allantoin was to be found on the chromatogram. This revealed a serious flaw in the technique not considered by Marsili *et al.* (1981), in that allantoin was eluted at exactly the same time as uric acid, and was detected as part of the peak identified as uric acid. The detection of uric acid was much more sensitive than that of allantoin, some 5.98 times greater. This means that if allantoin is present in milk at the same concentration as uric acid, uric acid concentration would be overestimated by approximately 17%. Rosskopf, Rainer and Giesecke (1991), however, reported milk allantoin concentrations to be over 7 times greater than uric acid

concentrations, which would make the contribution of allantoin to the "uric acid" peak approximately 50%., i.e. doubling the estimate of actual uric acid content.

This problem with the technique meant that whilst it was impossible to determine actual values of milk uric acid concentration with any confidence, it provided a rough estimate of the 'total' purine derivative concentration of milk (see section 3.2.9), assuming that the ratio of uric acid to allantoin was constant in milk.

A further difficulty arose with this technique after analyses were completed for the experiment described in Chapter 4. The column was replaced to achieve better separation of the lactic acid peak from surrounding peaks, for a purpose unrelated to these studies. A side effect of this was that citric acid and orotic acid coeluted, and an accurate quantification of these compounds was therefore not possible when milk organic acids were analyzed for a subsequent experiment (see Chapter 6).

2.4 DETERMINATION OF URINARY PURINE DERIVATIVES

2.4.1 Collection of Urine Samples

'Spot' urine samples were taken to allow the estimation of urinary purine derivatives (PD) using urinary creatinine as an internal marker for estimating volume (de Groot and Aafjes, 1960; Albin and Clanton, 1966; Erb, Surve, Randel and Garverick, 1977). Attempts to collect total urine samples with the use of stick-on external urine separators proved unsuccessful. Various devices constructed from vinyl, lay-flat tubing, washing machine hoses, sticky tape, Velcro and large plastic containers, all failed to provide satisfactory collections. They either leaked, came unstuck, got knocked off by the cow's tail, or simply fell apart. Furthermore, having a device glued over the vulva made it difficult to detect signs of oestrous and caused skin irritations in some cows, reducing feed intake and generally interfering with other aspects of the animals performance. It was therefore decided that spot urine collections would be used for the assessment of urinary PD. This imposed the least discomfort to the cows, eliminated the need for preservatives in the urine as it was collected, and simplified the sample preparation. Opposed to that, absolute daily yields of purine derivatives could not be calculated.

Spot urine samples were collected from cows using a combination of 'tickling' and behaviour. The animals were left alone for as long a period as possible before the sampling was due to take place, which meant that they had time to lie down and rest. When they were made to stand up for sampling, their first actions were often to urinate and defecate, making it much easier to collect a clean urine sample (and also a fresh sample of faeces). Cows which did not spontaneously urinate during the collection time were 'tickled'. Manual stimulation of the area immediately below the vulval, either side of the vulval, or on the vulva itself, induced the animal to urinate. This response was usually instant once the cow had become accustomed to the procedure (assuming there is urine in the bladder), and greater success was achieved once the tickler had become accustomed to the cow; knowing which areas of individual animals caused the best response meant that sampling time could be kept to a minimum. The sampling of 12 stall tied cows was usually completed in 10-30 minutes; if an animal had not urinated within about 1 hour of starting, it was abandoned on the assumption that she had probably urinated shortly before the start.

2.4.2 Determination of Urinary Purine Derivatives

The concentrations of urinary PD were determined directly in urine by HPLC. The method was a modified form of that used by Balcells, Guada, Peiro and Parker (1992). The individual PDs were separated and eluted using a gradient of acetonitrile with ammonium dihydrogen orthophosphate, and were detected at a wavelength of 205 nm.

2.4.2.1 Instrumentation

Column:

2 x S5ODS2 reverse-phase C18 (250 x 4.6 mm internal diameter, Phase Separations Ltd, Deeside, Clwyd, Wales) fitted in series, with a 25 mm guard column of the same specification.

Pump, Detector, and Integrator:

Ternary pump (model SP8000), UV detector (model Spectra 200), and integrator (model SP4600) all supplied by Spectra-Physics Ltd (Hemel Hempstead, Herts, England).

Autosampler:

Gilson model 232-401 (Gilson Medical Electronics (France) SA, Villier-Le-Bol, France).

2.4.2.2 Eluents

All water used was purified using a Fistreem Multipure de-ioniser (Fisons, Loughborough, UK), since it was much cheaper than commercial HPLC grade water and had acceptable UV absorption characteristics.

Stock buffer:

Ammonium dihydrogen orthophosphate, 11.503 g
(Sigma Chemical Co., Poole, UK)
This was dissolved in Multipure water and made up to 1.0 l.

The working eluents were prepared fresh daily from this stock buffer kept at 4°C. One litre of eluent A and 0.5 l of eluent B was sufficient for approximately 24 hours analysis.

Before and during use, both eluents were thoroughly degassed using a constant stream of helium.

Eluent A:

Stock buffer,	100 ml
Multipure water,	900 ml
Phosphoric acid, 0.1 M,	A few drops

Approximately 1 l was prepared by mixing the buffer and water and adjusting the pH to 4.0 with phosphoric acid.

Eluent B:

Stock buffer,	50 ml
Multipure water,	350 ml
Phosphoric acid, 0.1 M,	A few drops
Acetonitrile (Rathburn, Glasgow, UK; HPLC grade),	100 ml

Approximately 500 ml was prepared by mixing the buffer and water, adjusting the pH to 4.0 with phosphoric acid, and adding the acetonitrile.

2.4.2.3 Chromatographic Conditions

Urinary PD, creatinine and allopurinol were analyzed by a gradient of eluents A and B as shown in Table 2.1, run at 0.8 ml per minute. The effluent was monitored at a wavelength of 205 nm.

The total time taken for the complete analysis was 55 minutes, starting with a 6 minute isocratic period. By 27 minutes, all the required compounds had been eluted and the rest of the period was used for column re-equilibration.

Table 2.1 Eluent gradients programmed into the pump.

Time (min)	Eluent A	Eluent B
0	100	0
6	100	0
20	50	50
25	0	100
35	0	100
40	100	0
55	100	0

Approximately every 100 samples, or at the end of each week, the column was cleaned with 20% acetonitrile water for 4-5 hours at 1.0 ml/min.

2.4.2.4 Standard Preparation

A standard solution containing allantoin, uric acid, creatinine, xanthine, hypoxanthine and allopurinol was prepared for calibration purposes.

Standards:

Allantoin (Eastman Kodak Co., Rochester, NY, USA)	0.1581 g
Allopurinol (Sigma Chemical Co., Poole, UK),	0.1361 g
Creatinine (Aldrich Chemical Co. Ltd, Gillingham, Dorset)	0.1132 g
Hypoxanthine (Aldrich),	0.1361 g
Uric acid (Aldrich),	0.1681 g
Xanthine (Aldrich),	0.1521 g

The uric acid and xanthine were dissolved in hot alkalised water (pH > 10, using 0.1 M sodium hydroxide solution), allowed to cool, and pH readjusted with 0.1 M phosphoric acid to 6.0 before being made up to 500 ml in Multipure water. The allantoin, allopurinol, creatinine and hypoxanthine were dissolved in Multipure water and made up to 500 ml. The final standard solution containing 1 mM of each compound was made by adding together the two 500 ml solutions.

A number of 1.5 ml aliquots of this were frozen immediately in microtubes and stored at -20°C. Occasionally, a tube was thawed and diluted 1 in 10 with stock buffer ready for use. Diluted standards were refrozen in autosampler vials, and stored at -20°C.

2.4.2.5 Sample Preparation

Diluting buffer:

Ammonium dihydrogen orthophosphate,	11.503 g
Allopurinol,	0.1483 g

The $NH_4H_2PO_4$ and allopurinol were dissolved together in Multipure water, and the volume made up to 1 l.

A 75 μl aliquot of urine was diluted with 1.425 ml diluting buffer. This was done directly into an autosampler vial, which was then sealed with a teflon or teflon-coated rubber seal and crimped cap. Once diluted, the sample contained 1.0 mM allopurinol as an internal standard.

If more than one replicate of each sample was analyzed, the batch was analyzed completely once before being re-analyzed for a second time.

2.4.2.6 Calibration and Integration

The integrator was set to determine concentration by peak height, using a solution of standards to calibrate the machine at the start of each run. Peak height was found to be a more satisfactory measurement than peak area because several of the peaks were not completely separated at the base from neighbouring peaks. Allopurinol was originally included as an internal standard. However, due to the simplicity of the sample preparation, occasional misidentification of the peak by the integrator, and since the absolute concentrations of urinary PD were not required, it was kept as a standard for verification purposes only, and not used to correct for any sample dilution/concentration effects. Approximate retention times of the standard compounds are presented in Table 2.2.

Table 2.2 Retention times (minutes) of standard compounds.

Standard	Retention Time
Allantoin	7.42
Creatinine	14.51
Uric acid	21.10
Hypoxanthine	21.90
Xanthine	23.97
Allopurinol	24.62

2.4.3 Developmental Work

Initially, there were problems in running the technique of Balcells *et al* (1992) on the available equipment. Allantoin was one of the first compounds to be eluted from the column, and there were problems separating allantoin and another unidentified peak that was eluted shortly afterwards. The separation of these two peaks was improved by increasing the isocratic period to 6.0 minutes at the beginning of the run. Changing the pH of the eluents did not improve separation, and so this was left at 4.0, as in the original technique. Filtering the eluents and stirring eluent B, as advocated by Balcells *et al* (1992)

was not done, since no difference in the chromatograms was seen between solutions so prepared and solutions mixed and used without filtering or stirring.

The recovery of compounds in the sample was tested by spiking random samples of fresh urine with increasing concentrations of standards. The response of peak area was found to be linear in the range 0-12 mM added standard (i.e. 1 ml fresh urine + 1 to 12 ml of 1 mM solutions of standards, diluted to 20 ml in stock buffer), with $r^2=1.0$ for all compounds. The fact that some standards were lower in concentration than normal urinary concentrations (e.g. allantoin) whilst others were higher (e.g. xanthine and hypoxanthine) was considered unimportant due to the linearity of the system.

The run time of 55 minutes was rather long for when large numbers of analyses were required. Duplicate analyses of the same sample were carried out on the urine samples collected during the experiments described in Chapters 3 and 4, and the duplicates were found to closely agree, usually within 2-3%. For example, for urinary allantoin concentrations (Chapter 4), the mean proportion of first to second duplicate was 1.029, SE 0.009 (n=142). The slightly lower concentration found in the second duplicate is perhaps consistent with slight degradation of allantoin over time. This was tested by leaving standards and urine (fresh and diluted) on the laboratory bench and in a refrigerator for 24 and 48 hours but there was little apparent effect on the concentrations of purine derivatives. The differences of 2-3% between duplicates were more likely to be integration errors or errors associated with changes in the column binding characteristics over time. However, such errors were felt to be acceptable, and due to the small differences in duplicate analyses of early work, samples were analyzed once only during later work as it was felt that the HPLC system was better used for analysing more samples once than proportionally fewer samples more than once.

Chapter 3

The Influence of Dietary Energy Source and Dietary Protein Level on Milk Protein Concentration

3.1 SUMMARY

This chapter reviews some of the effects on milk protein of the two major dietary factors which influence it: dietary protein content and source of dietary energy (and their interaction). A 2x2 factorial experiment was carried out to investigate the effects of dietary protein level and energy source (barley versus sugar beet pulp). Urinary purine derivatives were used to estimate yields of microbial protein from the rumen in order to separate some of the effects of rumen and animal metabolism. Significant effects of diet on milk protein concentration were observed, with the high protein and barley-based diets producing higher milk crude protein concentrations than low protein and sugar beet pulp-based diets. No interaction effects were seen, although the barley high-protein diet produced significantly greater effects than the other three diets. The effect of barley-based diets was estimated to be in part at least due to increased supplies of metabolisable protein to the animal, with some evidence of effects which might have resulted from differences in rumen fermentation patterns between the two energy sources.

3.2 INTRODUCTION

Energy and protein have been identified as the two major attributes of the dairy cow diet which exert the greatest influence on the concentration of protein in milk (Emery, 1978; Thomas, PC, 1984; Spörndly, 1989; DePeters and Cant, 1992). This section aims to introduce in some detail the effects that these have on milk protein concentration, and to expose areas where knowledge is lacking.

3.2.1 Dietary Energy

An increase in the 'plane of nutrition' of the animal through an increased supply of energy yielding compounds has long been known to increase the quantity of milk produced by a dairy cow. Compounds which supply the majority of the energy to the animal can generally be classified as one of three types: fats, nonstructural (or storage) carbohydrates, and structural carbohydrates. As described briefly in Chapter 1, each of these has the potential to affect the concentration of milk protein, and therefore must be considered when formulating a dairy cow diet with the aim of increasing the milk protein concentration. Amino acids can be oxidised or used as a source of energy; this is biologically expensive and generally occurs in animals only as a last resort. However, milk production in the dairy cow has been increased to the extent where the high-yielding, early lactation animal cannot consume sufficient quantities of food to support lactation and has to rely heavily on 'alternative' sources of energy such as amino acids and body reserves at this time. Even at other times, when the animal is in positive energy balance, an increase in energy supply will often bring about an increase in milk production. Energy supply is clearly limiting at certain times of the lactation; there is some evidence with goats which suggests that in addition to limiting milk production, the relative rates of body protein synthesis and degradation change, releasing amino acids from labile protein stores when energy supply to the animal is very low (Riis, 1988; Champredon, Debras, Patureau Mirand and Arnal, 1990). It has been estimated that up to 27% of the dairy cow body protein may be utilised in this way (Botts, Hemken and Bull, 1979); this is discussed further in Chapter 6.

One of the two major energy types, dietary fat, is discussed in Chapter 4 and I do not intend to consider it further at this point. There are various characteristics of dietary carbohydrates, on the other hand, that are important with regards to the influence that the cow's diet has on the yield and composition of her milk, and of milk protein in particular.

As briefly mentioned in Chapter 1, energy quality as well as quantity must be considered. Ruminants, like almost all animals, lack cellulase and in order to utilise the energy locked up in cellulose (and other fibres) rely on their symbiotic relationship with rumen microorganisms. The principal end products of carbohydrates in the rumen are the volatile fatty acids (VFAs) acetate, butyrate and propionate, and methane and carbon dioxide (Figure 3.1). Methane and carbon dioxide, as well as heat, represent losses to the animal, whereas the VFAs and microbial cells serve as sources of energy and protein. Ruminant metabolism has therefore evolved to use VFAs for energy and since little, if any, dietary glucose gets past the tissues of gastrointestinal tract (Reynolds, 1992) ruminants rely on hepatic gluconeogenesis (from propionate, for example) to meet their glucose needs. This means that the high yielding dairy cow, which has a high requirement for glucose for lactose synthesis alone, may be more sensitive to changes in supply of gluconeogenic precursors than other ruminants.

The balance of products of fermentation is influenced by the type of dietary carbohydrate, depending on the metabolism and species of the microbial fermenter. Those fractions of the foodstuff which escape rumen fermentation pass into the duodenum and are available for digestion. The site of degradation, rumen or small intestine, influences the products

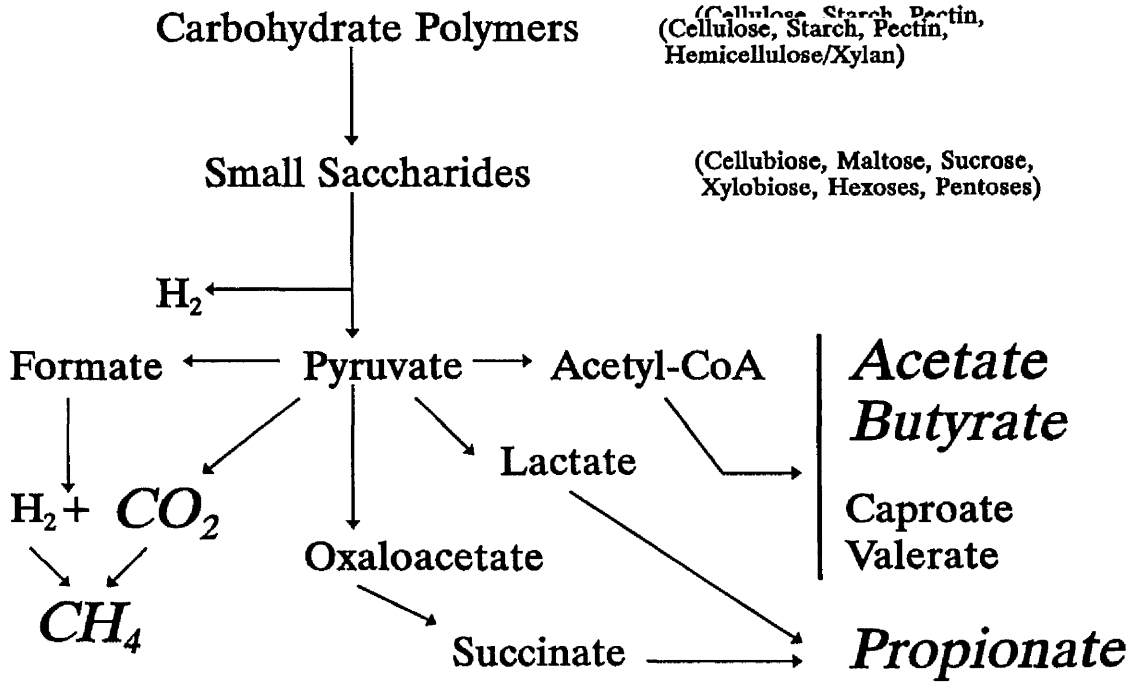


Figure 3.1 A generalised scheme of ruminal degradation of carbohydrates. Acetate, butyrate, propionate, CO_2 and CH_4 accumulate in the rumen as a result (after Russell and Hespell, 1981).

of that degradation. The degradation of starch and sugars in the rumen (see Nocek and Tamminga (1991) for an extensive review), leads to the absorption of a mixture of VFAs with a relatively high propionate:acetate ratio whilst the fermentation of fibres leads to a mixture that is relatively lower in propionate. In the duodenum and beyond, starch and sugars that escape rumen fermentation are degraded enzymatically and are absorbed as glucose. The rumen is the major site for fibre digestion and much of the fibre passed out of the rumen is indigestible. Furthermore, the rumen microbial population has its own requirements in terms of the quality of feedstuff, nitrogenous compounds as well as energy yielding compounds, and a correct balance must be achieved to gain the maximum benefit (Nocek and Russell, 1988).

3.2.2 Energy Quality

Energy quality, that is, the form of the energy-yielding compounds, can be as important in terms of the influence that the diet has on milk protein concentration as the level of energy intake by the animal. In their reviews both Emery (1978) and Spörndly (1989) found positive correlations between energy intake and milk protein concentration. Emery (1978), however, also found a negative correlation between the proportion of 'roughage' in the diet and milk protein concentration. There are a number of ways in which changes in the dietary energy quality can be brought about, two of which are: a) feeding concentrate supplements to a diet based on forage, and b) changing the sources of energy within those supplements. The consequences of these are that the forage to concentrate ratio may change and the products of rumen fermentation could be altered, both of which can influence milk protein production.

3.2.3 Forage to Concentrate Ratio

A common winter dairy cow management practice in the United Kingdom is to feed a forage-based diet (frequently grass-silage) supplemented with concentrates. These are often high in starch and relatively low in fibre, although this is not always so. Thomas, Aston, Daley and Bass (1986) found decreases in silage intake as the concentrate allowance was increased, and this partial substitution effect is commonly seen; the decrease in forage energy as forage intake is reduced is generally less than the increase in concentrate energy supply, with a net increase in energy supply to the animal. This increase in the plane of nutrition of the animal is a confounding factor in the investigation of changes in milk composition due to differences in energy quality (i.e. effects could be due to changes in

energy quality or net energy supply).

To investigate the effect of high-grain diets on the production of milk protein, Yousef, Huber and Emery (1970) compared *ad libitum* and restricted intakes of hay and maize silage with grain supplementation. They found significant increases in milk protein (principally casein) concentrations, with increasing grain allowances. These would have caused large increases in energy intake, as well as decreasing the forage to concentrate (F:C) ratio. Mosely, Coppock and Lake (1976), comparing diets with F:C ratios of 95:5, 80:20, 60:40 and 40:60, switched animals abruptly from one diet to another. Significant increases in milk protein concentration were found with increasing concentrate proportions. However, the effects were confounded with increases in net energy intake with decreasing F:C making it difficult to interpret the results. Likewise, MacLeod, Grieve and McMillan (1983) investigated four F:C ratios using dairy heifers, and found significant linear and quadratic increases in milk protein concentration ranging from 31.1 g/kg crude protein on an 80:20 diet to 32.6 g/kg on a 35:65 diet. However, although these diets were similar in digestible energy, dry matter intake increased with increased concentrate proportion, again making it difficult to attribute the effects to either changes in F:C ratio or increases in energy intake (or a combination), a problem with similar results found by Bartsch, Graham and McLean (1979). In one trial (Tessmann, Radloff, Kleinmans, Dhiman and Satter, 1991), a range of F:C ratios were tested, and a decrease in milk protein concentration, with a concomitant decrease in milk yield, was achieved with an increase in the proportion of forage in the diet. These results were almost certainly due to the effect of a decrease in dry matter intake with increasing forage, bringing about a decrease in metabolisable energy intake.

Several authors have studied the effect of dietary F:C ratio with isoenergetic diets (Evans, Buchanan-Smith, MacLeod and Stone, 1975; Grant and Patel, 1980; Hansen, Otterby, Linn and Donker, 1991). In an experiment comparing two diets of high and low fibre contents with similar digestible energy fed at similar rates, significant differences between the milk protein concentrations from the two diets were seen (Evans *et al.*, 1975). No significant differences were observed on milk yield and fat and lactose concentrations, but milk protein was found to increase from 27.8 g/kg on the high fibre diet to 34.4 g/kg on the low fibre diet. Hansen *et al.* (1991) compared 3 levels of concentrate feeding (40, 50 and 60% of diet DM) with two hays, and reported increasing milk yields with increasing

quantities of concentrates. Dry matter intake did not differ between treatments, and neither did milk protein concentration, although protein yields increased with milk yields. However, Grant and Patel (1980) compared two ratios (40:60 and 60:40) of a cereal based concentrate fed with hay whilst keeping the total digestible nutrient (TDN) contents of the diets similar. They found a decrease in crude protein content of milk on the 60% concentrate diet compared to the 40% diet. This was due to reductions in both the casein and whey components, and although the fat content of the diet was not reported, these trends are characteristic of increased dietary fat effects (see Chapter 4), and may well have resulted from increased supplies of fats in the soya bean and rapeseed meals included as protein supplements.

In conclusion, the effect of changes in forage to concentrate ratio remains unclear (Figure 3.2), although an increase in concentrate tends to lead to increasing energy intake. The interactions of dietary energy and protein are such that effects attributed to energy may well be brought about by changes in the protein supply, with increased efficiencies of utilisation of rumen degradable nitrogen leading to increased supplies of metabolisable protein to the animal and hence to increasing milk protein concentrations.

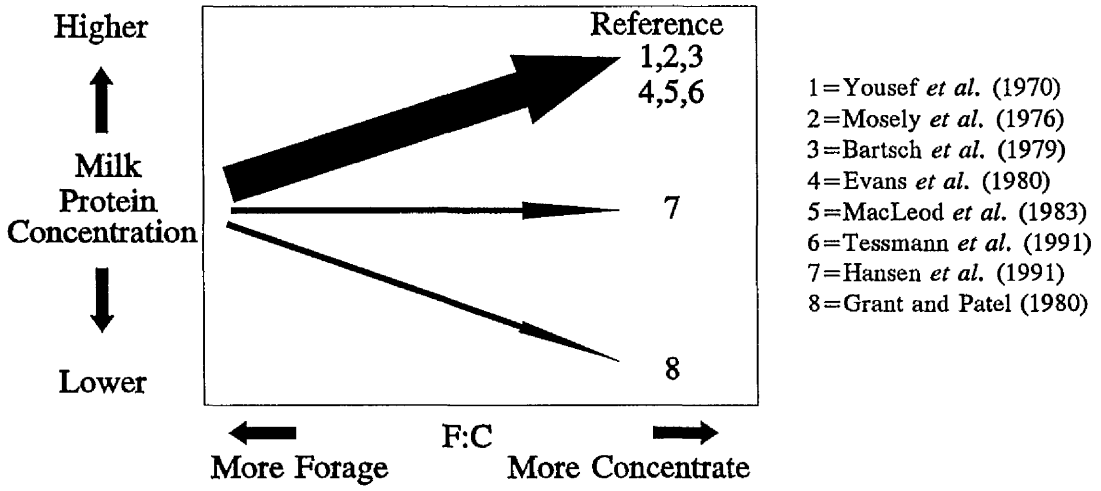


Figure 3.2 Diagram of effect of dietary forage:concentrate (F:C) ratio on milk protein concentration.

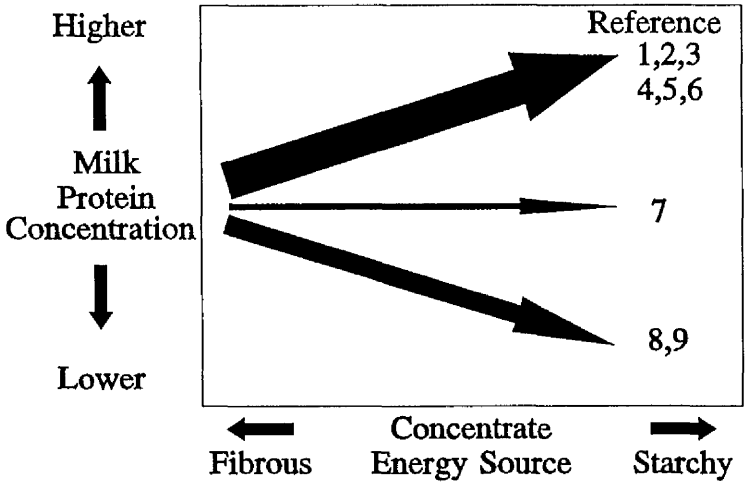
3.2.4 Concentrate Energy Quality

There is a large amount of interest in using the cheap sources of energy which can be found in by-products such as sugar beet pulp and spent distillers grains, making them attractive for inclusion in ruminant concentrates. Sugar beet pulp is relatively rich in residual sugars, particularly through the incorporation of molasses, although a high proportion of the energy yielded is due to the high fibre content. For this reason, a number of studies have investigated the feeding value of sugar beet pulp comparing it to supplements such as barley, a relatively expensive animal feed at present because of competition with human food markets and market subsidies.

Bhattacharya and Sleiman (1971) found that sugar beet pulp could replace barley (or maize) on an equal basis as a supplement to a forage-based diet (in this case hay) with similar metabolisable energy contents and dry matter digestibilities being reported, and they concluded that there were no significant differences between sugar beet pulp and barley in a lactation diet, although they did not measure milk proteins. Bhattacharya and Lubbadah (1971) measured milk protein in an experiment comparing the substitution of maize with sugar beet pulp at several rates in dairy cow diets, and found no significant differences between diets.

In experiments comparing starch versus fibre (from various sources such as beet pulp and citrus pulp) as supplements to silage, Sloan, Rowlinson, and Armstrong (1988) found significantly higher milk protein concentrations from dietary supplements low in neutral detergent fibre (i.e. higher in starch). MacGregor, Stokes, Hoover, Leonard, Junkins, Sniffen and Mailman (1983), comparing low and high starch supplements to silage, found nonsignificant increases in milk protein concentrations with high starch supplements.

Significant increases in milk protein concentration from dairy cows were observed on barley supplemented diets compared to sugar beet pulp supplemented diets (Lees, Oldham, Haresign and Garnsworthy, 1990; de Visser, van der Togt and Tamminga, 1990). Others have reported small but non-significant increases when sugar beet pulp was replaced by barley (Sloan, Rowlinson and Armstrong, 1987; Thomas *et al.*, 1984). In contrast, small decreases in milk crude protein (Figure 3.3), when comparing animals on barley supplemented silage-based diets to those fed a sugar beet pulp supplement, have been reported (Castle, Gill and Watson, 1981; Mayne and Gordon, 1984).



1=MacGregor *et al.* (1983)
2=Thomas *et al.* (1984)
3=Sloan *et al.* (1987)
4=Sloan *et al.* (1988)
5=Lees *et al.* (1990)
6=de Visser *et al.* (1990)
7=Bhattacharya and Lubbadah (1971)
8=Castle *et al.* (1981)
9=Mayne and Gordon, (1984)

Figure 3.3 Diagram of effects of concentrate energy source on milk protein concentration.

Acetate from the ruminal fermentation of dietary carbohydrates can be incorporated into fats (via acetyl-CoA); similarly amino acids from microbial sources are used for milk protein production. Because energy and protein metabolism are closely inter-related in the rumen (Nocek and Tamminga, 1991) differences in milk composition can be brought about by altering the source of energy in the diet. The mixtures of products of the fermentation of fibres and starch are often quite different, depending on the composition of the dietary input. A depression in milk fat is frequently seen with animals on diets where fibre, either from forage sources (Thomas, C, 1984; Sutton and Morant, 1989) or in supplements (Sutton, 1984), is replaced by starchy feeds (Grummer, Jacob and Woodford, 1987), probably because of the reduction in the rumen acetate:propionate ratio. The opposite is frequently seen with milk protein; in fact, changes in the concentrations of milk protein and milk fat tend to occur in opposing directions although this is usually a consequence of the different precursor requirements of the two milk components and not as a result of direct competition for precursors. Coulon and Rémond (1991), reported that the response of milk fat to diet was independent of those of milk yield and protein in a study of the results of sixty-six feeding trials; changes in the acetate:propionate ratio have frequently been found to be due to increases in propionate at relatively constant levels of acetate, propionate being a precursor for a different area of milk production. With diets based on hay, Lees *et al.* (1990) found significantly higher acetate + butyrate:propionate ratios on diets high in fibre (hay + sugar beet pulp) than from diets higher in starch (hay + flaked maize). They found that milk fat concentrations were significantly higher on the high fibre diets, as would be expected with more acetate available for fat synthesis. However, no

effects on milk protein concentrations were seen. This is in contrast to other work (Grummer, Jacob and Woodford, 1987; de Visser *et al.* (1990)) where high starch concentrates fed with silage caused an increase in milk protein concentrations (and yields) compared to high fibre concentrates; the high starch diets caused lower rumen acetate:propionate ratios (Grummer *et al.*, 1987; de Visser, van der Togt, Huisert and Tamminga (1992). Thomas and Chamberlain (1982), on the other hand, reported the absence of high rumen propionate:acetate ratios on silage-based diets supplemented with concentrates high in starch, supported by the results of Mayne and Gordon (1984) and Huhtanen (1988).

The effects of diet composition on the patterns of rumen fermentation are not clear (Figure 3.4), although much of the variation reported in the literature could well be due to differences in experimental procedure - feeding regimes, sampling times, sampling procedures - because propionate production tends to be much faster than acetate production and therefore a measurement of the A:P ratio depends very much on these factors in addition to feed composition.

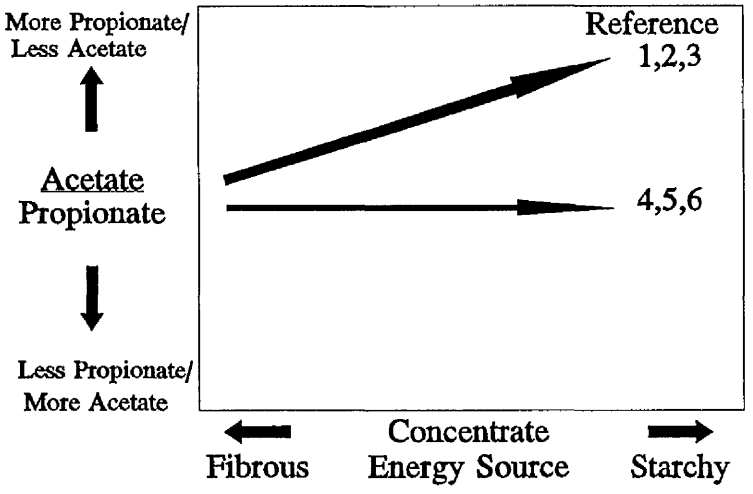


Figure 3.4 Diagram of effects of concentrate energy source on acetate (or acetate+butyrate) to propionate ratio.

3.2.5 Effects of Plasma Hormones

Lactation is under endocrine control. A complex interaction of hormones and growth factors are involved in the initiation and maintenance of lactation, and no single factor has absolute control over the synthesis of milk (Tucker, 1981). Those hormones which have

been identified as having a major influence on milk yield and composition are prolactin, insulin, growth hormone, and possibly glucagon. Relatively little is known about the influence of dairy cow nutrition on the endocrine partitioning of nutrients for lactation.

The potential endocrine influence on the yield and composition of milk is great, although the control of milk yield (i.e. volume) is not well understood. Milk is iso-osmotic with blood; water is drawn from plasma and ultimately into the lumen of the alveolus by diffusion as the osmotic potential of the mammary tissue secretion is increased by the secretion of ionic materials - principally K^+, Na^+, Cl^- and lactose (Linzell and Peaker, 1971). It is a commonly assumed that since lactose is present in milk at relatively high concentrations (40-50 g/kg), and its concentration is frequently seen to be more or less constant, lactose is the principal determinant of milk volume. While this partially correct, the mineral ions between them have almost as great a role in the osmotic control of milk as lactose since the lactose molecule is larger than the ions of potassium, sodium and chloride. However, lactose production may still be the major determinant of milk volume (by control rather than by default), and this is subject to the supplies of glucose reaching the mammary gland (Bickerstaffe, Annison and Linzell, 1974) and its utilization within the secretory cell (Kuhn, Carrick and Wilde, 1980). Current theories suggest that the rate limiting step of lactose synthesis, and hence milk production, may be the uptake of glucose across the cell membrane of the secretory cell and across the Golgi membrane within (CJ Wilde, personal communication). Although a number of hormones are needed for lactation, it is not known if lactation is controlled directly by the endocrine system, or if it is 'pushed' by indirect partitioning effects of factors such as prolactin, insulin and growth hormone.

Prolactin, although initially discovered through its association with lactation, is a highly conserved molecule that has been found in many animals (not just mammals) to play a major role in osmoregulation. It is not surprising that it has a role in the control of milk secretion in mammals considering that a high proportion of the water intake of the lactating mammal is used for milk production. Its main activity is the control of Na^+-K^+-ATPases, in the kidney (Pippard and Baylis, 1986) and mammary gland (Falconer and Rowe, 1977; Falconer, Langley and Vacek, 1983; Vonderhaar, 1987), but it has also been isolated from many other tissues of the body (Vonderhaar, Bhattacharya, Alhadi, Liscia, Andrew, Young, Ginsburg, Bhattacharjee and Horn, 1985). Recently, work at the Hannah

Research Institute (Bennett, Knight and Wilde, 1990) has revealed that the autocrine feedback inhibitor of lactation, which is present in milk and causes a local shutdown of milk secretion beyond a threshold concentration, acts to some extent by blocking the binding of prolactin to its receptors in the mammary tissue, possibly by decreasing the proportion of cell surface receptors. Plasma concentrations of prolactin have been seen to be affected by feeding, increasing slowly to a peak approximately 6 hours after a feed (Bines, Hart and Morant, 1983), but unlike growth hormone (see below), basal concentrations have not been correlated with cattle genetically selected for high milk yield (Kazmer, Barnes, Akers and Pearson, 1986). Furthermore, lactation has been seen to be unaffected by an artificial reduction in the plasma concentration of prolactin (Akers, 1985).

Lees *et al.* (1990) found high postprandial insulin peaks in the blood of animals on high starch diets. Postprandial insulin peaks in ruminants have been reported by several other authors (Trenkle, 1972; Hove and Blom, 1973; Bassett 1974). Insulin plays an important role in the regulation of energy metabolism - an increase in blood glucose concentration leads to an increase in insulin secretion which stimulates the uptake of glucose by extra-hepatic tissues causing, in the case of adipose tissue, the storage of energy (see Brockman and Laarveld, 1986). Conversely, an increase in plasma insulin has been shown to reduce milk yield, by reducing the availability of glucose for lactose production whilst increasing the concentrations of protein and fat (Schmidt, 1966). Insulin also stimulates the uptake of amino acids from the blood by tissues, and while it is needed absolutely (together with hydrocortisone and prolactin) for milk protein synthesis (Lockwood, Turkington and Topper, 1966; Gertler, Weil and Cohen, 1982; Rosen, Rodgers, Couch, Bisbee, David-Inouye, Campbell and Yu-Lee, 1986; Meisler and Howard, 1989; Vonderhaar and Ziska, 1989), it was not seen to significantly increase the rates of extraction of amino acids or glucose from the blood (Laarveld, Christensen and Brockman, 1981; Metcalf, Sutton, Cockburn, Napper and Beever, 1991; Tesseraud, Grizard, Makarski, Debras, Bayle and Champredon, 1992) by the mammary gland. However, neither Laarveld *et al.* (1981) or Tesseraud *et al.* (1992) measured amino acids associated with erythrocytes nor small peptides (Backwell, Bequette, Wilson, Walker, Calder, Wray-Cahen, Metcalf, Lobley and MacRae, 1993; Webb, Dirienzo and Matthews, 1993) which means that they may have underestimated the amounts of amino acids taken up by the mammary tissue (Baumrucker, 1985), and Metcalf *et al.* (1991) was only measuring five specific amino acids. Despite this, the evidence strongly suggests that insulin does not directly affect amino acid

extraction rates by the mammary gland. Even if insulin has no direct effect on the uptake of amino acids and glucose by the udder, it may have indirect effects by (at reduced levels) reducing the uptake of these by other tissues, thereby partitioning them towards the udder for milk production. Insulin resistance of non-mammary tissues during lactation may help to increase the partitioning of glucose towards the udder (Grizard, Champredon, Aina, Sornet and Debras, 1988), although the mechanism responsible for this is unknown (Vernon, 1989). Lower basal plasma concentrations of insulin have been seen in low-yielding compared to high-yielding dairy cows in support of this theory (Hart, Bines, Morant and Ridley, 1978; Bines *et al.*, 1983). Bines *et al.* (1983) also found postprandial peaks in plasma insulin concentrations, with absolute increases being higher in low yielding animals.

Bovine growth hormone (somatotrophin, or bST) has been the subject of study in many investigations with dairy cows because of its ability to bring about increases in milk production with little change in milk quality and initially without increases in feed intake, although this does occur after a time lag. Whereas insulin generally stimulates anabolic processes that are directed towards the storage of excess energy in the body, bST tends to stimulate anabolic processes directed towards growth, inhibiting cellular glucose uptake, and promoting fatty acid mobilization, cellular uptake of amino acids, and protein synthesis. Milk yields from high and low yielding dairy cows have been positively correlated to (natural) basal plasma concentrations of growth hormone (Hart, Bines and Morant, 1979; Kazmer *et al.*, 1986), and also to the ratio of growth hormone to insulin (Hart *et al.*, 1979). No consistent diurnal variation in bST was seen with regard to feeding patterns (Bines *et al.*, 1983), although increases in plasma concentrations of nonesterified fatty acids (NEFA) and of ß-hydroxybutyrate were seen in early- or mid-lactation dairy cows treated with injections of bST (Miller, Reis, Calvert, DePeters and Baldwin, 1991b), whereas triacylglyceride concentrations were seen to fall. The plasma concentrations of NEFA, ß-hydroxybutyrate and triacylglycerides were found to be the major determinants of their rate of uptake by mammary glands (Miller, Reis, Calvert, DePeters and Baldwin, 1991a). An increase in the extraction rate of certain amino acids by the mammary gland has been observed on bST-treated versus control dairy cows (Hanigan, Calvert, DePeters, Reis and Baldwin, 1992) although bovine growth hormone receptors have not been found in bovine mammary tissues (Akers, 1985; Bauman and McCutcheon, 1986) and Sejrsen and Knight (1993) recently found no local galactopoietic

effect of intramammary infusions of growth hormone in goats. Therefore, the effects of growth hormone are likely to be indirect, either through some intermediary, e.g. somatomedins (Ronge, Blum, Clement, Jans, Leuenberger and Binder, 1988), and/or through a partitioning effect, perhaps in conjunction with insulin. Growth hormone has been shown to increase cardiac output and mammary blood flow (Davis and Collier, 1985; Davis, Collier, McNamara, Head and Sussman, 1988), thereby increasing the effective delivery of nutrients to the mammary gland.

Dietary energy quality has been shown to influence the patterns of rumen fermentation, and through this to influence the endocrine status of the animal. Since milk synthesis is under endocrine control, the manipulation of hormones by dietary means offers a possible mechanism for the regulation of milk protein synthesis. Little, however, is known about the effect of dietary protein supply on the endocrine status of the dairy cow, and if partitioning of amino acids may occur in this manner. Since many hormones are proteins and polypeptides, the possibility exists that many of the degradation products of dietary proteins themselves have a direct endocrine effect on the animal. The potential for this is large and, from a research point of view, rather daunting.

3.2.6 Dietary Protein

This is the ultimate source of nitrogen for milk protein synthesis, and an adequate supply is therefore critical in order to achieve efficient utilisation. There is, however, no simple relationship between dietary protein supply and milk protein concentration, with the energy supply and source influencing protein utilisation by the rumen and also by the mammary tissue. Lactose and mammary gland derived protein are both produced in Golgi vesicles (Linzell and Peaker, 1971), and for a long time this was thought to link the production of the two milk components since an increase in milk protein production which could increase the protein concentration was frequently observed to be countered by an increase in yield; milk protein concentration was therefore considered to be more or less constant and unchangeable through dietary manipulation. However, differences in mammary energy partitioning can lead to changes in milk yield independently of protein utilisation (see Chapter 4). Furthermore, the body can quickly buffer changes in energy supply to the animal, storing or releasing energy as required. This sort of buffering is not seen with nitrogen, at least not in the short term (i.e. over the course of hours rather than days and weeks), and physiological processes involved with milk protein production may be more

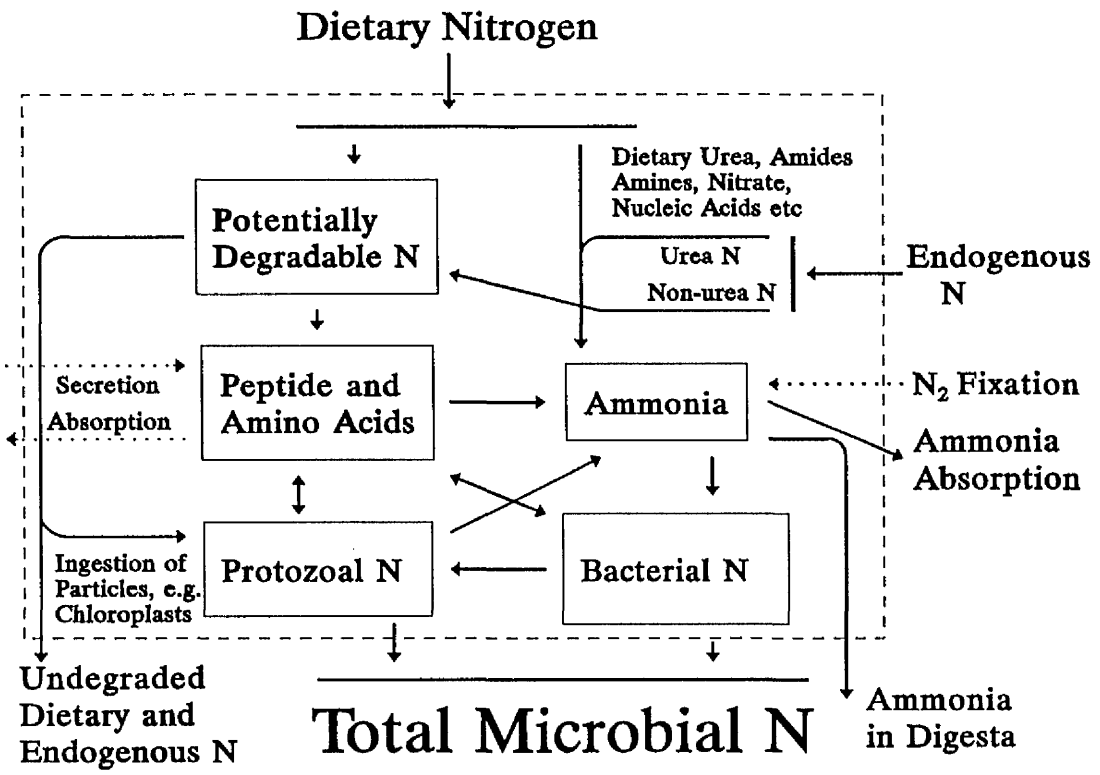


Figure 3.5 A generalised scheme of nitrogen flow within the rumen (from Leng and Nolan, 1984).

conservative if the supply of nitrogen is not as secure as the supply of energy.

The link between nitrogen intake at the mouth and output in the milk is not a direct one. Nitrogen may pass through the rumen unaltered, to be digested in the small intestine. Alternatively, it may arrive at the small intestine as microbial protein; in this way the conversion of non-protein nitrogen (NPN) sources of CP (e.g urea) into true protein can be achieved. Figure 3.5 gives a general overview of the flow of nitrogen within the rumen. Protein quality, in terms of the eventual relative value of the diet (i.e. the balance of amino acids), may be important when a certain amino acid becomes limiting.

The precursors of milk proteins are passed to the mammary secretory tissues from blood plasma as free amino acids, amino acids associated with the erythrocytes, and small peptides (Backwell *et al.*, 1993). It is only relatively recently that the significance of small peptides as a source of α-amino nitrogen has been recognised (see Webb *et al.*, 1993). An increase in the supply of these precursors to the mammary tissue is a function of their concentration in the blood reaching the secretory cells and the rate at which they are delivered, i.e. the rate of mammary blood flow. Also important is the relative value of

the mixture of precursors, with methionine, phenylalanine, histidine, valine, leucine and lysine having been identified as amino acids most likely to be limiting in dairy cows (Broderick, Satter, and Harper, 1974; Foldager, Huber and Bergen, 1980; Rulquin and Vérité, 1993), although the most limiting depends on the animal's diet, e.g. lysine on a diet of maize silage. Input-output studies have shown that amino acids can be classified into three distinct functional groups: group 1, essential amino acids (EAA) which are taken up by the mammary gland in approximately the same quantities as they are secreted in milk; group 2, EAA which are taken up in larger quantities than they are secreted; and finally a third group which are taken up in smaller quantities than secreted (Mepham, 1982). A certain amount of interconversion between the two latter groups takes place to make up the balance, and some amino acids (group 2) may be used for energy production.

The protein requirements of ruminants have been characterised by a number of systems in different countries throughout the world. That which is currently relevant to this work in Britain is the Metabolisable Protein system.

3.2.7 The UK Metabolisable Protein System

The presently recommended system in the United Kingdom, as outlined in the report of the Agricultural and Food Research Council Technical Committee on Responses to Nutrients (TCORN, 1992), regards dietary CP as consisting of four fractions - quickly degradable protein and slowly degradable protein - the two constituting the effective rumen degradable protein (ERDP), that used by the rumen - and digestible undegraded protein (DUDP), which escapes degradation in the rumen and is digested in the small intestine, and finally the indigestible undegraded protein, which is unavailable to the animal. Metabolisable protein, therefore, is the protein which is actually available to the animal for utilization, and consists of microbial protein and DUDP. The metabolisable protein system builds on and refines the previous recommendations (Agricultural Research Council (ARC), 1980 and ARC, 1984) which took into account the nitrogen supply needed to optimise the growth of the rumen microbes - the rumen degradable protein (RDP) - and which was supplemented with rumen undegradable protein (UDP) since microbial protein alone is insufficient to support high levels of milk production in the dairy cow.

Differences in the rumen degradability of dietary protein will ultimately determine the route by which nitrogen gets from the feedstuff to milk. Rumen degradable nitrogen

sources will be incorporated into microbial protein - subject to the availability of sufficient fermentable metabolisable energy (FME) for microbial utilisation - which will later be digested in the small intestine. Any rumen degradable nitrogen supplied in excess of microbial requirements will effectively be lost.

It is not sufficient, therefore, to simply increase dietary crude protein concentrations in order to increase milk protein concentration, and this will be discussed later. A number of authors have investigated the effects of altering the dietary supply of one or other of RDP or UDP. To dissociate the effects of RDP and UDP from one another experimentally, the requirements of the rumen microbes must be satisfied both in terms of nitrogen and energy. Unfortunately, what these requirements are, and how they change with different supplies of protein and energy sources, is still unclear. What is clear, however, is that dietary nitrogen cannot be considered without taking into account the supplies of dietary energy, both with regards to the rumen (Nocek and Russell, 1988) and to the animal itself (Oldham, 1984). An excess of RDP will be wasted, when, for example, energy supplies are limiting, since the animal is unable to utilise the nitrogen (typically urea or NH_3) which is the major end product of microbial degradation of protein, whilst a shortage of RDP will mean that the full potential of the rumen will not be realised, resulting in reduced digestibility of carbohydrates. Furthermore, when the microbial capture of RDP is low, the quantities of α-amino nitrogen available to the animal may be further reduced by the hepatic utilisation of substantial amounts of amino acids for ureagenesis (Reynolds, Tyrrell and Reynolds, 1991), potentially reducing the quantities that are available for milk protein production. Similarly, an excess in the DUDP supply to the animal beyond the rumen will be wasted, and has a cost associated with deamination and excretion. It is not clear, however, that the high yielding dairy cow would ever normally be in this position due to the requirements of lactation.

The effect of decreasing the effective protein degradability (i.e. supplying more UDP at the same level of CP) has been seen to be variable, and may increase milk protein concentration (Majdoub, Lane and Aitchison, 1978; Winsryg, Arambel and Walters, 1991), decrease it (Forster, Grieve, Buchanan-Smith and MacLeod, 1983), and to have little effect (Robinson and Kennelly, 1988; Casper and Schingoethe, 1989a). In some instances (Robinson and Kennelly, 1988), increasing the supply of UDP increased both milk yield and milk protein yield with no net effect on protein concentration, although the

effect on lactation in general was positive. Since the rate of protein degradation is determined by the activity of the rumen microbes and their interaction with the rate of protein intake, the latter has important effects on milk protein concentration.

3.2.8 Protein Intake

This is a function of protein concentration in the diet and dry matter intake and there is contradictory evidence in the literature about its influence on milk protein concentration. Protein intake is often correlated with metabolisable energy intake since extra dietary protein often stimulates forage intake. Emery (1978) found a significant relationship between increasing dietary protein concentration (i.e. CP g/kg DM) and milk protein concentration, but notes that dietary protein intake influences total milk production to a much greater extent than milk protein production. This is supported by Spörndly (1989) who found a significant positive correlation between milk yield, but not milk protein concentration, and dietary CP concentration, and a significant positive correlation to both with increasing CP intake. A concomitant increase in both milk yield and milk protein yield will obviously produce no net change in milk protein concentration.

Increasing the CP concentration of mixed rations from 127 g/kg DM to 193 g/kg DM caused a small, but non-significant, increase in milk yield from high-yielding dairy cows (Claypool, Pangborn and Adams, 1980). No change was seen in milk protein concentration, although with the increase in milk yield, protein yield was increased. Blauwiekel and Kincaid (1986) also reported similar results when increasing the CP concentration of the diet from 144 g/kg DM to approximately 190 g/kg DM. Gordon (1977), on the other hand, found significant increases in both milk yield and milk protein concentration when increasing the CP content of the concentrates from 122 g/kg DM to 181 g/kg DM, but achieved no further gains by increasing it still more (to 236 g/kg DM); greater effects were seen at higher levels of concentrate feeding which implies that energy supply may have been limiting at lower concentrate levels. Lees *et al.* (1990) also found significant increases in milk yield and milk protein yields with increasing dietary CP concentration, but, as with Gordon (1977), no difference in milk protein concentration was observed. Significant increases in milk protein concentration have, however, been observed with increasing dietary CP concentration (Forster *et al.*, 1983; Kung, Huber, Bergen and Petitclerc, 1984) even with concomitant increases in milk yield, although these were seen on maize based diets where dietary protein is generally in shorter supply than

metabolisable energy.

The effect of increasing the dietary crude protein concentration appears, therefore, to be an increase in milk production with concomitant increases in milk protein yield, resulting in small, if any, changes in the protein concentration. Without knowledge of the contribution that the rumen makes to the protein supply to the animal, it is difficult to determine the reasons for this effect. Therefore, what is required is a simple index of microbial protein production which can be used on intact animals used for normal feeding experiments. The measurement of purine derivative excretion goes some way towards achieving this objective.

3.2.9 Purine Derivatives as Estimates of Microbial Protein Yield

In the United Kingdom, grass pasture and grass silage are presently the dominant feedstuffs of dairy cows. The yield of microbial protein from the rumen on diets composed exclusively of grass silage is, however, low compared to other diets (e.g. grass hay), but can be increased by supplementation (Thomas and Rae, 1988). There are a number of methods for estimating the protein supply from the rumen using the flow of external markers such as $^{15}NH_3$, $^{35}SO_4$, and ^{32}P-phospholipids, or internal markers such as 2,6-diaminopimelic acid (DAPA), D-alanine, DNA, RNA, and purines (Broderick and Merchen, 1992). Of increasing interest is the use of purines derivatives excreted by the animal. Under normal circumstances nucleic acids (NA) in feedstuffs are rapidly subjected to microbial degradation (Smith and McAllan, 1970; McAllan and Smith, 1973) and any NA reaching the duodenum are either from the diet, protected from degradation, or have been synthesised by the rumen microbes.

In the duodenum, microbial cells are digested and the NA released are degraded to nucleosides and free purine and pyrimidine bases; these are absorbed from the small intestine (Bergen, 1978; McAllan, 1980). The urinary excretion of the end products of purine degradation (Figure 3.6) in ruminants - purine derivatives (PD) - has been shown to correlate highly with exogenous supplies of NA infused into the rumen in sheep (Chen, Hovell, Ørskov and Brown, 1990a) and cattle (Verbic, Chen, MacLeod and Ørskov, 1990). The PD excreted in bovine urine are uric acid and allantoin, with very low concentrations of hypoxanthine and xanthine; in ovine urine there are higher concentrations of hypoxanthine and xanthine due to lower tissue concentrations of xanthine oxidase

(Chen, Ørskov and Hovell, 1990b). Measurement of urinary excretion of PD therefore offers a totally non-invasive method of estimating microbial protein yield from the rumen.

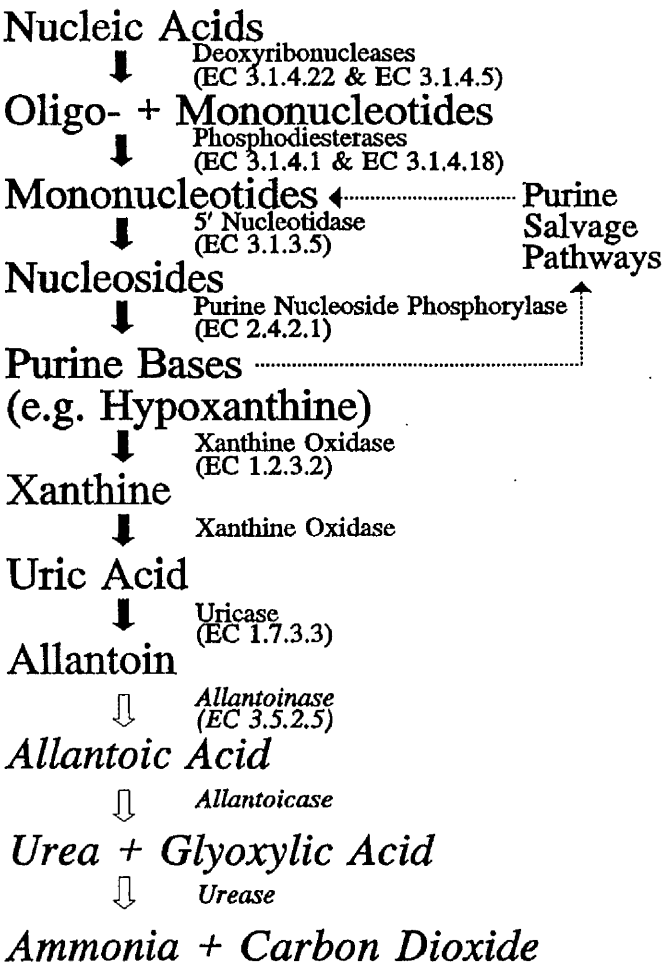


Figure 3.6 Degradation of purines. In ruminants, this does not continue beyond allantoin. The high activity of xanthine oxidase in bovine plasma means that purine salvage is minimal outside the intestinal mucosa.

Not all PD excreted are derived from exogenous sources; some are produced as a result of endogenous turnover of body tissue NA. A certain amount of salvage of the purine ring occurs in the production of the two purines, adenine and guanine, in body tissues which may have important consequences on the relationship between microbial protein and urinary PD excretion. Animals nourished by intragastric infusions (eliminating the effect of microbial rumen fermentation) of NA-free nutrients allowed the estimation endogenous contributions of tissue NA to urinary PD excretion (Chen, *et al.*, 1990b), with cattle being found to excrete three times as much PD as sheep per kilogram metabolic weight ($W^{0.75}$). It was concluded that this is due to lower purine salvage rates in cattle (other than in the intestinal mucosa (Verbic *et al.*, 1990)) because of the high activity of xanthine oxidase in bovine plasma. Once the purines have been degraded to xanthine and beyond, salvage of the purine ring is not possible and therefore it is excreted. With negligible activity of xanthine oxidase in sheep plasma, purines are available for salvage in tissues other than the intestinal mucosa. The result of this is that more sensitive measurements may be made in cattle than in sheep. The contribution that endogenous NA makes to the total PD excretion will be very low compared to the NA derived from rumen microbes, particularly for a lactating dairy cow with a high rate of feed intake and microbial cell production.

In lactating animals, PD are excreted in milk (Wolfschoon-Pombo, 1983; Tiemeyer, Stohrer and Giesecke, 1984; Rosskopf and Giesecke, 1992), and some are lost via saliva and subsequent degradation in the rumen (Chen, Hovell and Ørskov, 1990c). Chen *et al.* (1990c) estimated that as much as 10% of the urinary excretion of PD may be lost via saliva, although more recent work puts this figure much lower (Surra, Guada, Balcells and Castrillo, 1993).

By making assumptions about the ratio of NA-nitrogen (NA-N) to protein N in microbial cells (microbial protein) and rates of recovery of purines yielded to the duodenum, an estimate of microbial protein yield can be produced using urinary purine excretion. Clark, Klusmeyer and Cameron (1992) point out that there is much variation in the ratio of purine:microbial protein in the literature, and cite unpublished data which have been found to alter with factors such as source of CP, amount of CP, F:C ratio and time after feeding. If this phenomenon usually occurs in intact animals, it would be impossible to determine whether changes in urinary PD excretion were due to changes in microbial cell numbers, or changes in NA-N:microbial protein ratios. Smith and McAllan (1970) found diurnal changes in NA-N:microbial protein, and also significant changes in NA-N:total microbial N in relation to feeding (Smith and McAllan, 1974), which is expected with increased NA production for mitosis. On the other hand, other authors have found the NA-N:total N to be constant across a range of diets (Stokes, Hoover, Miller and Blauweikel, 1991).

One of the potentially most valuable aspects of the purine derivative technique for estimating microbial protein yield is its application to intact animals which are not affected by the presence of intestinal fistulae and cannulae. Not only is this an important welfare aspect, it makes the procedure a very cheap one. It can be used on animals which may be drawn from, and returned to, a large pool, as happened for the experiments presented in this thesis, thereby overcoming the problems of repeatedly using the same animals on several different experiments. In order to measure the total daily purine derivative excretion, total urine collections must be performed which, for female animals, means the use of a urine separator or a urethral catheter. This makes the large scale collection of samples from uninhibited individual animals impossible, but the problem can be avoided by the use of an internal marker such as urinary creatinine. The rate of creatinine excretion from the body is constant for individual animals, even over long periods of time (months) (De Groot and Aafjes, 1960; Albin and Clanton; 1966), although body mass

(protein mass) can influence the rate of excretion (De Groot and Aafjes, 1960), perhaps contributing to the differences in rates of excretion between individuals. Albin and Clanton (1966) found differences in urinary creatinine excretion in the same individuals at different stages of reproduction. Creatinine excretion from cattle has been found to be unaffected by feeding (De Groot and Aafjes, 1960; Albin and Clanton, 1966; Kertz, Prewitt, Lane and Campbell, 1970). The use of urinary creatinine concentration as a marker against which other urinary metabolites (e.g. PDs or hormones (Erb, Surve, Randel and Garverick, 1977)) can be measured is potentially very efficient, provided the between-animal variation is taken into account with the use of experimental design techniques such as the Latin Square and experimental periods for covariate measurements.

3.2.10 Conclusions

In conclusion, an increase in dietary metabolisable energy from carbohydrates tends to bring about an increase in milk protein concentration. The form of this energy is also important and a decrease in the forage:concentrate ratio tends to increase milk protein concentration, although this is confounded somewhat with changes in the plane of nutrition. Concentrate energy quality is also important, with starchy concentrates tending to increase milk protein concentration compared to those with a high fibre content. This may be a result of differences in rumen fermentation patterns, leading to higher propionate absorption and hence a sparing of amino acids from gluconeogenesis, and it may also be a result of changes in microbial protein production. The latter can be monitored by the use of urinary purine derivative excretion. Protein supply to the animal is important for milk protein synthesis, although the responses seen in the literature have been variable. Part of the problem may be due to imbalances between rumen degradable protein and fermentable metabolisable energy leading to a poor microbial protein production.

3.3 EXPERIMENTAL AIMS

A 2x2 factorial experiment was conducted to investigate the effects of dietary energy quality and the level of dietary DUDP on milk protein concentration and quality. Two simple diets were formulated to compare barley with molassed sugar beet pulp as supplements to grass silage at a constant F:C ratio of 40:60, together with two levels of DUDP: one above and one below predicted requirements, with all diets formulated to provide the same adequate supply of ERDP. In order to determine the relative effects of diet on rumen microbial yield, urinary purine derivative excretion was measured.

3.4 MATERIALS AND METHODS

3.4.1 Animals and Their Management

Twelve multiparous Holstein-Friesian cows in the third month of lactation were used for the experiment. These were drawn from the college dairy herd at Auchincruive and housed in a metabolism unit in individual stalls fitted with de Boer yokes. The animals were milked with the rest of the herd in the main dairy unit, using a 20/20 Herringbone milking parlour, having been released from their yokes and left in a collecting yard shortly before milking at approximately 0800 h and 1700 h. They were returned to the yard after milking where they stayed until the metabolism unit had been prepared for their return, and thus gained approximately 2-3 hours of exercise each day. As part of the Auchincruive dairy cow management programme, the animals were weighed approximately every 2 weeks.

3.4.2 Experimental Design

The experiment was designed as a 2x2 changeover, based on three 4x4 orthogonal Latin Squares. Each experimental period consisted of a 3 week adjustment period followed by a 1 week collection period. The mean milk yields during the 7 days prior to the start of the experiment were used to allocate animals to Latin Squares, with the top 4 yielders in the first square, the next 4 in the second square, and the 4 lowest yielders in the last square.

The experimental data obtained were analyzed statistically using analysis of variance with Genstat 4 (Lawes Agricultural Trust, 1980) and Genstat 5 (Lawes Agricultural Trust, 1990). The blocking structure was Period*(Square/Cow) and treatment structure was diet Energy*Protein. Probabilities of $P<0.05$ were considered significant; where $0.10>P>0.05$, the effect was regarded as an interesting trend worth further consideration. Since the dietary combination of starch and high protein was expected to produce the greatest effects on milk protein production, the data were also analyzed using orthogonal contrasts to compare the barley high-protein diet with the other three diets. Residual maximum likelihood (REML; Patterson and Thompson, 1971) was used to calculate variance components (see Box, Hunter and Hunter, 1978), using dietary treatments, Protein*Energy, as fixed effects, and Period/Day/Time/Cow as random effects. REML was also used to test for carry-over effects from one diet to the next. Analysis of variance was not suited to this analysis because of the unbalanced nature of the design.

3.4.3 Diet Formulation and Production

The four diets consisted of silage fed *ad libitum* together with a concentrate ration mixed on farm. The concentrate rations were formulated to meet predicted ME requirements, and in accordance with early recommendations of the UK metabolisable protein system (ARC, 1980; ARC 1984; TCORN, 1992), using SAC Advisory Service rationing software (NW Offer, personal communication). This was used to predict nutrient requirements for the animals at the start and finish of the experiment on the basis of the group's mean milk yield and weight at the start, and the diets were formulated to meet these requirements over the range of predicted intakes throughout the experimental period.

The mean initial milk yield of the group was 28 kg/d and the mean yield at the finish was predicted to be 20 kg/d, based on a reduction of 1.5% per week over the 18 weeks of the experiment. The initial mean live weight of the group was 577 kg, with no predicted change in weight over the duration of the experiment. With this information four concentrates were formulated, two based on barley (B), two on molassed sugar beet pulp (S), representing starch and fibre sources respectively.

For convenience during formulation, and in the absence of true figures, the metabolisable energy and metabolisable protein levels of B and S were assumed to be sufficiently similar as to be considered equal, i.e., it was assumed that the only significant difference was in the source of energy (Bhattacharya and Sleiman, 1971; Castle, 1972; Castle *et al.*, 1981). Mineral and vitamin supplements (McLellan Animal Health, Ayr, UK) were incorporated in the rations at the rate 25 g/kg DM; a general purpose in-feed supplement (230 g/kg Ca, 35 g/kg P) for the B diets, and a high-phosphorus (190 g/kg Ca, 105 g/kg P) supplement for the S diets.

The diets were formulated (including silage) to keep all four isoenergetic. The two protein levels were formulated to supply equal quantities of RDP to each of the diets, with the high (H) and low (L) protein levels being brought about by high and low levels of DUDP. The high protein concentrate corresponded to 132%, and the low protein concentrate to 87%, of predicted requirement for DUDP (TCORN, 1992). High and low protein levels were achieved by the addition of soya and urea to the B or S, and represent the highest and lowest possible rates of soya bean meal incorporation whilst keeping other factors constant.

Table 3.1 Summary of the ingredient composition of high and low protein concentrates (g/kg fresh weight).

	High Protein	Low Protein
Barley/Sugar Beet Pulp	836.9	959.8
Soya	140.3	0.0
Urea	0.4	13.0
Mineral & Vitamins	22.4	27.2

The concentrate part of the diets (Table 3.1) were prepared on-farm at various times throughout the experiment. Home grown barley was ground using a hammer mill to pass through a 1.6 mm sieve, and mixed in a 1.5 tonne mixer. One tonne batches of the B-based concentrates were pre-mixed at intervals throughout the experiment, and bagged ready for feeding.

The S-based concentrate mix was prepared in individual feed bins shortly before feeding, since the difference in particle size between the pellets and the soya/urea/minerals was too great to achieve an evenly mixed product in the mixer. Attempts were made to mill the pellets - which was possible - but the process was very slow with a large potential for damaging the milling equipment. More importantly, it was not known what effect the milling process would have on the fibre fraction of the sugar beet pulp pellets nor how consistent any effect would be. Therefore, the 'protein' (soya, urea and mineral) portions of the S concentrates were premixed by hand and added to the S for individual animals daily.

Some of the cows refused to eat sugar beet pulp pellets, probably because of the pellet hardness. These cows, therefore, received sugar beet pulp shreds in their place, on the assumption that the pellets and shreds were identical in composition.

3.4.4 Animal Feeding

Cows were individually fed fresh first-cut grass-silage daily after the evening milking; this was topped up during the following day if necessary. Silage intakes were recorded daily by weighing silage offered and refused. The amounts of silage that were offered were 5-10% above *ad libitum* requirements, using previous intakes as a guide.

The daily concentrate ration for each cow was calculated according to the rolling average

of silage intake over the previous 3 days, keeping to a forage:concentrate ratio of 40:60 on a dry matter basis. This was calculated using the silage fresh weights, with factors calculated from silage DM contents obtained before the start of the experiment and from tables (Givens and Moss, 1990). The concentrates were fed on top of the silage in three roughly equal portions, one each after evening and morning milkings, and one at midday.

In order to avoid problems with the high level of concentrate feeding during the experiment, changes from one concentrate to another (i.e. at the start and end of the experiment and during the transition from one experimental period to the next) were done in steps of 25:75, 50:50 and 75:25 of each of the 'old' and 'new' concentrates respectively. Each mix was fed for 2 days.

In addition to and regardless of the amounts of silage and concentrates fed to the cows, a token amount of parlour concentrate (approximately 200 g) was fed at each milking via the automatic in-parlour feeders. Prior to the experiment, the cows received 7.2 kg/d of concentrate fed in parlour, and approximately 3 kg/d of a barley/soya mix (2.5 kg barley + 0.5 kg soya bean meal) fed at midday.

3.4.5 Sample Collection and Analysis

Samples were collected during the last seven days of each experimental period. During each collection period, silage and concentrate samples were collected for feed analysis. Samples of the silage refusals were collected from each cow for dry matter analysis. These samples were frozen immediately after collection and stored at -20°C until analyzed.

Spot urine samples of approximately 100 ml were collected by vulval stimulation at about 1030 h and 1530 h. These were frozen and stored at -20°C until analyzed for creatinine and the purine derivatives allantoin and uric acid using composite morning and afternoon samples. All urine samples were analyzed from 4 of the animals for which all (except 1 out of 224) samples had been obtained.

Over the same 7 days as urine samples were collected, fresh uncontaminated samples of faeces were collected from the floor behind each cow for the determination of indigestible acid detergent fibre (IADF) as an estimate of whole tract OM digestibility of the diets (Penning and Johnson, 1983). Approximately 100 ml were collected before the animals

were released for milking and were frozen and stored at -20°C. Later, these were dried at 60°C, milled, and stored at room temperature until analyzed.

Morning and evening milk samples were collected over 4 days at the end of each experimental period. These were preserved using Lactab milk preservative tablets (Thompson and Capper Ltd, Runcorn, Cheshire, UK), and kept refrigerated until analyzed. Analysis of milk protein, fat and lactose concentrations was done by the Dairy Technology Department, SAC, Auchincruive, using a Milko-Scan 203 analyzer (Foss Electric Ltd, Denmark). Morning and evening samples were also collected on one of the 4 days for more detailed analyses of milk nitrogenous constituents (Kjeldahl nitrogen, casein and NPN). For this, the samples were kept refrigerated without the use of a preservative before analysis the following day; the samples for each cow were bulked according to the milk yield at the time of collection. Concentrations of milk sodium, potassium and chloride ions, and milk freezing point depression, were determined by Hadjira Mohammedi, a fellow student who took milk samples from the animals on my experiment for investigative purposes (H Mohammedi, in preparation). Determination of milk urea and minerals were performed on milk samples from 4 consecutive milkings per experimental period, and the weighted means of these analyses was used in the statistical analysis.

Milk Kjeldahl nitrogen, casein and NPN fractions were measured by the Dairy Technology Department at SAC Auchincruive using the procedures outlined in Section 2.3.

Mean milk yields taken over the 8 milkings were used to calculated daily yields of milk constituents, using weighted (or bulked) mean values of each constituent.

3.5 RESULTS

Samples were collected from all cows on all treatments except for one cow that was ill during period 2 of the experiment with severe laminitis. She was housed separately on straw in a recovery box until a few days before the start of the collection week of period 3. She was fed her allotted experimental diet and had free access to fresh water; straw intake was not measured.

Tables 3.2 and 3.3 show the mean composition of concentrates and silage fed during the experiment respectively.

Table 3.2 Composition of the concentrate portions of the diets. B=Barley, S=Sugar beet pulp, H=High protein, L=Low protein, PC=Parlour concentrate.

	BH	BL	SH	SL	PC
Dry Matter, g/kg	870	890	882	886	882
Organic Matter, g/kg DM	943	941	892	886	894
Crude Protein, g/kg DM	181	161	165	126	216
Metabolisable Energy, MJ/kg DM	13.2	12.9	12.8	12.5	13.5
Fermentable Metabolisable Energy*, MJ/kg DM	12.2	11.9	12.3	12.0	11.5
Neutral Detergent Fibre, g/kg DM	128	132	264	290	275
Acid Detergent Fibre, g/kg DM	68	68	153	168	158
Starch, g/kg DM	477	517	0	0	151
Water Soluble Carbohydrates, g/kg DM	20	20	196	208	68
Acid Hydrolysis Ether Extract, g/kg DM	29	29	15	14	61
In vitro Organic Matter Digestibility, g/kg OM	893	858	897	858	751
K, g/kg DM	8.3	5.2	19.1	17.9	13.4
Ca, g/kg DM	8.2	9.8	13.5	15.4	14.3
P, g/kg DM	5.6	5.1	3.5	3.2	7.1
Mg, g/kg DM	3.5	3.6	1.9	2.8	8.8
Na, g/kg DM	2.4	2.8	5.9	6.7	4.9

*Estimated, FME = ME - 0.33 x AHEE.
† Not determined.

Table 3.3 Composition of the silage used (mean of 3 samples, each bulked over 7 days).

	First Cut Grass-Silage
Dry Matter, g/kg	207
Organic Matter, g/kg DM	925
Crude Protein, g/kg DM	180
Metabolisable Energy, MJ/kg DM	11.3
Acid Hydrolysis Ether Extract, g/kg DM	62
Water Soluble Carbohydrates, g/kg DM	14
Neutral Detergent Fibre, g/kg DM	482
Acid Detergent Fibre, g/kg DM	324
Rumen Degradable Protein, g/kg DM	154
Undegradable Protein, g/kg DM	26
In vitro Organic Matter Digestibility, g/kg OM	774
D-Value, g/kg DM	704
NH_4-N g/kg Total N	128
pH	3.8
Calcium, g/kg DM	5.2
Phosphorus, g/kg DM	3.3
Magnesium, g/kg DM	2.3

Concentrate batches mixed at different times were very similar in composition (e.g. for BH and BL respectively, mean crude protein content was 181 g/kg DM, SD 12.7, and 161 g/kg DM, SD 7.6, and mean ME was 13.2 MJ/kg DM, SD 0.14, and 12.9 MJ/kg DM, SD 0.35). Analyses of the S diets were done on the sugar beet pulp and 'protein' portions separately; the analyses given in Table 3.2 are calculated values assuming additivity. The sugar beet shreds fed to some of the animals were very similar in composition to the pellets fed to others (for pellets and shred respectively example mean values were, 102 and 118 g CP/kg DM; 13.0 and 13.0 MJ ME/kg DM and *in vitro* OM digestibilities of 888 and 911 g/kg OM).

Silage composition did not change greatly over the duration of the experiment, with, for example, 182, 174, 176 and 189 g CP/kg DM and 11.1, 11.3, 11.3 and 11.4 MJ ME/kg DM for periods 1-4 respectively.

Table 3.4 gives treatment mean values of daily dry matter intakes (DMI) of silage and concentrate, daily crude protein intake (CPI), daily metabolisable energy intake (MEI) with the contributions of ME from silage and concentrates assumed to be additive. There were no significant treatment effects on DMI. Apparent whole tract OM digestibilities (estimated using the IADF content of feed and faeces; digestibility=1-(feed IADF/faecal IADF)), and allantoin/creatinine ratios (A/C) are also given in Table 3.4. Rumen fermentable ME intakes (FMEI) were estimated from the ME and acid hydrolysis ether extract (AHEE) contents of the concentrates, using the equation for concentrate FME = ME - 0.033 x AHEE, and from silage, using FME = 0.71 x ME (TCORN, 1992), again assuming additivity. From Table 3.4 it can be seen that the initial specification of a dietary F:C ratio of 40:60 on a dry matter basis was met for all four diets, despite the unavailability of true DM figures for the concentrates until the conclusion of the trial.

In this work the A/C ratio was used as a simple index of microbial protein yield with the basic assumption that an increased A/C indicates an increased microbial protein supply. Uric acid was also determined in the urine, but the results were unreliable because uric acid crystals precipitated out upon thawing. The urine was stirred in an attempt to redissolve the crystals, but with little success. The outcome was that the concentration of uric acid in the urine was relatively constant compared to both the concentrations of allantoin and creatinine - presumably because the urine was saturated with uric acid at the conditions in which it was redissolved. Methods in subsequent experiments were changed to overcome this problem. Both energy source and protein level were found to significantly affect the urinary A/C ratios, with increased purine excretion on the barley and high protein diets.

Table 3.5 gives a summary of the mean milk yield and composition obtained by Milko-Scan analysis from each of the four diets, and Table 3.6 gives the treatment means of the major nitrogenous constituents in the milk. Milk yields were far lower than predicted by the ration formulation software, although differences were seen with respect to the four experimental diets fed. Milk yield was significantly increased by the barley based diets ($P<0.05$), but was unaffected by source of energy. Milk protein concentration, like the urinary A/C ratios, was significantly increased on the barley and high protein diets ($P<0.05$). There was a trend ($P=0.082$) for milk fat concentrations to be increased on

the sugar beet pulp based diets. Milk casein concentrations were not significantly affected by dietary treatment, and differences in true protein concentration were brought about by changes in whey protein concentration. There was no effect of diet on the proportion of milk true protein as casein (nor, therefore, as whey).

Table 3.4 Mean daily dry matter intakes (DMI), and intakes of crude protein (CPI), metabolisable energy (MEI) and estimated intakes of ERDP (ERDPI), DUDP (DUDPI) (TCORN, 1992) and fermentable metabolisable energy (FMEI). Whole tract apparent OM digestibilities and allantoin/creatinine ratios. Calculated probabilities for effect of dietary protein (P), dietary energy (E) and their interaction (P.E).

	Diet					F Probability		
	BH	BL	SH	SL	SED	P	E	P.E
Silage DMI, kg/d	6.8	6.4	6.5	6.8	0.31	0.908	0.715	0.189
Concentrate DMI*, kg/d	10.1	9.7	9.8	10.2	0.46	0.908	0.715	0.189
Total DMI*, kg/d	16.9	16.1	16.4	17.0	0.77	0.908	0.715	0.189
CPI, kg/d	3.1	2.8	2.9	2.6	†			
ERDPI, kg/d	2.03	1.84	1.63	1.42	†			
DUDPI, kg/d	0.46	0.27	0.65	0.52	†			
MEI, MJ/d	216	203	205	210	†			
FMEI, MJ/d	183	171	177	182	†			
Whole Tract Apparent Digestibility of OM, g/g	0.81	0.83	0.83	0.84	0.001	0.167	0.074	0.601
Allantoin/Creatinine	2.00	1.90	1.90	1.79	0.059	0.021	0.018	0.789

* Not including 0.4 kg/d (fresh weight) parlour concentrate.
† Not determined.

Table 3.5 Mean values for milk yield, protein, fat and lactose concentrations and yields, with the calculated F probability of dietary protein (P), energy (E), and protein.energy interaction (P.E) effects.

	Diet					F Probability		
	BH	BL	SH	SL	SED	P	E	P.E
Milk Yield, kg/d	18.2	17.6	18.5	17.2	0.57	0.021	0.873	0.601
Protein, g/kg	36.3	35.1	35.1	34.9	0.40	0.020	0.022	0.110
Fat, g/kg	40.4	41.6	42.7	42.0	1.09	0.739	0.082	0.249
Lactose, g/kg	45.7	45.6	45.2	45.8	0.27	0.284	0.355	0.084
Protein Yield, g/d	656	617	647	595	21.7	0.007	0.334	0.672
Fat Yield, g/d	737	737	774	720	22.0	0.095	0.526	0.097
Lactose Yield, g/d	837	805	840	786	27.0	0.035	0.682	0.575

Table 3.6 Effects of dietary treatments on major milk nitrogenous constituents.

	Diet					F Probability		
	BH	BL	SH	SL	SED	P	E	P.E
Crude Protein, g/kg	35.6	34.4	34.2	34.1	0.51	0.075	0.028	0.116
True Protein, g/kg	33.8	32.4	32.5	32.5	0.55	0.113	0.144	0.104
Casein, g/kg	27.1	25.6	26.1	26.2	0.61	0.120	0.667	0.104
Whey, g/kg	6.68	6.81	6.29	6.34	0.176	0.051	0.041	0.457
Non-Urea NPN, g/kg	0.132	0.139	0.120	0.101	0.0144	0.550	0.021	0.197
Urea, g/kg	0.348	0.341	0.333	0.345	0.0247	0.482	0.254	0.736
Crude Protein Yield, g/d	645	605	630	583	22.9	0.014	0.259	0.848
True Protein Yield, g/d	611	572	604	556	22.1	0.012	0.479	0.788
Casein Yield, g/d	489	453	481	448	19.3	0.018	0.631	0.921
Whey Yield, g/d	122	119	117	109	4.7	0.101	0.042	0.379
Non-Urea NPN Yield, g/d	2.49	2.49	2.30	1.81	0.270	0.209	0.035	0.208
Urea Yield, g/d	6.19	5.89	5.95	5.14	0.444	0.090	0.129	0.428

The A/C ratio (Table 3.4) gives an indication of the supply of microbial protein to the animal. In order to correct for this and to differentiate between effects due to microbial protein supply and effects due to other factors (i.e. animal effects), production variables can be corrected for treatment differences in A/C ratio by dividing the variable by the ratio (with the assumption of linearity). When this was done, the effects of dietary protein level were lost for all variables. The significant effect of dietary energy on milk crude protein was also lost, although the effects of energy on milk yield and milk fat yield remained significant ($P<0.05$), with higher yields of milk and of milk fat per unit A/C on the S diets than on the B diets (mean values of B vs S: 9.35 vs 9.83 kg milk/d per unit A/C and 383 vs 414 g fat/d per unit A/C).

Urinary purine derivative excretion was found to differ with time of sampling, with higher A/C ratios obtained in the morning than in the afternoon (1.94 vs 1.86, sed 0.034). No interactions between time of sampling and diets were seen. However, these results were based on morning and afternoon samples bulked for each animal over 7 days and could possibly have been due to bulking errors or exceptionally high or low PD excretions on one of the days or times. Significant differences in the diurnal variation (DV) of the A/C

ratios due to experimental period were seen, with DV assessed as the ratio of the am value divided by the pm value. The am/pm values were 1.00, 1.10, 1.05 and 1.02 (sed 0.047) for periods 1, 2, 3 and 4 respectively. The value for period 2 was significantly different ($P<0.05$) to that of the period 1.

In order to determine the variance components due to each of the sampling variables (i.e. period, day, time and cow), all urine samples from 4 of the animals were analyzed. The animals were selected for completeness of the collections, which meant that the dietary treatment were not balanced. Variance components for each of the random effects are given in Table 3.7, and shows that the greatest variance was due to the effect of each animal, and the smallest due to each sampling time. This lends greater confidence to the effects of DV seen in the bulked samples.

Table 3.7 Estimated components of variance in sampling urinary PD excretion.

Effect	Component of Variance
Period	0.0088
Period.Day	0.0044
Period.Day.Time	0.000016
Period.Day.Time.Cow	0.1574

Table 3.8 Treatment effects on milk sodium, potassium, and chloride ion concentrations and yields.

	Diet					F Probability		
	BH	BL	SH	SL	SED	P	E	P.E
Na, g/kg	0.398	0.389	0.416	0.419	0.0316	0.908	0.293	0.804
K, g/kg	1.369	1.348	1.352	1.309	0.0170	0.018	0.034	0.366
Cl, g/kg	1.031	1.004	1.013	1.002	0.0170	0.127	0.384	0.504
Na, g/d	7.46	6.79	8.05	7.52	0.679	0.233	0.193	0.880
K, g/d	25.91	24.65	25.92	23.49	0.886	0.011	0.373	0.366
Cl, g/d	18.76	17.46	18.73	17.01	0.609	0.002	0.581	0.633

The mean treatment effects on the concentrations and daily yields of sodium, potassium, and chloride ions are presented in Table 3.8. The ratio of potassium to sodium (K/Na) in the milk tended to be higher ($P \approx 0.099$) on the B diets, due to the significant increase in K on the B diets, despite small and non-significant increases in Na concentration on the S diets. Similarly, there was a strong tendency ($P \approx 0.056$) for the lactose/Cl ratio to be higher on the low protein diets. Overall, however, there was no difference in the ratios of ratios of these compounds (i.e. K/Na / lactose/Cl), which are the main factors influencing milk osmosity. This was supported by little difference in the milk freezing point depression measured from each dietary treatment (grand mean -545.6 °Hortvert), differences that were seen being less than the accuracy of the instrument.

The ratios of protein to fat and protein to lactose give an indication of the partitioning of nutrients for the synthesis of these milk constituents. These ratios are given in Table 3.9.

Milk proteins are derived ultimately from dietary crude protein. Table 3.10 shows the relative protein efficiencies for each diet (i.e. milk protein output/crude protein intake).

Table 3.9 Ratios of milk protein to fat and lactose, and of fat to lactose.

	Diet					F Probability		
	BH	BL	SH	SL	SED	P	E	P.E
Protein/Fat	0.911	0.856	0.838	0.839	0.0209	0.077	0.005	0.069
Protein/Lactose	0.794	0.770	0.777	0.763	0.0082	0.003	0.053	0.413
Fat/Lactose	0.883	0.911	0.946	0.920	0.0247	0.967	0.051	0.137

Table 3.10 Relative protein efficiencies of milk nitrogenous constituents (milk protein output/crude protein intake, g/kg).

	Diet					F Probability		
	BH	BL	SH	SL	SED	P	E	P.E
Crude Protein	205	218	220	226	12.1	0.276	0.183	0.697
True Protein	194	206	208	216	12.0	0.253	0.181	0.836
Casein	156	163	168	174	9.9	0.365	0.107	0.903
Whey	38.6	43.1	40.5	42.2	2.51	0.098	0.788	0.449
Non-Urea NPN	1.00	1.11	1.04	0.96	0.005	0.678	0.106	0.010
Urea	1.98	2.15	2.13	1.99	0.19	0.948	0.969	0.269

The treatment means from the BH diet were significantly higher than those of the other three diets for milk true protein concentration, casein concentration, potassium concentration, and A/C ratio ($P<0.05$), and milk total nitrogen concentration and the ratio of protein/fat ($P<0.01$). The treatment means of the BH diet were lower than the other diets for milk fat concentration, although this did not quite reach significance ($P=0.066$).

The effect of experimental period was significant on milk yield ($P<0.05$) and milk urea concentration ($P<0.001$) (Figure 3.7). No effects of period were seen on other nitrogenous constituents on milk.

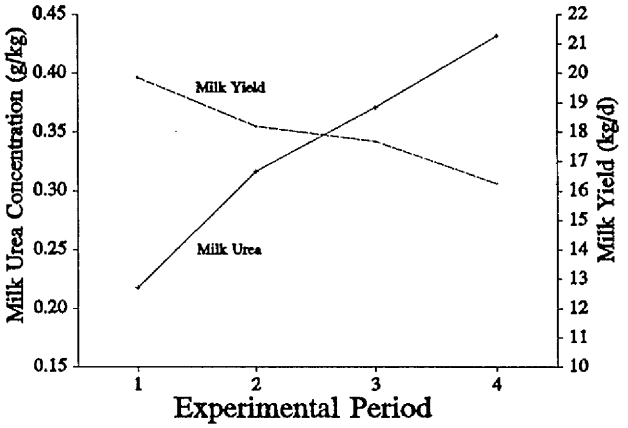


Figure 3.7 Effect of experimental period on milk yield and urea concentration.

Figure 3.8 shows the mean milk yields of the whole group over the duration of the experiment. After an initial drop in the milk yield at the start of the experiment (corresponding to the reduction in parlour fed concentrate), the decline in yield was at a rate which is to be expected for the stage of lactation of the group.

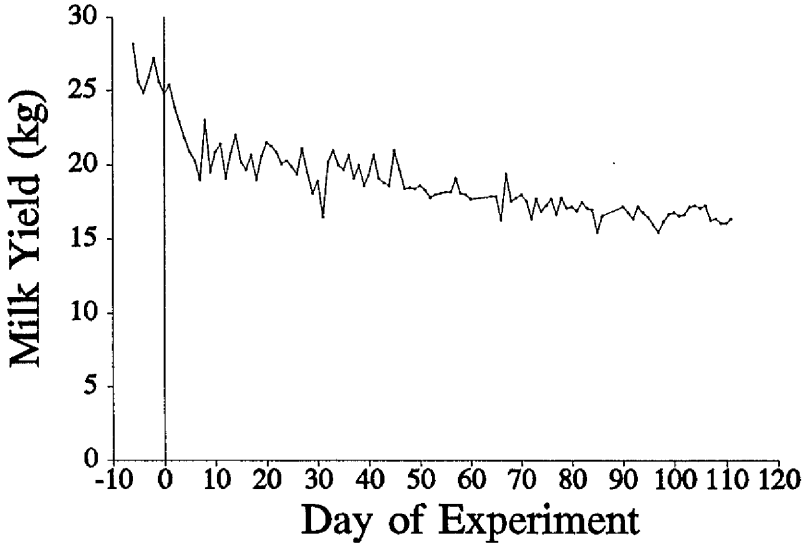


Figure 3.8 Mean daily milk yields of the group of experimental cows.

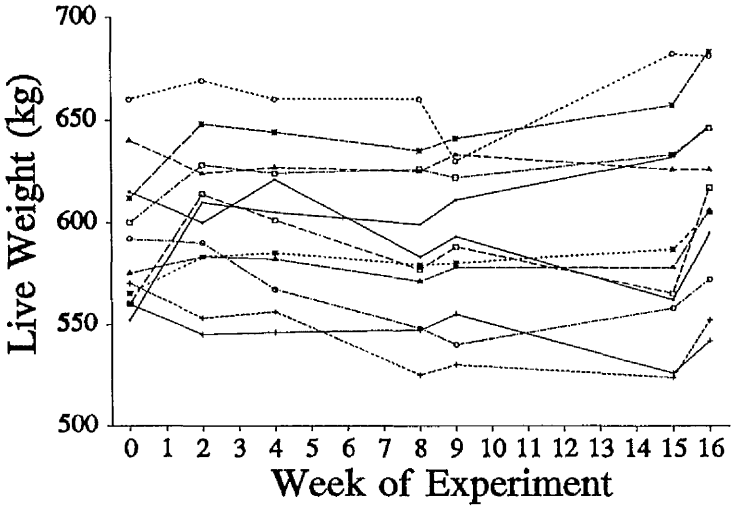


Figure 3.9 Individual live weights of the experimental cows.

Figure 3.9 shows the live weights of the experimental cows. The identity of individual animals is not shown since there was little change in the weight of individuals or of the group as a whole.

No significant residual effects of diet were seen (not shown); in other words, changing from diet BH to BL was found to be no different from changing from diet BH to SH or SL, and so on. Likewise, apart from the initial drop in milk yields at the start of the experiment (Figure 3.8), which, it is assumed, was associated with an increase in protein concentrations, no residual effect of the experiment was detected once the animals had been returned to the main herd. The Scottish Milk Recording Association (SMRA, Paisley, Scotland) took samples before, during and after the experiment as part of the normal Auchincruive herd management. Increases in milk protein concentration were recorded over the duration of the experiment, consistent with samples taken independently by myself, and these high protein concentrations were seen to continue after the experiment. Milk yields were seen to continue to decline as expected for animals nearing the end of the lactation. The milk yields and milk composition recorded by the SMRA are consistent with figures normally seen for dairy cows at that stage of lactation, and without more detailed sampling it is not possible to attribute the milk compositions seen to anything other than stage of lactation.

3.6 DISCUSSION

The two factors which are generally acknowledged to have the greatest influence on milk protein concentration are forage to concentrate ratio, and metabolisable energy intake. In this experiment, the F:C ratio was kept constant throughout on all four diets. The mean silage intakes were slightly higher on the BH and SL diets than on the BL and SH diets, but not significantly so. It is assumed, therefore, that any effects seen due to diet must have been caused by differences in the concentrate portion of the diets.

Increasing dietary protein level caused significant increases in both milk crude protein concentration and yield. An increase in dietary protein also caused significantly higher milk yields. Barley as an energy source, as opposed to sugar beet pulp, caused significantly increased milk protein concentrations, but not yields. The interaction between energy source and protein level was not significant, although the barley-based high protein diet produced significantly higher milk protein concentrations than the other three diets. Similar effects were seen with the Kjeldahl analysis of milk samples from a single day, though the effect did not attain significance owing to the larger SEDs associated with the sampling method. In retrospect, it would have been better to have analyzed a sample bulked over all four days rather than just the one.

Inspection of Table 3.6 reveals that the high and low protein diets based on sugar beet pulp produced milk with very similar concentrations of both casein and whey proteins, whereas the two barley-based diets caused quite different results; the difference between the barley and sugar beet pulp based-diets was in the relative yields of milk that each supported compared. Several studies have shown that certain essential amino acids (group 1 EAA) taken up by the udder are secreted in milk with high efficiencies, and that the milk protein concentration can be increased by supplying greater quantities of those which appear to be limiting (Broderick, Satter and Harper, 1974; Clark, 1975). It is assumed that the output of milk proteins observed in the present study was related to the total supply of precursors from the diet.

The milk yields from all animals on this experiment were very low compared to the yields immediately prior to the start. This was shown in Figure 3.8 and is despite reasonable feed intakes. Holstein-Friesian milk typically has a protein concentration of about 32-33 g/kg; the concentrations obtained on this experiment were all well above this figure but

the yields were perhaps half typical daily protein yields. This suggests that there may be a maximum production rate of protein relative to milk volume and that milk volume was limited due to a shortage of some other precursor.

Increasing the level of protein in the diet from a formulated deficit of DUDP to a formulated excess apparently increased milk crude protein concentration. However, when the milk protein concentration was corrected for microbial protein supply by dividing by the A/C ratio, the effects of dietary protein level were lost, suggesting an effect of RDP rather than DUDP even though all four experimental diets were formulated to supply equal quantities of RDP. This implies that the effects seen were either due to an increase in rumen nitrogen efficiency, or to differences in supplies of ERDP. Estimates of the actual intakes of ERDP suggest the latter to be important. Milk urea concentration has been shown to correlate highly with blood urea concentrations (Oltner and Wiktorsson, 1983; Roseler, Ferguson, Sniffen and Herrema, 1993), and these in turn with rumen ammonia concentration (Lewis, 1957). Milk urea concentration was found to be independent of milk yield (Gustafsson and Palmquist, 1993). These reports suggest that milk urea concentration can be used as a rough estimate of the rumen ammonia status, and therefore to the efficiency of utilisation of rumen degradable nitrogen sources. Oltner, Emanuelson and Wiktorsson (1985) conclude that milk urea concentration is less affected by absolute levels of dietary protein intake, and more by the ratio of crude protein to metabolisable energy. This is as one would expect since the utilisation of energy and nitrogen in the rumen are closely linked. Milk urea concentrations from this experiment were similar to those found by Oltner *et al.* (1985), and far lower than those reported by Roseler *et al.* (1993) suggesting an efficient use of the available N. No effect of experimental diet was seen on milk urea concentration, nor were there any differences in the 'efficiency' of production of milk urea concentration per unit CPI. This suggests that the differences seen in A/C ratios were not due to differences in the efficiency of utilization of RDP from the different diets, but rather in differences in supply of RDP that were used with the same efficiency in creating microbial protein. It was this extra microbial protein that was more important in affecting milk crude protein than the extra DUDP supplied in the feed.

Microbial protein yield is dependent on the availability of RDP and rumen fermentable ME (FME). If RDP is limiting, fermentation of ME will be reduced. Conversely, if FME is limiting, fermentation of CP will be reduced. Figures 3.10 and 3.11 show the response

of the A/C ratio to increasing CPI and FMEI respectively. The fact that a consistent increase in the A/C ratio was achieved with increasing CPI but not with increasing FMEI indicates that RDP was limiting, rather than energy. Figure 3.12 shows the response of A/C ratio to the estimated intake of ERDP (TCORN, 1992), and adds support to this theory. Further support is provided by the evidence from milk urea concentration because if FMEI was limiting, differences in the efficiency of rumen N utilization would be expected.

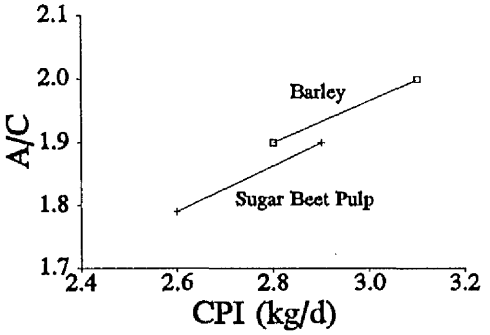


Figure 3.10 Response of allantoin/creatinine (A/C) to crude protein intake on barley- and sugar beet pulp-based diets.

The mean milk urea concentration was seen to increase significantly with experimental period (Figure 3.7). The mean milk yield dropped during the course of the experiment, as was expected from cows at that stage of lactation. The drop in milk yield was not sufficient to explain the increase in milk urea concentration, which must, therefore, have been at least in part due to a decrease in the efficiency of RDP utilization as the experiment progressed; changes in silage protein content and degradability are not enough to explain the effect either. No effects due to experimental period were seen on the concentrations of either the casein or whey fractions of the milk protein, and reductions in the yields of these can be explained by the reductions in milk yields.

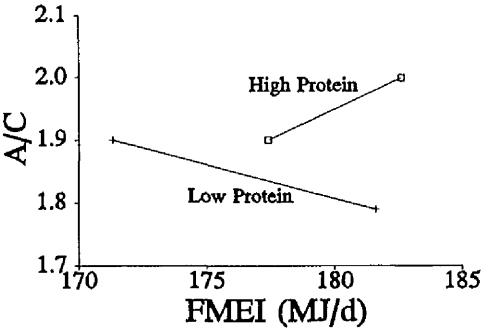


Figure 3.11 Response of allantoin/creatinine (A/C) to increasing supply of fermentable ME on high and low protein diets.

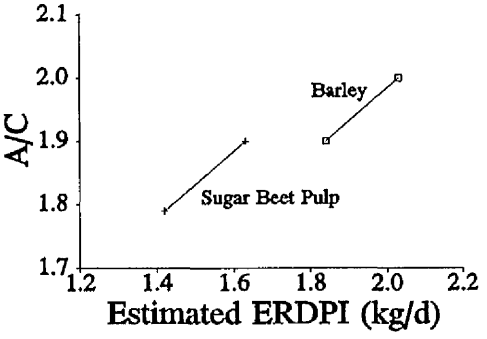


Figure 3.12 Response of allantoin/creatinine (A/C) to estimated intake of effective rumen degradable protein on barley- and sugar beet pulp-based diets.

Dietary energy source had a much clearer effect on milk crude protein concentrations, with higher concentrations on the B diets than on the S diets. CPI was slightly higher on the B diets, as were the A/C ratios. Again, this indicates an overall greater supply of protein

to the animal which resulted in the increased milk protein production. No significant effects on casein concentration were seen due to either energy source. There were, however, slightly higher concentrations of whey proteins on the B diets (statistically significant, but in fact an increase of only about 0.4 g/kg), and a strong trend (P=0.051) towards higher whey protein concentrations on the low protein diets. The non-urea NPN (NUNPN) fraction of milk was also higher on the B diets, which, in combination with the whey proteins, led to the increase in milk crude protein concentration. The identities of the NUNPN compounds are unknown; they were, however, the only N fraction of milk which was significantly affected by dietary energy source after correction for the estimated supply of microbial protein. Bovine milk contains factors such as orotic acid and uric acid in relatively large quantities. Uric acid is likely to change in parallel with urinary allantoin excretion and indeed, apart from that of the SL diet, the NUNPN yields, when corrected for A/C, were all similar.

The concentration of whey protein was significantly higher on the B diets than on the S diets. The identity of individual whey proteins was not determined, although those present in greatest quantities are ß-lactoglobulin, α-lactalbumin, serum albumin and various immunoglobulins. The increase in whey proteins on the B diets was large enough to be the main cause in the increase the true protein concentration of milk, although the difference in the effect of energy source was lost when the supply of dietary crude protein was taken into account (Table 3.10). Indeed, there was a slight trend (P=0.098) for the two low protein diets to increase whey protein yields to a greater extent than was accounted for by the level of dietary crude protein fed. Owing to their relatively low importance from a nutritional and processing aspect, little work has been done to study the effect of diet on milk whey protein production. Sutton, Beever, Aston and Daley (1993) found that increasing the amount of crude protein fed increased the milk crude protein concentration by increasing both casein and whey protein fractions. Lactose and whey protein production was seen to be broadly correlated, and it is possible that α-lactalbumin production was increased in response to increased supplies of glucose and hence lactose production. In this study, the production of lactose and whey proteins follow broadly similar patterns, although it may be complicated by the form in which energy was supplied to the mammary tissue, which would have been quite different for the barley and sugar beet pulp based diets. This is an area of research which clearly requires further investigation.

Differences in milk fat concentration were not significant. However, there was a trend (P=0.082) towards higher fat concentrations on the sugar beet pulp diets and there were near significant differences in the fat/lactose ratios (P=0.051) due to energy source. Although rumen or blood VFA concentrations were not measured, an increase in the acetate:propionate ratio has been seen to bring about increases in the fat concentrations of milk (Lees *et al.*, 1990; de Visser *et al.*, 1990) on diets high in fibre from concentrates. The present results suggest that higher acetate:propionate ratios were achieved on the two S diets compared to the two B diets.

Lactose concentrations were not affected significantly by diet; they were, however, rather lower than normally expected for bovine milk. Lactose production is probably limited by the availability of glucose, both in terms of absolute supply, and in competition with the needs of other synthetic pathways. Responses of milk yield to increased dietary crude protein supply are often mediated through more efficient use of dietary energy sources by the rumen with an increased supply of N to the rumen. No differences were seen in the apparent whole tract OM digestibilities of the four diets of the present study, which suggests that absolute improvements in energy source utilization were not the driving force behind the observed results, particularly since the diet with the lowest CPI (diet SL) was associated with the highest apparent whole tract OM digestibility. The greater protein/lactose ratios on the two S diets suggests that an increase in propionate supply to the animal may have led to a sparing of amino acids which would otherwise have been used for gluconeogenesis. Reynolds, Huntington, Tyrrell and Reynolds (1988) calculated that amino acids may contribute about 16.5% to hepatic glucose production in dairy cows on a maize-silage and concentrate diet, a diet on which one might expect a relatively high rate of propionate production.

Two of the diets, BH and SL, were frequently seen to cause significantly different effects to the other diets in some way. Although few significant interaction effects were seen between dietary protein level and energy source, the effects of these two diets offer some evidence for their existence. It is interesting to speculate, for instance, that the high true milk protein concentration obtained on the BH diet (achieved because of a high casein concentration) could have been due to a combination of a greater supply of amino acids for casein production and a greater supply of energy. Within the secretory cells of the mammary gland, there is a general pool of milk constituent precursors; how they are

partitioned between the different constituent depends on their relative value for different constituents. Glucose is needed for lactose synthesis, acetate for fat synthesis, and amino acids for protein synthesis. All can also be used as energy sources. The high amino acid and high propionate supplies which it is postulated were provided by the BH diet were more appropriate for milk protein synthesis than the (notionally) low amino acid, low propionate levels provided by the SL diet. The influence of diet on endocrine factors, and the subsequent control of metabolism by them is strongly suspected, but with no way of measuring their activities at Auchincruive, it was an area of research which could not be followed.

One of the more striking features of the results of this experiment is the remarkably low mean milk yield obtained from the four diets, even though the animals achieved relatively high ME intakes. One of the features of the experimental diets compared to the pretrial diet was their lower fat contents, the concentrates containing less than 30 g/kg AHEE. Dietary fat usually causes a significant increase in milk yield, with a dilution of the milk protein (see Chapter 4). High inclusion rates of dietary fat can reduce rumen fermentation of fibre. However, these diets were relatively low in fat, and the apparent whole tract OM digestibilities were not different between diets. During the 7 days before the start of the experiment, the group of cows gave a mean milk yield of 27.5 kg/d, with 31.1 g/kg milk protein, on a parlour concentrate ration with an AHEE content of 61 g/kg.

The diets were originally formulated to meet ME requirements when yielding some 27 kg/d, with no weight change. Because of the low yields, the same formulation program was used to predict ME requirements of the animals at the production levels actually achieved. These were 155, 154, 160 and 152 MJ/d for the production levels from diets BH, BL, SH and SL respectively. Comparison with the ME intakes calculated from feed analyses given in Table 3.4 (which assume additive effects of ME supply from concentrates and silage) shows that there were apparent losses of 50-60 MJ ME per day. This should be enough energy for the production of approximately 10 kg of milk, or a weight gain of approximately 0.4 kg/d. Therefore, either the estimation of ME was incorrect, or there were large inefficiencies in the system. Since 50-60 MJ of energy is a lot to lose as heat and methane (JD Oldham, personal communication), is most likely that the major errors came from the estimations of ME contents of the diets. Prediction of the ME contents of the diets was done using the *in vitro* digestibility estimations. The whole

tract OM digestibility measurements using IADF can be used to perform a similar calculation. Taking the conservative estimate of ME = 0.16 x DOMD using the whole tract OM digestibilities, the daily ME intakes of the animals are very similar at 209, 195, 200 and 210 MJ ME/d for diets BH, BL, SH and SL respectively. The answer to the problem therefore remains unclear.

3.7 CONCLUSIONS

Milk protein concentration was significantly increased by an increase in protein supply to the animal. This was achieved through an increase in the production of microbial protein, as estimated by urinary purine excretion, with greatest production (excretion) rates being seen on the barley-based diets and the high protein diets. By using purine derivative excretion data in conjunction with milk urea concentrations, it is concluded that all diets were deficient in effective rumen degradable protein, with the sugar beet pulp diets more deficient than the barley diets, and that these differences in the supply of ERDP were the cause of the effects seen on milk protein. The milk protein concentrations were relatively high from all four experimental diets, and this was possibly due to a combination of low fat, low ERDP contents of the diets causing the relatively low milk yields. Small differences in the concentrations of milk fat and near significant differences in the fat/lactose ratio indicate that there were differences in the rumen fermentation patterns from the two dietary energy sources. Increased protein/lactose ratios indicate that amino acids may have been spared from gluconeogenesis on the barley and high protein diets.

Chapter 4

Effects of Dietary Fat on the Yield and Relative Proportions of Different Milk Protein Fractions

4.1 SUMMARY

In Chapter 3, a 2x2 factorial experiment was described in which mid-lactation dairy cows were fed four diets differing in source of energy and level of protein in order to investigate the effect of these on milk protein concentration. The results of that experiment indicate that an increase in the crude protein supply to the animal resulted in an increase in milk protein production. All four diets produced relatively high milk protein concentrations and relatively low milk yields. A factor common to all diets was their low fat content. This chapter describes an experiment which was designed to investigate the effect of dietary fat on milk protein concentration. Three diets were fed to mid-lactation dairy cows in a complete Latin Square changeover design, each at one of two levels of metabolisable energy and one of two levels of fat. The concentration of protein in milk was significantly higher from the low energy, low fat diet, due to lower milk yields. Yields of milk protein were highest from the high fat, high energy concentrate, and may have been a result of sparing of amino acids from hepatic gluconeogenesis. Microbial protein yield, as estimated by urinary purine derivative excretion, was highest on the low fat, low energy and high fat, high energy concentrates, which both supplied similar amounts of fermentable metabolisable energy. Evidence for increased *de novo* synthesis of fatty acids on the low fat diet indicated that there was a reduced availability of glucose for lactose synthesis and ATP production, leading to the reduced milk yields and hence increased milk protein concentrations.

4.2 INTRODUCTION

The modern dairy cow typically produces more milk during early lactation than can be supported by the amount of food that she can eat and therefore goes into negative energy balance at this time, utilising body stores of energy. Since the animal is limited by the quantities of feed she can eat, an increase in the supply of energy must be achieved by increasing its density; high quality carbohydrate energy sources are relatively expensive and excessive intakes of cereal grains can lead to metabolic disorders. Much attention is therefore paid to the use of fats and oils (referred to from now on collectively as fat) in dairy rations, which have a high energy density and can be much cheaper than carbohydrates, particularly when derived from by-products such as tallow and cotton seed. The use of fat in lactation rations for dairy cows has been discussed in a number of recent reviews (Palmquist and Jenkins, 1980; Coppock and Wilks, 1991) although until recently (Coppock and Wilks, 1991) the importance of the effects of dietary fat content on milk protein were not stressed. In Chapter 3, it was seen that high milk protein concentrations were obtained on four experimental diets that were all low in fat. This Chapter describes an experiment that was conducted to investigate the effects of dietary fat on milk protein concentration in the light of that observation.

4.2.1 Dietary Fat as an Energy Source

As discussed in Chapter 3, an increase in the supply of dietary energy to the dairy cow will tend to increase milk protein concentration. This is true when the energy is in the form of carbohydrates, but generally not when the energy is supplied by fat. In this case, the reverse is the frequently seen (Emery, 1972; Spörndly, 1989; DePeters and Cant, 1992), with an increase in dietary fat causing a reduction in milk protein concentration.

There are several metabolic mechanisms by which dietary fat may influence milk protein concentration, both at the rumen level and at the animal level. Fat can have a toxic effect on micro-organisms, and fibre fermentation in the rumen has been seen to be reduced at high levels of dietary fat inclusion. Triglycerides, as fats are absorbed by the gut, can be incorporated directly into milk fats, thereby reducing the load on energy sources needed for *de novo* synthesis of fatty acids, i.e. principally glucose, acetate and ß-hydroxybutyrate, releasing glucose to be used for the synthesis of lactose and ATP (for use by the Na^+/K^+-ATPase) and hence bring about an increase in milk yield (see Figure 4.1).

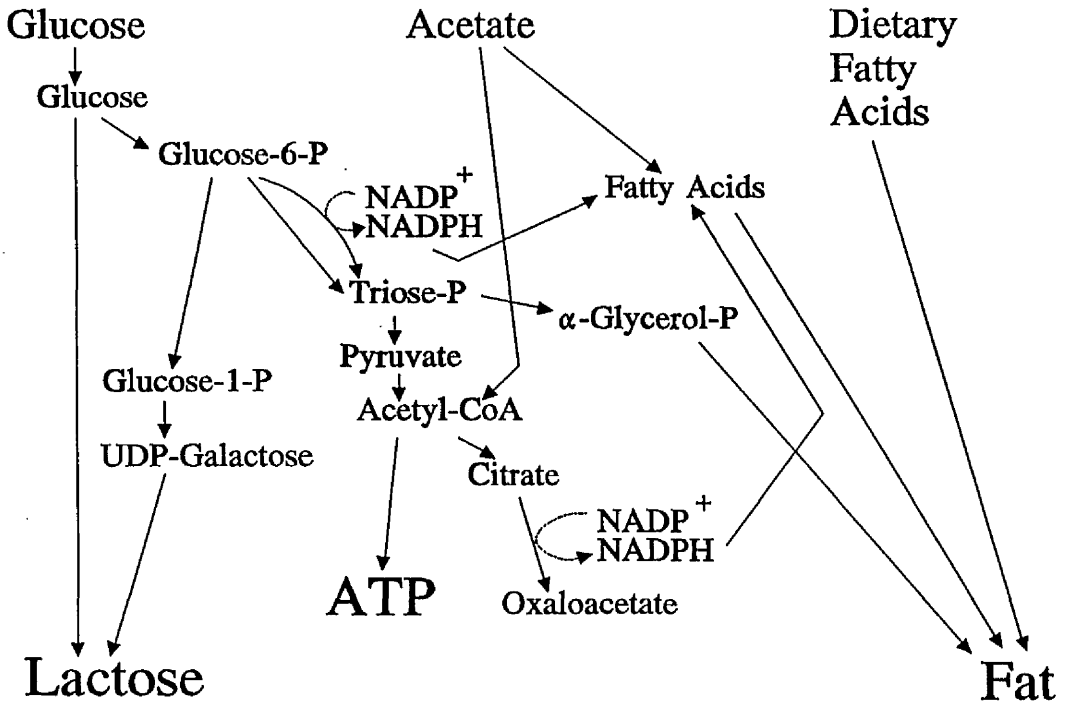


Figure 4.1 Simplified diagram of flow of precursors and energy within the mammary epithelium to milk lactose and fat. ß-Hydroxybutyrate has much the same fate as acetate.

4.2.2 Dietary Fat and Digestibility

In their reviews, Palmquist and Jenkins (1980) and more recently, Coppock and Wilks (1991), concluded that an increase in the unprotected (i.e. ruminally available) fat content of the diet can adversely affect fibre fermentation in the rumen. It has been proposed that this is due to either physically coating the fibre and hence reducing its availability, modifying the microbial population through selective toxicity, affecting microbial activity by interfering with the cell membrane, or reducing the availability of cations by forming insoluble complexes (Devendra and Lewis, 1974). There is, therefore, a large amount of interest in the use of ruminally inert fats that are either protected physically (e.g. as whole oil-seeds) or chemically, by saponification with calcium rendering the fat insoluble. There is a great deal of information in the literature on this subject, and the majority of the more recent work includes some detail of the protein concentration of milk produced on various experimental diets; little work has been done with the effect on milk protein as the major topic. Some of the more recent work that illustrates the factors which may be involved is discussed below.

Emanuelson, Murphy and Lindberg (1991) compared tallow with raw and heat-treated full-fat rapeseed (canola). No effect on milk yield or composition was reported, despite reduced organic matter (OM) digestibility in the rumen on diets supplemented with tallow. Whole tract apparent digestibility of OM, on the other hand, was unaffected by dietary treatment. This hindgut compensation for reduced rumen digestibility has frequently been found on a number of diets that varied in level and quality of fat (Klusmeyer, Lynch, Clark and Nelson, 1991b; Jenkins and Jenny, 1992). This has several possible implications since the absolute ME supply to the animal may not be affected by differences in the site of digestion, but its availability to rumen microbes and hence the form in which that energy is supplied to the animal can be. Jenkins and Jenny (1992) found a decrease in the concentrations of rumen VFAs, primarily through a reduction in acetate (with a subsequent decrease in the acetate:propionate ratio), on diets with supplements that varied from 100% prilled fat (assumed to be ruminally inert) to 100% rapeseed oil, indicating a reduction in fibre degradation with increasing ruminally available fat. They found small increases in milk protein concentration as milk yield decreased; protein yield was unchanged. Interestingly, they also reported small but significant increases in the milk NPN concentrations, the majority of which was probably in the form of urea. This, together with increased whole tract apparent nitrogen digestibility on diets with added fat indicates a possible reduction in the supply of amino acids for milk protein production due to reduced microbial protein production and increased ureagenesis. Emanuelson *et al.* (1991) in contrast, found no difference in duodenal flows of microbial N, rumen ammonia-N, nor milk protein yields. Khorasani, de Boer, Robinson and Kennelly (1992) fed increasing levels of processed rapeseed from 0% to 17.4% of the feed DM. An increase in the level of fat incorporation reduced rumen acetate concentrations, reduced the acetate:propionate ratio but did not affect the whole tract apparent DM, OM, CP, or fibre digestibilities. Plasma glucose and insulin concentrations were seen to decline with increasing dietary fat, and reduced yields of milk protein with similar milk yields caused reduced milk protein concentrations. Fat, therefore, may in some circumstances, slightly shift the site of digestion from the rumen to the hind gut without significantly altering the amount of energy that is supplied to the animals, but merely the form in which it is absorbed.

Several authors have reported the use of protected fats in dairy cow rations with no adverse affects on the rumen or whole tract digestibility of feedstuffs (Schauff and Clark,

1989; Ohajuruka, Wu and Palmquist, 1991; Palmquist, 1991; Drackley and Elliot, 1993). Drackley and Elliot (1993) observed increased milk yields with increasing fat supplementation; similar protein yields on all diets caused a dilution of milk protein and hence a decrease in its concentration. Palmquist (1991) fed two formulations of calcium soaps at two levels in the diet, and found decreased DM intakes as fat supplementation increased; the protein concentration of milk was reported to be unaffected by source or level of fat, but milk yield decreased. Grummer (1988), feeding prilled fat at two daily intake levels similarly found no effect on rumen metabolism or milk composition; whole tract digestibility of DM and NDF was, however, lower on the low fat diets, an effect that could not be explained. The mixed response of milk yield and composition to protected fat supplementation may be partly due to the tissue availability of other energy sources such as glucose and acetate following changes in rumen fermentation and the supply of nutrients from the gut. This is discussed further in Section 4.2.5.

4.2.3 Dietary Fat and Rumen Microbial Efficiency

Part of the effect of dietary fat on milk protein concentration may be due to a change in rumen efficiency of protein capture. Metabolisable energy supplied in the form of fat rather than carbohydrate is useful to the animal in either form. However, the fermentable ME (FME) content of the diet will be lower because of fat's low solubility and its potential of inhibition of fibre fermentation. This could lead to changes in the efficiency of rumen degradable nitrogen capture both through a deficiency in energy for the microbes and also through changes in microbial populations.

Klusmeyer, Lynch, Clark and Nelson (1991a) found decreased organic matter digestion in the rumen of dairy cows fed calcium soaps of long chain fatty acids when comparing fish meal and soya bean meal as protein sources, although there was no change in supply of microbial-N to the duodenum. This led to improvements in efficiency of microbial-N capture per unit OM digested, an effect similarly reported by Emanuelson *et al.* (1991), although this was not seen when fat replaced forage (Klusmeyer *et al.*, 1991b). Sutton, Knight, McAllan and Smith (1983) found a similar effect in sheep, with decreases in OM digestibility on diets supplemented with oils. They also found an increase in the flow of microbial-N to the duodenum, and attributed the effect to defaunation of the rumen, leading to less predation of bacteria by protozoa, a finding also reported in the work of Ikwuegbu and Sutton (1982). Other authors (Ohajuruka *et al.* (1991); Palmquist,

Weisbjerg and Hvelplund, 1993), in contrast, found no difference in ruminal OM digestibility between diets varying in fat content and quality, with no difference in microbial-N flow to the duodenum and hence no difference in microbial efficiency.

4.2.4 Dietary Fat and Fermentation Patterns

If dietary fat has an effect on digestion, then it follows that the products of digestion which are absorbed from the gut may be changed by the inclusion of supplementary fat in the diet. In Chapter 3, the effects of the ruminal acetate:propionate molar ratio (A:P) on milk protein concentration were discussed; if the inclusion of supplementary fat in the diet reduces fibre digestibility in the rumen - for whatever reason - then the products of rumen fermentation may be expected to change, either absolutely, or in proportion to one another. This can occur, for instance, if acetate production is reduced because of reduced fibre degradation. In the recent literature, ruminal A:P ratios obtained from various dairy cow rations have been found to change in both directions when rumen fibre digestibility was decreased (Emanuelson *et al.*, 1991; Klusmeyer *et al.*, 1991b; Ohajuruka *et al.*, 1991) but not if there were no changes in digestibility (Grummer, 1988; Schauff and Clark, 1989; Drackley and Elliot, 1993). Some authors found changes in the A:P but not in whole tract digestibility (Klusmeyer *et al.*, 1991b; Jenkins and Jenny, 1992; Khorasani *et al.*, 1992) indicating that the supply of ME to the animal was the same across different diets, but the form in which the energy was presented to the animal would have been different depending on the site of digestion.

4.2.5 Dietary Fat and Changes in Animal Metabolism

In their paper, Casper and Schingoethe (1989b) proposed a model to describe the depression of milk protein concentration when dairy cows are fed high fat diets. They argued that milk protein output (i.e. daily yield) is regulated by a combination of milk yield and protein concentration; a reduction in the concentration (regardless of the milk yield) would therefore be a 'depression'. The alternative view is that milk protein concentration is a function of milk yield and total daily protein production; in this case, an increase in milk yield without an increase in protein production would be a 'dilution'. Casper and Schingoethe (1989b) proposed that additional dietary fat blocks the release of growth hormone (somatotrophin, bST) from the anterior pituitary gland, reducing its plasma concentrations and thus reducing its (indirect) effect of stimulating amino acid uptake by the mammary glands. This in turn, they postulated, causes a reduction in the

supply of amino acids for incorporation into milk protein. Amino acids are taken up by mammary tissue by a number of transport systems (Baumrucker, 1985), most of which normally operate below saturation point. Several of these transport mechanisms are sodium dependent, utilising a gradient of Na^+ ions to concentrate amino acids inside the cell. This ionic gradient is maintained by the Na^+/K^+-ATPase on the plasma membrane, the same gradient that, together with lactose production, controls the flow of water into milk (Linzell and Peaker, 1971), and which is regulated by plasma prolactin (Falconer and Rowe, 1977; Falconer, Langley and Vacek, 1983). This could be part of the reason why milk protein concentration is relatively constant.

The work by Casper and Schingoethe (1989b) showed a reduction in the mammary arterio-venous (A-V) differences of certain amino acids with cows fed on diets with added fat. Their figures, however, were based on estimated blood flows (a ratio of 400:1 litres of blood/kg milk production, from Drackley and Schingoethe, 1986) and the A-V differences reported may not have reflected an overall change in delivery of amino acids to the mammary tissue; total daily milk protein yields were unaffected by dietary fat. Cant, DePeters and Baldwin (1993) reported a 7% decrease in blood flow in dairy cows fed additional fat (yellow grease) which reduced the delivery of amino acids to the mammary tissue; total daily milk protein yields were again unaffected by dietary fat since increased apparent extraction rates of plasma amino acids compensated for the lower delivery rates on the high fat diets. However, their estimates of blood flow were flawed since they were based on the Fick principal using essential amino acids but did not take into account mammary protein turnover. DePeters, Taylor and Baldwin (1989) evaluated the effects of dietary fat in isoenergetic diets on milk protein content and concluded that the effect of dietary fat was to reduce the concentration of milk protein by causing significant differences in the crude protein intakes (CPI) of the animals, with lower CPIs on the high fat diets; increased milk protein yields reflected increased CPIs by animals on the low fat diets.

Protein is not, of course, the only constituent of milk; fat and lactose are the other two major constituents that are produced by the secretory cells and are more likely to be affected by dietary fat. Palmquist and Moser (1981) reported an decrease in blood glucose and insulin levels after feeding a protected lipid supplement; blood lipid concentration was, however, increased by more than a factor of 2, together with milk fat production. Milk

protein concentration was decreased, as expected, because of a slight increase in milk yield; protein yield was unaffected by dietary fat. Schmidt (1966) showed that an increase in plasma insulin reduced milk yield (and increased the concentrations of fat and protein) by reducing the availability of glucose for lactose synthesis. Correlations have been found between basal plasma insulin levels and milk yields (see Section 3.2.5). Milk fatty acids are synthesised in the mammary tissue using the energy from glucose oxidised by the pentose phosphate pathway and from citrate by the isocitrate dehydrogenase pathway, both yielding supplies of the reducing equivalent NAPDH (Pethick, Bell and Annison, 1987). By increasing the precursors of milk fat, e.g. acetate and triacylglycerides, *de novo* synthesis of milk fat is reduced, decreasing the utilisation of energy from glucose for this purpose, and thus allowing a greater proportion of the glucose taken up by the lactating mammary gland to be used for lactose production (Faulkner and Pollock, 1989) and hence increased milk yields. Absorbed fatty acids can be incorporated into milk fat without change (Storry, Hall and Johnson, 1968; Steele, Noble and Moore, 1971; Storry, Hall and Johnson, 1973; Smith, Dunkley and Franke, 1978; Banks, Clapperton and Kelly, 1980). An increase in the output of long-chain fatty acids - originating from the diet - is associated with a decrease in the *de novo* synthesis of short chain fatty acids in the mammary gland (Banks *et al.*, 1980; Faulkner and Pollock, 1989). The use of milk soluble citrate concentrations has been proposed as an indication of the rate of fatty acid synthesis in the mammary secretory tissue (Faulkner and Clapperton, 1981; Faulkner and Pollock, 1989), with decreased milk citrate concentrations being an indication of the flux of isocitrate to oxaloacetate in the production of NADPH for intramammary fatty acid synthesis purposes (Bauman, Brown and Davis, 1970). Isocitrate is derived from the citric acid cycle, ultimately from acetate, ß-hydroxybutyrate, or glucose (see Collier, 1985). An increased delivery of acetate to the mammary tissue will therefore reduce the load on glucose utilization, and allow an increase in its use for lactose synthesis. The work of Miller, Reis, Calvert, DePeters and Baldwin (1991a and 1991b), discussed briefly in Chapter 3, indicated that the plasma concentration of triacylglycerides and acetate (and other fat 'derivatives') is the overriding factor that influences their uptake by the bovine mammary gland. Increased plasma concentrations of triacylglycerides, acetate, free fatty acids, NEFA, and/or ß-hydroxybutyrate have been observed by a number of authors feeding supplemental dietary fat to dairy cows (Palmquist and Conrad, 1971; Wrenn, Bitman, Waterman, Weyant, Wood, Strozinski and Hooven, 1978; Smith *et al.*, 1978; Palmquist and Conrad, 1978; DePeters *et al.*, 1989; Erickson, Murphy and Clark, 1992).

Several authors have reported work in which various treatments have been used in attempts to reduce the depressing or diluting effect of fat on milk protein concentration. The use of nicotinic acid (niacin) and increased dietary protein allowances are two such treatments. Erickson *et al.* (1992) found elevated levels of blood plasma NEFA and ß-hydroxybutyrate on dairy cow rations containing calcium salts of long-chain fatty acids compared to controls; milk yields were increased with the added fat, protein concentrations were decreased, but protein yields were unaffected. It was reported that when nicotinic acid was added to the diets, the activity of adenyl cyclase was depressed, or the activity of cAMP was increased, thereby reducing lipolysis and ketogenesis (Erickson *et al.*, 1992). The overall effect of nicotinic acid was to slightly increase protein yields, and significantly increase protein concentrations, an effect attributed by the authors to increased rumen microbial efficiency. Cant, DePeters and Baldwin (1991) infused casein into the rumen or abomasum with or without supplemental fat in the diet. Added fat increased milk yield, decreased milk protein concentration but not did not affect protein yields. Infusing casein at the abomasum increased milk protein (casein and whey) concentration, compensating to some extent for the additional dietary fat. Recent attempts by others to reduce the effect of dietary fat on milk protein concentration, for example by the use of protein-fat bypass supplements (Holter, Hayes, Kierstead and Whitehouse, 1993) or high levels of undegradable protein (Palmquist, Weisbjerg and Hvelplund, 1993) have met with poor success.

4.2.6 **Conclusion**

It has been seen that dietary fat can affect both rumen function and animal tissue metabolism. High concentrations of fat in the diet can lead to a reduction in feed digestibility, particularly of fibre, and hence lead to a reduction in acetate production. Hindgut compensation for this is frequently seen however, resulting in similar supplies of metabolisable energy, although differences in the form in which that energy is presented to the animal. Because of the low solubility of fat, it has a low FME value and this can lead to a decrease in rumen fermentation and a reduction in the production of microbial protein, although paradoxically an increase in microbial efficiency may be achieved due to rumen defaunation. Most, if not all of the post-ruminal effects seen to have been caused by dietary fat on milk protein concentration may be attributed to dilution of the protein by increasing milk yields. The increased milk yields may be due to changes in the intramammary partitioning of nutrients, decreasing the *de novo* synthesis of fatty acids,

reducing the utilization of glucose for energy and α-glycerol-P production, and hence allowing an increase in the use of glucose for lactose synthesis. The high concentrations of milk protein found during the experiment described in Chapter 3 were attributed in part to low milk yields. Protein yields were also rather low, however, and it is not clear from the literature what effect changes in the supply and form of energy has on the production (i.e. yield) of protein in milk since it is apparently a combination of the yields of milk and protein that controls protein concentration, not concentration and yield that controls protein yield.

4.3 EXPERIMENTAL AIMS

A complete changeover design experiment was conducted to investigate the effect of source and level of concentrate metabolisable energy on the concentration and yield of protein in milk from dairy cows. Three concentrates were fed that supplied two levels of fat and two levels metabolisable energy with similar contents of dry matter and crude protein. Purine derivative excretion was measured to estimate the relative effects of diet on rumen microbial yield, and milk urea and organic acid concentrations were measured in order to obtain information about nutrient partitioning.

4.4 MATERIALS AND METHODS

4.4.1 Animals and Their Management

Twelve multiparous Holstein-Friesian cows in mid-lactation were drawn from the SAC Auchincruive herd for use in the experiment. They were housed in a metabolism unit in individual stalls fitted with de Boer yokes, with stall length adjusted to each animal. They were milked *in situ* using a vacuum line and bucket units at approximately 0530 h and 1530 h. Milk yields were recorded at each milking by weighing. During adjustment periods the animals were occasionally released from their stall for a few hours exercise in a collecting yard.

4.4.2 Experimental Design

The experiment was a complete changeover design based on four 3x3 Latin Squares. Each experimental period was divided into adjustment and collection periods of 3 weeks and 1 week in length respectively. The mean milk yields from the 7 days prior to the experiment were used to allocate animals to Latin Squares, with the 3 lowest yielding cows assigned to square 1, the next 3 to square 2, and so on to the 3 highest yielders in square 4. Within squares, the three treatments were randomly allocated to each animal.

The data obtained were analyzed statistically using analysis of variance with Genstat 5 (Lawes Agricultural Trust, 1990). A treatment structure of Period*(Square/Cow) and a blocking structure of experimental Diet were used. In the analysis of urinary purine derivative excretion data, a treatment structure of Diet*Day*Time was used. Differences between diets were assessed by subsequently using a t-test because of the non-orthogonal design of the experiment in terms of levels of fat and energy. The t statistic (calculated the differences of two means divided by the standard error of the difference of those means) was tested against the two-tailed t-distribution using the 14 residual degrees of freedom of the stratum from which the treatment means were calculated in the analysis of variance table.

Probabilities calculated from the F and t statistics are given in the results section below. The effects were considered significant where $P<0.05$. Where $0.10>P>0.05$, the effect was considered interesting and worthy of further consideration.

4.4.3 Diet Formulation and Production

The experimental diets were based on *ad libitum* first cut grass-silage. This was supplemented with concentrates fed at a flat rate of 12 kg per day. No attempt was made to keep the F:C ratio constant.

The concentrates were formulated and produced by Dalgety Agriculture Ltd (Aztec West, Bristol, UK) to guideline specifications. The 3 concentrates were formulated to provide between them two levels of acid hydrolysis ether extract (AHEE) and two levels of metabolisable energy (ME). Dry matter content, crude protein content, protein degradability and the ratio of starch to digestible crude fibre were all formulated to be similar across the three concentrates. Digestible crude fibre is a parameter calculated by the Dalgety formulation software from the values held on their database of feedstuffs. The composition of each of the three concentrates, low energy, high fat (LEHF), low energy, low fat (LELF) and high energy, high fat (HEHF) is given in Table 4.1. The lower ME level was intended to be moderate in terms of dairy cow requirements. In essence, the concentrates consisted of a relatively low quality carbohydrate energy source plus added fat (mainly palm oil) (LEHF), a high quality carbohydrate energy source with very little added fat (LELF) or the LELF energy sources plus the LEHF added fat (HEHF). This meant that in addition to two contrasting energy levels and two contrasting fat inclusion rates, the 3 concentrates offered two contrasting fermentable metabolisable energy (FME) levels.

The formulation of the concentrates allowed three comparisons to be made between the mean effects of the diets. The effect of dietary ME (and FME) density at the same fat content was tested by comparing the mean effect of LEHF with the mean effect of HEHF; the effect of concentrate fat content (and FME density) at the same ME level by comparing LEHF with LELF; and the effect of concentrate fat content at the same FME density by comparing LELF with HEHF. The logical fourth concentrate that would have allowed a 2x2 factorial investigation would have been a high energy, low fat one. An increase in ME beyond that achieved on the LELF concentrate, however, was not practicable.

The concentrates were produced as a single batch for the whole experiment shortly before the commencement, and were stored under cover until used.

Table 4.1 Composition of the three experimental concentrates.

	Concentrate		
Raw Material (%)	LEHF	LELF	HEHF
Barley	-	24.1	25.3
Wheat	-	10.4	14.0
Wheat Feed	29.7	-	-
Rice Bran	22.3	-	-
Molassed Sugar Beet Pulp	11.7	23.3	9.2
Prairie Meal	2.5	-	-
00-Rapeseed Meal	12.4	-	20.0
Sunflower Seed Meal	-	17.9	3.5
Field Beans	-	17.5	2.5
Toasted Soya Hulls	-	-	7.5
High Protein Soya Bean Meal	-	-	2.3
Fat	1.0	-	1.0
Palm Oil	4.0	0.6	4.0
Molasses	10.0	4.0	7.7
Minerals and Vitamins	6.4	2.2	3.0

4.4.4 Animal Feeding

Cows were offered fresh silage *ad libitum* daily at approximately 0930 h. The concentrate allowance was fed in two equal portions of 6 kg at each milking.

Silage was prepared (put in feed bins) using a feeder wagon on Mondays, Wednesdays and Fridays, with the storage, under cover, of 1 day's worth of silage during the week and 2 over the weekend. No deterioration of the stored silage was apparent as it was cold during the months of the experiment (January to April).

Silage fed during period 1 was first cut silage prepared with a formic acid silage additive (Add-F, BP Nutrition (UK) Ltd, Northwich, Cheshire). A new silage clamp was opened at the end of Period 1 and silage that was prepared using a bacterial inoculant (EcoSyl, ICI Bio Products, Billingham, Cleveland) was fed during Periods 2 and 3.

4.4.5 Sample Collection and Analysis

During the last 10 days of each experimental period, silage intake was measured by weighing the silage offered and weighing back the refusals the following morning. Small samples (approximately 200 g) of the silage offered were collected daily and bulked for analysis. Similarly, approximately 200 g of silage refusals were collected from each cow and bulked over the 10 days for dry matter analysis. Silage samples were frozen and stored at -20°C until analyzed.

Spot urine samples were taken by vulval stimulation at approximately 1030 h and 1430 h on each of two consecutive days at the end of each collection period. To avoid the problem of uric acid precipitation from frozen undiluted urine experienced in the first experiment, the samples were immediately diluted 1 in 20 (75 μl urine in 1.5 ml) with 0.1 M ammonium dihydrogen orthophosphate solution containing 0.1 M allopurinol as an internal standard. This was done directly into autosampler vials that were sealed with a crimped cap. The samples were either analyzed immediately using a high performance liquid chromatography (HPLC) technique for measuring the urinary purine derivatives or were frozen upright and stored at -20°C until analyzed. Problems with urine dilution at the end of the first experimental period meant that urine sampling was repeated over the following two days.

Faeces samples were collected at the same times as urine samples. Fresh faeces was collected from each cow, preferably as they defecated, or from the floor directly behind each one, taking care to avoid contamination. The samples were frozen immediately, and were stored at -20°C until being dried at 60°C for storage before later analysis.

Milk samples for Milko-Scan analysis were taken at four consecutive milkings, starting with an afternoon milking. The samples were preserved using Lactab milk preservative tablets (Thompson and Capper Ltd, Runcorn, Cheshire, UK) and stored at 4°C until analyzed. Samples from the first two milkings of the four were also taken and kept unpreserved at 4°C for subsequent analysis of milk crude protein, casein, non-protein nitrogen and urea as described in Section 2.3. Subsamples of this were frozen and stored at -20°C for later analysis of milk minerals (Section 2.3).

Milk yields were recorded daily throughout the experiment. To obtain a mean figure for the milk yield of each animal, the yields recorded over the last 7 days of each experimental period were used. Rook, Fisher and Sutton (1992) found no significant advantage in collecting milk samples over more than 4 consecutive milkings; despite this, pooling a larger number of data points (where the data is easily available) will lead to a more accurate mean.

4.5 RESULTS

During the experiment two of the cows developed mild mastitis, and although both animals were on diet LELF at the time of infection, this was considered to be a coincidence. The animals were treated and recovered. With the exception of one cow, the infections did not occur during collection periods. For that one cow, the infection occurred at the end of the first experimental period, but not until after the collection of all the milk samples and 2 of the 4 spot urine samples. The subsequent drop in milk yield and corresponding increase in urine output caused an increase in the dilution of the urine constituents. Therefore, only the results of the first two urine collections were used for this cow. Milk composition is affected by mastitis, but the composition of the milk collected from this animal did not appear abnormal and so the results were included in the statistical analyses.

Table 4.2 shows the mean composition of the silage used during the experiment. Silage dry matter changed from one period to the next (202, 259 and 301 g DM/kg for periods 1, 2 and 3 respectively) although the analysis of the dry matter was within the expected range for silage from, for instance, different parts of a clamp (e.g. 171, 187 and 188 g crude protein/kg DM, and predicted energy content of 11.4, 11.5 and 11.5 MJ ME/kg DM for silages from periods 1, 2 and 3 respectively). Slightly greater differences were seen in the DM composition of silages used in periods 1 and 2 than between silages used in periods 2 and 3, and this can be explained to some extent by the different additives used on the grass in different clamps.

The mean composition of the concentrates fed is given in Table 4.3. No differences in concentrate composition were seen between samples from the different experimental periods (i.e. concentrates did not change with storage).

Table 4.4 shows the mean daily intakes of silage dry matter, total dry matter (DMI), crude protein (CPI), metabolisable energy (MEI) and estimated FME (FMEI). FME was estimated from the ME and acid hydrolysis ether extract (AHEE) contents of the concentrate (with concentrate FME = ME - 0.033 x AHEE) and silage ME (with silage FME = 0.71 x ME), assuming additivity (TCORN, 1992). There was a significant increase in the DMI of animals fed the LEHF concentrate due to a significant increase in the silage intake.

Table 4.2 Composition of silage used (mean of 3 samples, each bulked over 10 days).

	First Cut Grass-Silage
Dry Matter, g/kg	254
Organic Matter, g/kg DM	932
Crude Protein, g/kg DM	182
Metabolisable Energy, MJ/kg DM	11.5
Rumen Degradable Protein, g/kg DM	155
Undegradable Protein, g/kg DM	27
Neutral Detergent Fibre, g/kg DM	435
Acid Detergent Fibre, g/kg DM	261
Water Soluble Carbohydrates, g/kg DM	40
Ether Extract, g/kg DM	45.7
Acid Hydrolysis Ether Extract, g/kg DM	56.3
In vitro Organic Matter Digestibility, g/kg OM	784
D-Value, g/kg DM	718
NH_4-N, g/kg Total N	89
pH	3.7
Calcium, g/kg DM	6.2
Phosphorus, g/kg DM	3.4
Magnesium, g/kg DM	2.5
Potassium, g/kg DM	20.8
Sodium, g/kg DM	4.0

Differences in silage DMI between diets (Table 4.4) meant that the forage:concentrate ratio (F:C) was 47:53, 46:54 and 46:54 on a DM basis for the diets LEHF, LELF and HEHF respectively. Therefore, even though there were significant differences in the dry matter intakes of the animals on different diets, the F:C ratio was very similar for each diet.

Table 4.3 Composition of the three experimental concentrates. L=Low, H=High, E=Energy, F=Fat.

	Concentrate		
	LEHF	LELF	HEHF
Dry Matter, g/kg	858	857	860
Organic Matter, g/kg DM	864	918	913
Crude Protein, g/kg DM	191	185	182
Metabolisable Energy (E3), MJ/kg DM	12.1	12.1	13.6
Fermentable Metabolisable Energy*, MJ/kg DM	9.1	11.1	10.8
Acid Hydrolysis Ether Extract, g/kg DM	92.0	30.6	84.2
Ether Extract, g/kg DM	84.5	18.7	77.8
Starch, g/kg DM	128	238	240
Water Soluble Carbohydrates, g/kg DM	105	97.4	93.4
Acid Detergent Fibre, g/kg	128	139	128
In vitro Organic Matter Digestibility, g/kg OM	698	811	779
Calcium, g/kg DM	16.7	10.5	14.3
Phosphorus, g/kg DM	9.6	6.1	7.0
Magnesium, g/kg DM	4.6	3.4	3.5
Potassium, g/kg DM	17.1	14.4	12.7
Sodium, g/kg DM	4.3	3.2	3.1

*Estimated.

Milk constituent concentrations were calculated as the mean of the four samples taken over consecutive milkings, weighted for the yields at the time of each collection. Constituent yields were calculated using the weekly mean milk yield for each animal (i.e. weighted mean constituent concentration multiplied by mean yield to give a mean constituent yield). Table 4.5 gives a summary of the results obtained by Milko-Scan analysis of the protein, fat and lactose concentrations and yields for each of the three diets. Significantly increased milk solids concentrations were found on the LELF concentrate. Milk yields, however, were lowest on this, the low fat diet. No significant difference due to treatment was seen in the ratios of protein/fat, protein/lactose or fat/lactose.

Table 4.4 Mean daily dry matter intakes and intakes of crude protein, metabolisable energy and acid hydrolysis ether extract, and estimated intakes of effective rumen degradable protein, digestible undegraded protein (TCORN, 1992), and fermentable metabolisable energy; whole tract apparent organic matter digestibilities, and allantoin plus uric acid to creatinine ratios (AU/C).

	Diet				t Probability		
	LEHF 1	LELF 2	HEHF 3	SED	1-2	1-3	2-3
Silage DMI, kg/d	9.1	8.6	8.7	0.19	0.013	0.026	0.724
Total DMI, kg/d	19.4	18.9	19.0	0.19	0.011	0.033	0.588
CPI, kg/d	3.6	3.5	3.5	†			
ERDPI, kg/d	2.4	2.3	2.3	†			
DUDPI, kg/d	0.62	0.48	0.48	†			
MEI, MJ/d	229	223	240	†			
FMEI, MJ/d	168	184	182	†			
AHEEI, kg/d	1.5	0.8	1.4	†			
Whole Tract Apparent Digestibility of OM, g/g	0.79	0.81	0.82	0.003	<.001	<.001	0.296
AU/C	3.24	3.51	3.51	0.104	0.024	0.022	0.978

†Not determined.

Table 4.5 Mean values and SEDs for milk yields (mean of 7 days) and milk protein, fat and lactose concentrations and yields (weighted mean of 2 days).

	Diet				t Probability		
	LEHF 1	LELF 2	HEHF 3	SED	1-2	1-3	2-3
Milk Yield, kg/d	32.8	31.0	33.8	0.63	0.012	0.178	<.001
Protein, g/kg	31.4	32.5	31.0	0.45	0.019	0.486	0.005
Fat, g/kg	32.5	34.4	31.9	1.07	0.090	0.616	0.035
Lactose, g/kg	48.3	49.0	47.6	0.30	0.028	0.053	<.001
Protein Yield, g/d	1022	1002	1038	15.9	0.223	0.341	0.040
Fat Yield, g/d	1068	1064	1075	40.8	0.923	0.866	0.791
Lactose, g/d	1584	1517	1612	34.0	0.069	0.424	0.014

The effects of diet on the major milk nitrogenous fractions are summarized in Table 4.6. The concentrations of the protein fractions were greatest on the low fat diet. There was no effect of diet on casein as a proportion of true protein, with a grand mean of 0.82.

Table 4.6 Treatment mean values of major milk nitrogenous compounds.

	Diet				t Probability		
	LEHF 1	LELF 2	HEHF 3	SED	1-2	1-3	2-3
Milk Yield, kg/d	32.8	31.0	33.8	0.63	0.012	0.178	<.001
Crude Protein, g/kg	30.4	31.5	30.0	0.37	0.008	0.332	0.001
True Protein, g/kg	28.6	29.8	28.2	0.39	0.009	0.354	0.001
Casein, g/kg	23.4	24.3	23.0	0.30	0.008	0.281	<.001
Whey*, g/kg	5.21	5.45	5.18	0.153	0.137	0.852	0.099
Non-Urea NPN, g/kg	0.086	0.062	0.070	0.0119	0.071	0.214	0.523
Urea, g/kg	0.415	0.441	0.447	0.0194	0.202	0.126	0.774
Crude Protein Yield, g/d	990	971	1005	16.5	0.247	0.378	0.053
True Protein Yield, g/d	931	917	945	16.0	0.385	0.408	0.102
Casein Yield, g/d	763	750	773	15.3	0.401	0.494	0.139
Whey Yield, g/d	169	168	172	3.4	0.727	0.388	0.233
Non-Urea NPN Yield, g/d	2.8	1.9	2.2	0.41	0.048	0.193	0.432
Urea Yield, g/d	13.7	13.5	15.3	0.86	0.819	0.083	0.058

*Whey calculated as True Protein - Casein

Mean milk concentrations and yields of milk citric, orotic, lactic, and hippuric acids are presented in Table 4.7. The effect of additional dietary fat caused significant changes in the concentrations of all milk organic acids measured. Milk uric acid was also determined and presented in Table 4.7, although this was a composite of uric acid and allantoin, and was regarded as a measure of milk purine derivative content. There was no difference in the milk PD excretion data between the two diets with similar FME concentrations.

Mean concentrations and yields of potassium, sodium, calcium, phosphorus, magnesium, chlorine and zinc are presented in Table 4.8. Copper, iron and manganese were also measured, but were close to the lower limits of detection of the technique with no differences between samples being found; copper concentrations were 0.01 mg/kg or less, iron concentrations averaged 0.02 mg/kg, and manganese concentrations were all less than 0.01 mg/kg.

Table 4.7 Treatment mean effects on milk organic acid concentrations and yields.

	Diet				t Probability		
	LEHF 1	LELF 2	HEHF 3	SED	1-2	1-3	2-3
Orotic, mg/kg	91.2	80.9	90.9	3.83	0.018	0.939	0.021
Citric, mg/kg	1023	842	1040	18.9	<.001	0.375	<.001
Lactic, mg/kg	347	514	409	42.1	0.001	0.163	0.026
PD*, mg/kg	17.0	21.0	19.9	0.66	<.001	<.001	0.105
Hippuric, mg/kg	13.5	12.0	12.1	0.42	0.003	0.003	0.888
Orotic Yield, g/d	2.99	2.49	3.02	0.117	<.001	0.833	<.001
Citric Yield, g/d	33.6	26.1	35.1	0.96	<.001	0.144	<.001
Lactic Yield, g/d	11.5	16.2	13.9	1.47	0.006	0.117	0.142
PD Yield, g/d	0.56	0.66	0.68	0.026	0.003	<.001	0.436
Hippuric Yield, g/d	0.45	0.37	0.41	0.019	0.001	0.091	0.043

*PD, purine derivatives, measured by the technique of Marsili *et al.* (1981) as uric acid, but also assumed to contain a contribution of allantoin. See Section 2.3.7.

Table 4.8 Treatment mean milk mineral concentrations and yields.

	Diet				t Probability		
	LEHF 1	LELF 2	HEHF 3	SED	1-2	1-3	2-3
Sodium, g/kg	0.40	0.39	0.41	0.011	0.516	0.153	0.047
Potassium, g/kg	1.64	1.61	1.68	0.031	0.490	0.152	0.043
Chlorine, g/kg	0.94	0.96	0.98	0.031	0.572	0.209	0.472
Calcium, g/kg	1.12	1.13	1.11	0.025	0.691	0.780	0.501
Phosphorus, g/kg	0.94	1.00	0.93	0.021	0.011	0.843	0.007
Magnesium, g/kg	0.11	0.10	0.11	0.002	0.001	0.936	<.001
Zinc, mg/kg	0.27	0.28	0.29	0.011	0.440	0.102	0.357
Na Yield, g/d	12.8	12.0	13.8	0.43	0.069	0.043	<.001
K Yield, g/d	53.8	50.0	56.9	1.43	0.020	0.046	<.001
Cl Yield, g/d	30.8	29.9	33.1	1.01	0.393	0.044	0.008
Ca Yield, g/d	36.6	34.8	37.4	0.87	0.062	0.383	0.011
P Yield, g/d	30.1	30.8	31.5	0.75	0.875	0.293	0.367
Mg Yield, g/d	3.49	3.04	3.59	0.087	<.001	0.276	<.001
Zn Yield, mg/d	9.04	8.77	9.99	0.430	0.540	0.044	0.013

Despite significant differences between the effects of diets LELF and HEHF on K and Na concentrations, the mean K/Na ratios of the three diets were not significantly different with means of 4.2, 4.2, and 4.1 g/g (SED 0.11) for diets LEHF, LELF, and HEHF respectively. Likewise, the lactose/Cl ratios were not significantly different between diets with means of 51.7, 51.4 and 49.2 g/g (SED 1.86) for diets LEHF, LELF and HEHF respectively, despite differences in effects on lactose concentrations between all three diets.

There were no problems of precipitation of uric acid from the thawed urine samples in this experiment (unlike those described in Chapter 3) because the samples were frozen diluted 1 in 20 in buffer. The treatment mean ratios of the combined urinary uric acid and allantoin concentrations to urinary creatinine (AU/C) are given in Table 4.4 for comparative purposes. These ratios were used as a simple index of microbial protein yield on the assumption that an increased AU/C ratio indicates an increased microbial protein supply. The effects of diet on allantoin/creatinine and uric acid/creatinine ratios were assessed separately and are summarized in Table 4.9, together with the effects of diet on the ratio of urinary allantoin to uric acid. The combined urinary PD excretion (AU/C) was very similar on diets LELF and HEHF, which had similar dietary FME contents. The combined urinary PD excretion from animals fed the LEHF concentrate was significantly lower than that of the other 2 diets.

Table 4.9 Summary of effects of diet on purine derivative excretion; allantoin/creatinine (A/C), uric acid/creatinine (U/C), allantoin + uric acid/creatinine (AU/C), and allantoin/uric acid (A/U).

	Diet				t Probability		
	LEHF 1	LELF 2	LEHF 3	SED	1-2	1-3	2-3
A/C	2.92	3.14	3.20	0.062	0.034	0.008	0.480
U/C	0.32	0.37	0.31	0.022	0.036	0.471	0.009
AU/C	3.24	3.51	3.51	0.104	0.024	0.022	0.978
A/U	12.0	11.1	12.6	0.69	0.226	0.379	0.047

Table 4.10 Summary of effects of sampling time and day on purine derivative excretion; allantoin/creatinine (A/C), uric acid/creatinine (U/C), allantoin + uric acid/creatinine (AU/C), and allantoin/uric acid (A/U).

	Time			Day			F Probability	
	AM	PM	SED	1	2	SED	Time	Day
A/C	2.76	3.41	0.067	2.96	3.21	0.067	<.001	<.001
U/C	0.27	0.40	0.031	0.42	0.25	0.031	<.001	<.001
AU/C	3.03	3.81	0.081	3.38	3.46	0.081	<.001	0.311
A/U	11.9	11.9	0.64	9.8	14.0	0.64	0.931	<.001

Since the urine samples were taken twice daily over two days, the effect of time and day of sampling could also be assessed. These effects are summarized in Table 4.10, and some interesting results were seen. Both A/C and U/C differed with time and day of sampling, although the changes were in opposite directions on the two days of collection such that the combined PD excretion data (AU/C) was not significantly affected by day of sampling.

In order to take into account the effects of microbial supply to the animal, yields of milk and its constituents can be corrected using the AU/C. This was done, and the results are summarized in Table 4.11 and 4.12. Unlike in Chapter 3, where dietary effects were removed by taking microbial protein supply into account by this procedure, dietary effects still remained for milk yields, and for the yields of the proteins and lactose.

Relative protein efficiencies (i.e. milk protein output/crude protein intake) for each diet are presented in Table 4.13. Similarly, by taking the dietary crude protein supply into account in this way, differences due to dietary treatment were still seen in the protein components of the milk.

Table 4.11 Mean values for allantoin+uric acid/creatinine corrected milk yield, protein, fat and lactose yields (kg/d (milk yield) or g/d (protein, fat and lactose) per unit AU/C).

	Diet				t Probability		
	LEHF 1	LELF 2	HEHF 3	SED	1-2	1-3	2-3
Milk Yield / AU/C	10.2	9.0	9.6	0.29	<.001	0.078	0.042
Protein / AU/C	318	290	297	9.2	0.009	0.038	0.479
Fat / AU/C	331	308	308	14.2	0.129	0.127	0.995
Lactose / AU/C	491	439	459	13.9	0.002	0.037	0.172

Table 4.12 Mean values of allantoin+uric acid/creatinine corrected yields of major milk nitrogenous constituents.

	Diet				t Probability		
	LEHF 1	LELF 2	HEHF 3	SED	1-2	1-3	2-3
Crude Protein / AU/C	308	281	288	8.9	0.008	0.035	0.457
True Protein / AU/C	290	265	270	8.4	0.011	0.035	0.554
Casein / AU/C	237	217	221	6.8	0.010	0.032	0.559
Whey / AU/C	53	48	49	2.0	0.053	0.127	0.628
Non-Urea NPN / AU/C	0.87	0.54	0.67	0.115	0.012	0.094	0.291
Urea / AU/C	4.2	4.0	4.3	0.25	0.278	0.782	0.180

Table 4.13 Relative protein efficiencies of milk nitrogenous constituents (milk protein output/crude protein intake, g/kg).

	Diet				t Probability		
	LEHF 1	LELF 2	HEHF 3	SED	1-2	1-3	2-3
Crude Protein	274	280	291	4.0	0.150	<.001	0.016
True Protein	257	264	273	3.9	0.094	0.001	0.039
Casein	211	216	223	3.7	0.158	0.004	0.071
Whey	46.7	48.3	49.9	1.07	0.160	0.009	0.148
Non-Urea NPN	0.77	0.54	0.66	0.114	0.060	0.358	0.293
Urea	3.78	3.94	4.41	0.218	0.477	0.011	0.046

A test of linear regression of milk purine derivative concentration on urinary purine derivative excretion (divided by milk yield to remove autocorrelation effects, AU/C / milk yield, AU/C/M) was performed using the model:

$$\text{Milk PD} = \text{constant} + \text{AU/C/M} + \text{Period} + \text{AU/C/M.Period}$$

to take into account the effect of experimental period.

An increase in the AU/C/M was found to be associated with an increase in milk purine derivative concentration (Figure 4.2; $P=0.099$); the effect of AU/C/M in the regression was slightly greater ($P=0.105$) than the effects of the constant, experimental period, and the interaction of period and urinary purine excretion/milk yield, which were not significant.

Using a model similar to that above, the regression of milk hippuric acid yield on silage dry matter intake was tested. Neither the concentration (P=0.826) nor yield (P=0.473) of hippuric acid was related to the silage dry matter intake of the animals, despite the mean concentration being highest for the diet which produced the highest mean silage DMI.

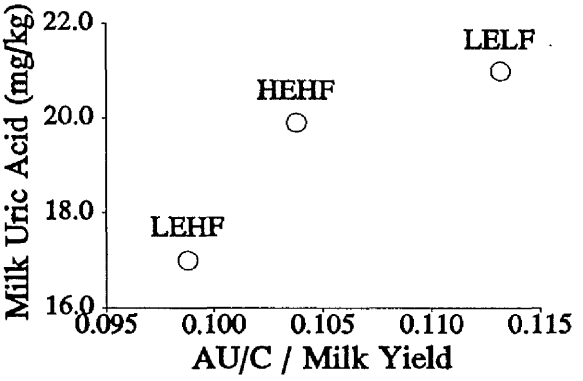


Figure 4.2 Relationship between urinary allantoin+uric acid/creatinine (divided by milk yield) and milk uric acid concentration.

4.6 DISCUSSION

The three concentrates fed allowed the investigation of three combinations of factors: a) the effect of increasing concentrate fat content at similar ME densities (LELF versus LEHF), b) the effect of increasing concentrate ME at similar fat levels (LEHF versus HEHF), and c) the effect of increasing ME at similar FME densities (LELF versus HEHF). The increases in concentrate fat and ME in cases (a) and (b) respectively were associated with an increase in potential FME, and the increase in ME in case (c) was through an increase in fat. All three concentrates had similar crude protein contents. Although the three concentrates differed quite markedly, as they were formulated to do, the silage intakes were not restricted. As a result, silage intakes were seen to be highest for animals fed the LEHF concentrate, and this may have been due to the carbohydrate quality of that concentrate, although numerically the differences were small. The whole tract apparent organic matter digestibility of the diet fed to these animals was significantly lower than those of the two high FME concentrate diets, and the increase in silage intake may have resulted to compensate. This compensation may have reduced treatment differences, and although the effect of adding fat to the dairy cow diet has variable effects on forage intakes (see Coppock and Wilks, 1991) the F:C ratios were very similar for all three diets. These were too similar in the present study to have been the cause of effects on milk composition such as those discussed in Section 3.2.3. The increase in silage intake caused increased daily intakes of crude protein, and a somewhat higher MEI than animals on the other low ME concentrate. Increases in FME intake were not enough to make up for the difference between concentrate FME densities. Dietary fat has been shown to decrease rumen digestibility of fibre, and hence decrease nitrogen utilisation within the rumen. The striking similarity between the AU/C ratios of diets LELF and HEHF would indicate that even if this did occur, the microbial yield to the duodenum was not affected.

The silage fed as a basal ration to the animals changed between periods 1 and 2 as a new clamp was opened. Differences between the two clamps were small, and the design of the experiment meant that variation in the basal ration was removed in the statistical analysis as an effect of experimental period. Detailed comparisons of the effects of the silage were not performed because the aim of the experiment was to investigate the effects of the concentrate supplements. For this reason, the daily concentrate allowance was high and constituted more than 50% of the dry matter intakes of the animals.

Increasing fat in the diet caused a decrease in the concentration of milk crude protein (Table 4.5). This was due to significant increases in milk yields by the inclusion of supplemental fat in the concentrate; there was no significant difference in the yield of milk protein between the two low ME diets. Increasing the ME supply at the high fat level (HEHF) increased the milk yields still further (though not significantly), and in this case, a small but non-significant increase in the milk protein yield was seen above the other high fat diet. The results of milk protein analyzed by Kjeldahl techniques (Table 4.6) are similar: true protein concentrations were reduced significantly by adding fat to the diet, with the effect being greater for the caseins than for whey proteins. Overall, the two methods of crude protein analysis gave good relative agreement and differences in absolute figures can be explained by differences in analytical technique.

On all three treatments, the concentration of milk protein was quite high, whereas the concentration of milk fat was quite low and was seen to decrease on the high fat diets. This has been reported by other authors (Wrenn *et al.*, 1978; de Visser, Tamminga and van Gils, 1982) and may be a consequence of the high levels of concentrates fed. The mean total daily AHEE intakes by animals on the two high fat concentrates were almost double that of animals on the low fat concentrate, and yet the total daily milk fat production was almost exactly the same on all three diets. There must, therefore, have been a significantly higher rate of *de novo* synthesis of fatty acids by the mammary secretory tissue to achieve the milk fat production by the animals on the low fat diet. This is indicated by the significant reduction in milk citrate output (Faulkner and Pollock, 1989). Citrate occurs in milk as a soluble ion with Ca^{+} (Larson, 1985), both of which may also be bound to casein (Linzell and Peaker, 1971). Although the citrate measured in this study was total milk citrate, as opposed to the soluble citrate studied by Faulkner and Pollock (1989), it is reasonable to assume that the (highly significantly) reduced output of total milk citrate was due to its use in the cytosol of the secretory cells for the production of NADPH by the isocitrate dehydrogenase pathway.

The addition of palm oil to dairy cow diets has been shown to increase the palmitic acid content of milk fat and reduced the *de novo* synthesis of fatty acids (Banks *et al.*, 1980). Changes in the fatty acid profile of milk fat, and the sources of those fatty acids, will confound the effect of diet on milk fat production since the incorporation of dietary fats such as palmitic acid (C_{16}) is more efficient energetically than the *de novo* synthesis of

shorter chain fatty acids (e.g. C_8). Glucose is used in the production of fats for NADPH generation and α-glycerol-P formation. The incorporation of preformed fatty acids reduces the need for NAPDH, and also for the α-glycerol-P units per unit weight of fat since 1 g of milk fat contains fewer molecules of long chain fatty acids than 1 g of fat with a greater proportion of short chain fatty acids.

The daily production of lactose was increased by the addition of fat to the diet, as is expected with increased milk yields since lactose synthesis and secretion draws water into milk. Lactose concentrations, however, were significantly reduced by the addition of fat in the diet. This has also been reported by several other workers investigating the effect of dietary fat on milk composition (DePeters, Taylor, Finley and Famula, 1987; DePeters *et al.*, 1989). The highly significant reduction in lactose concentration between diets LELF and HEHF was balanced by significant increases in the concentrations of potassium and sodium, coupled with a slight increase in chlorine concentration. Milk is iso-osmotic with blood and the flow of water from blood to milk is controlled mainly by these four compounds, sodium balanced by potassium, lactose balanced by chlorine. Lactose is only slightly dissociated in water and therefore, despite the relatively large quantities in which it is present, has little more of an effect than the individual effects of potassium, sodium and chlorine, which are completely ionised in water. Neither the K/Na nor the lactose/Cl ratios differed due to dietary treatment, therefore neither did the (K/Na)/(lactose/Cl) ratios. In other words, the individual concentrations of osmotically active factors in milk changed, but their balancing ratios did not. Lactose synthesis is controlled by the availability of glucose and its passage across the plasma and Golgi membrane. The increase in lactose yields on the high fat diets, and in particular on the HEHF diet suggests that more glucose was available for the production of lactose on these diets. This would go some way to explaining the increased milk yields on the two high fat diets. Lactose concentration was lower on these diets, the large part of the balance of osmotically active components in the milk being made up of K^+, Na^+, and Cl^-. The K^+/Na^+ balance is controlled by the K^+/Na^+-ATPase in the basal membrane of the mammary secretory cell (Falconer and Rowe, 1977) producing a ionic gradient that notionally extends past the apical membrane (Linzell and Peaker, 1971) causing the efflux of ions into the intraluminal space due to differences in the electrical potential (Shennan, 1992). These change the osmotic potential of mammary epithelial secretion, which pulls water out of the blood. The K^+/Na^+-ATPase is regulated by prolactin (Falconer *et al.*, 1983), the binding of which to sites in the

mammary secretory cell has been shown to be reduced by the autocrine feedback inhibitor of lactation (FIL; Addey, Peaker and Wilde, 1991) in milk (Bennett, Knight and Wilde, 1990). I am unaware of any reports in the literature directly linking the FIL to changes in the activity of the K^+/Na^+-ATPase. However, it could be a mechanism by which milk volume is acutely controlled.

Potassium concentrations within the mammary secretory epithelium are maintained by the Na^+/K^+-ATPases on the basal membrane of the cell. This, obviously, has a requirement for ATP, the main source of which is the hydrolysis of glucose by glycolysis. The intracellular competition for glucose between lactose synthesis and the production of energy for fatty acid synthesis has been discussed, but a further, and by no means less significant need for glucose is the requirement for ATP. If energy from glucose (ATP from glycolysis and NADPH from the pentose phosphate pathway) is not used for *de novo* synthesis of fatty acids, it is possible that a further proportion of that energy is available for use by the Na^+/K^+-ATPases on the basal membrane of the mammary epithelium. If this is the case, the relative importance of competition between the transport of glucose into the Golgi apparatus - the rate limiting step to lactose synthesis (CJ Wilde, personal communication) - and the passage of glucose through glycolysis for ATP production may increase. Since both the rate of glucose uptake by the Golgi apparatus (Kuhn, Carrick and Wilde, 1980) and glycolysis follow classic Michaelis-Menten kinetics, the partitioning between these two processes depends on the a) the numbers of 'reaction sites' (enzymes or transporters) available, and b) the inhibition of glycolysis (at pyruvate kinase) by intracellular concentrations of ATP. Given a limited supply of glucose, with the demands of lactose production and the pentose phosphate pathway more easily met, it may be envisaged that glycolysis could proceed at a faster rate, with the production of more ATP. This would allow the membrane bound ATPases to either function at a faster rate or increase in numbers and increase the membrane transport of sodium and potassium. No direct evidence for this action was seen in the present study, apart from the differences in milk ion and lactose secretion, but it deserves further investigation.

Apart from K, Na and Cl, most minerals present in milk are bound to solids such as casein, and are probably there for nutritional purposes. Differences in the calcium, phosphorus and magnesium content of the milks can be explained by differences in the concentrations of the 'carrier' compounds, i.e. fat and protein.

Dairy cow breeding for milking characteristics over the last hundred years or more has been done with a view to increasing milk yield and increasing fat concentration. It is seems reasonable to assume that one of the effects of breeding for increased fat concentration is to have given milk fat a relatively greater sink capacity than lactose when there is competition for nutrients. Adding fat to the diets in this study reduced the apparent utilisation of nutrients for fatty acid synthesis within the mammary tissue, increasing the availability of these nutrients for other uses, namely glucose for lactose synthesis. This experiment was not designed to address this problem, but future work would be useful in determining the proportions in which different precursors are used for milk production by the mammary gland.

Turning to the effects of diet on milk protein concentration, the results suggest that this was controlled by a combination of protein supply to the mammary tissue and milk volume. The daily yields of milk protein were lowest on the LELF concentrate although the large differences in milk protein concentration were due to a combination of protein production and milk volume. All three concentrates provided similar quantities of crude protein although the LEHF diet was associated with higher silage intakes and as a result, higher daily crude protein intakes than the other two treatments. The urinary purine excretion was lowest on this diet, and although the difference was not significant, the milk urea concentrations were also lowest on this diet. These two factors and the reduced apparent OM digestibilities indicate that the rumen protein utilization was lowest on the LEHF diet, i.e. less protein was degraded and captured by the rumen. The relative efficiency of protein utilization was least on this diet, with a reduced digestibility and microbial protein production (Figure 4.3) being compensated for to some extent by increased silage intakes. The reduction in microbial protein capture (as estimated by AU/C) was related not to intake of ME and but to intake of FME (Figures 4.4 and 4.5). Dietary protein which escaped rumen degradation would have been digested further down the gut to provide some of the milk protein precursors necessary for the levels of protein production that were achieved. The relative protein efficiencies were significantly less on both low ME diets than on the high ME diet. Hepatic utilisation of amino acids for gluconeogenesis may have been reduced on the high ME diet, which would explain the statistically significant difference in milk protein production between the two high FME diets since both dietary crude protein supply and microbial protein capture were similar for these two diets.

Metcalf (1992) proposed that milk lactate could be used as an indication of the availability of precursors of the citric acid cycle, i.e. glucose, acetate and ß-hydroxybutyrate. There were significant differences in the lactate concentrations of milk due to diet (Table 4.8), with the greatest concentrations found on the LELF diet. This supports the hypothesis that the mammary gland on the low fat diet was relatively short of energy yielding compounds and glucose would therefore have been less available for lactose production, helping to explain the lower milk yields on this diet. On the other hand, the fat/lactose ratios in the milk were not affected by diet, which suggests that the mammary gland was not limited by energy supply on any of the diets, despite differences in the form of that energy, and that 'target' production rates of fat and lactose were met. What controlled these 'targets' is a different question that warrants further investigation.

Milk orotate may provide an estimation of the intramammary production of milk protein in the same way that citrate indicates

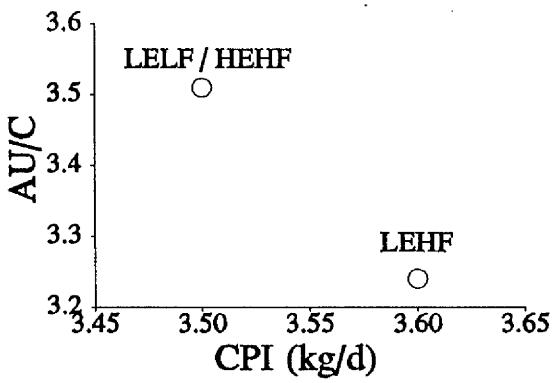


Figure 4.3 Response of urinary purine derivative excretion (AU/C) to dietary crude protein intake (CPI).

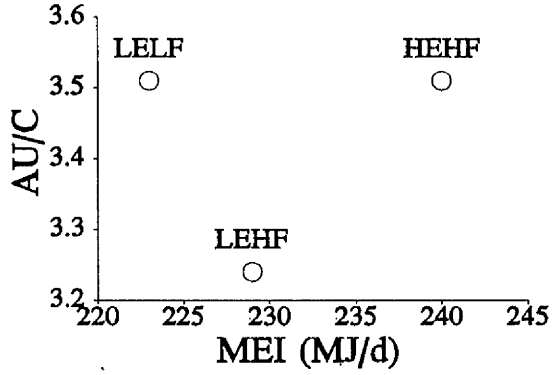


Figure 4.4 Response of urinary purine derivative excretion (AU/C) to metabolisable energy intake (MEI).

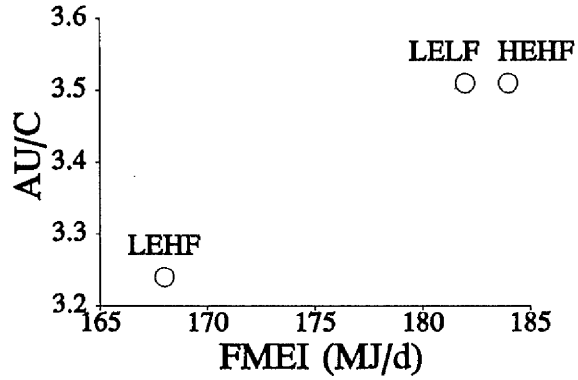


Figure 4.5 Response of urinary purine excretion (AU/C) to estimated fermentable energy intake (FMEI).

intramammary fatty acid production. Orotic acid is an intermediary in the synthesis of pyrimidine nucleotides (see Chen and Larson, 1971), and its presence is characteristically high in ruminant milk (Larson and Hegarty, 1977; Larson and Hegarty, 1979). Orotic acid in milk has been shown to be synthesised almost exclusively in the mammary gland, the rates of which were increased with additions of amino acids (Ehle, Robinson, Clark and Baumrucker, 1981). An increased concentration of orotic acid in milk may therefore be an indication of a reduction in its utilisation for the nucleic acid (NA) synthesis that is necessary for protein production (i.e. production of mRNA and tRNA). In the present study, milk nitrogen fractions were measured directly, and although casein yields did not differ significantly between diets whilst orotic acid concentrations and yields did, the direction of change was the same with the highest levels of production of both on the high fat diets.

Herbivores excrete large amounts of aromatic acids in their urine, the source of which is dietary since there is no evidence for the synthesis of aromatic compounds by the mammal. Aromatic amino acids such as phenylalanine and other phenolic compounds such as lignin are the major sources of aromatic rings in the diet. Hippuric acid (HA), a conjugate of glycine and benzoic acid, was found to be present in the milk at concentrations which differed significantly with diet, the highest concentration being found on the LEHF diet. It is assumed that HA diffused into the milk from plasma and therefore reflected the plasma concentrations. Silanikove and Brosh (1989), feeding goats on straw, suggested that the urinary excretion of HA was correlated with a) dry matter intake (of lignaceous compounds) and b) rumen retention time. In this study, the excretion of HA in milk was not correlated to silage dry matter intake. Furthermore, it is unlikely that differences in rumen retention time would account for the differences in HA concentration seen because a decrease in the outflow rate of rumen contents would tend to reduce the forage intake, not increase it, as was seen on diet LEHF. All three concentrate supplements fed had similar protein contents, and although it is possible that the amino acid profiles were different, it is unlikely that the LEHF concentrate had a sufficiently greater content of aromatic amino acids to have caused the increase in HA seen. Very similar HA concentrations were, however, found on the two diets that had similar (high FME) carbohydrate sources. It is most likely, therefore, that the increase in HA concentrations seen on the LEHF diet was due to the increased fibre supply to the animals, both from the increased silage intake and from the concentrate.

Purine derivative excretion expressed as a ratio to creatinine was found to vary with intake of FME (Table 4.20 and Figure 4.4). Across the three diets, PD excretion was also found to differ significantly with time of collection, and, in the case of individual levels of allantoin and uric acid excretion, with day of collection (Table 4.11). Total PD excretion (allantoin + uric acid) did not differ between days. In Chapter 3, the greatest component of variance of urinary PD excretion as measured by allantoin/creatinine was found to be associated with individual animals over a 7 day, twice daily collection regime. Differences between morning and afternoon samples were also found on that experiment. In this study, A/C and U/C were 23% and 48% higher respectively in the afternoon than in the morning. Rosskopf, Rainer and Giesecke (1991) reported significantly higher plasma allantoin concentrations from dairy cows at 2 hours after feeding than at 8 h. Rosskopf and Giesecke (1992) reported large fluctuations in blood plasma allantoin concentrations in steers fed twice daily. Chen, Grubic, Ørskov and Osuji (1992), on the other hand, concluded that diurnal variation of plasma PD concentrations and urinary PD excretion from young steers was very small and not significant, although the daily DMI was much lower than in the present study, with only 4.1 kg/d per animal compared to an average of 19.1 kg/d in the present study, approximately a quarter of which was fed at each of two milkings (6 kg fresh weight concentrate). When sheep were offered urea treated barley straw *ad libitum*, with no 'meals' of concentrates, the A/C ratio was found to be very constant throughout the day (Surra, Guada, Balcells and Moreno, 1991). It is most likely that the differences in morning and afternoon PD excretion in the present study were due to concentrate feeding patterns, since concentrates were consumed within 5-10 minutes of being offered. The reported lack of diurnal variation by other authors is probably due to the lack of variation in the feeding regimes of the animals used.

The significance of diurnal variation on the estimation of microbial protein is important when spot urine samples are used, and it is not well defined at present. The results of this experiment suggest that comparison of spot urine samples must be done taking time of feeding into account otherwise confounding may occur. Chen *et al.* (1992) suggested taking spot samples at particular times of the day to gain a representative estimate of PD excretion for the whole day. This is not necessarily a good thing to do since a) it is not possible to tell what the 'mean' value is without taking more than one sample, and b) diurnal variation may yield information that would be lost with only one sampling time, for example, the speed of ruminal degradation of different feedstuffs (e.g. differences

between starch and certain fibres).

Interestingly, significant differences due to day of sampling were also observed for A/C and U/C (Table 4.11), although the effect of day was the opposite for each (hence a significant difference in the ratio of urinary allantoin to uric acid on different sampling days), with a net result of no difference between days for AU/C. Uricase activity in the plasma is responsible for the rate of conversion of uric acid to allantoin (Figure 3.6), and the present results therefore suggest a possible difference in the plasma uricase activity between the two sampling days. The difference between sampling days cannot be explained; if the results were random, one would not expect the high degree of significance that was found, bearing in mind that the present results were taken over three experimental periods each 4 weeks apart. The first sampling day was a Monday and the second a Tuesday, except for the first period's results that were collected on a Wednesday and Thursday. Care was taken to ensure that the animals received the same treatment on each of the two days (i.e. time of milking and feeding, and time of sampling). The midweek collections of the first period reduce the likelihood of 'weekend' effects, e.g. differences in milking procedures by dairy staff during the week and over the weekend, which may have influenced the subsequent days' performance, and also of effects of introduction of the animals to the sampling technique, because the animals had been sampled for 2 days previously. Analysis of the urine was done immediately for the first sample of each collection period (subsequent samples were frozen until analyzed) and in order of animal number each time. Degradation of uric acid to allantoin whilst in the vial therefore seems to have been unlikely. Regardless of the reasons for the day to day differences in the molar proportions of urinary uric acid and allantoin, the sum of the two compounds, which did not differ between sampling days, was used as the estimate of rumen microbial protein yield.

Milk purine derivative concentrations (a combination of uric acid and allantoin) were correlated to the urinary excretion of AU/C (when divided by milk yield to remove autocorrelation effects), and varied independently of milk yield. PD excretion in milk may therefore offer an even more convenient and practicable method of estimating rumen microbial yields than the use of urine, with the potential for large scale and routine monitoring of rumen protein function. One potential problem is the contribution that the mammary tissue may make to the purine derivatives excreted in milk because it has a high

metabolic rate, and the turnover of NA is likely to be high compared to other tissues. The concentration of xanthine oxidase in milk is high (about 160 mg/l; Ball, 1939) - bovine milk is frequently used as a source of xanthine oxidase by chemical companies - and in addition to any expression by mammary tissue, it has been shown to be taken up from blood (Blakistone, Sisler and Aurand, 1978). It is not known to what extent purines produced within the mammary gland are salvaged - exogenous purine sources are largely unavailable for use by the animal other than in the intestinal mucosa due to the high plasma activity of xanthine oxidase (Verbic, Chen, MacLeod, and Ørskov, 1990) - but considering the activity of xanthine oxidase in milk, it is unlikely that much salvage can occur and therefore the contribution that mammary derived purines make to total purine excretion may be higher in milk than in urine. Furthermore, if diffusion is the means by which allantoin and uric acid enter milk, as indicated by the high correlation between milk and plasma concentrations of allantoin (Rosskopf *et al.*, 1991; Rosskopf and Giesecke, 1992), the level of endogenous purine production by mammary tissue is relatively unimportant because they will diffuse out as well as in.

4.7 CONCLUSIONS

In this experiment, supplementary dietary fat was seen to reduce the concentrations of milk solids. The milk protein concentration, like that of fat and lactose, was reduced on high fat concentrates, despite significant increases in daily yields of protein and lactose on the high ME concentrate. The reductions in solids concentrations were brought about mainly through significant increases in milk yields, as the *de novo* synthesis of fatty acids for milk fat was reduced and lactose production was increased. Dietary fat has a low FME value, and this was observed in the low urinary purine derivative excretion from animals fed the low ME, high fat concentrate. Animals fed the high ME, high fat concentrate yielded more milk protein than those animals fed the low ME, low fat (but equal FME) concentrate, indicating that amino acids may have been spared from gluconeogenesis by the extra supply of ME. It is therefore concluded that the concentration of protein in milk depends not only on the supply of precursors for milk protein production, but also on the supply of precursors for fat and lactose production and which will ultimately determine milk yields.

Chapter 5

Effects of Additional Undegradable Dietary Protein Intake During the Dry Period on Milk Yield and Composition During the Subsequent Lactation

5.1 SUMMARY

In Chapters 2 and 3 experiments were described that investigated short term dietary effects on the milk protein concentration from dairy cows. This chapter describes an experiment that was designed to investigate longer term effects of diet on milk protein concentration by manipulating the animal's tissue reserves during the dry period, and allowing a typical dairy cow lactation management system to operate during the following lactation. A group of 22 multiparous dairy cows were paired at the end of lactation according to condition score and predicted calving date. One animal from each pair received the usual dry cow treatment of *ad libitum* access to second-cut grass-silage, whilst the other animal received a restricted quantity of the same silage, together with *ad libitum* access to straw and a daily ration of 0.5 kg prairie meal (high protein maize gluten meal). Both groups of animals calved at about the same condition score of approximately 2.5-3.0. All animals were given access to the same diet during the subsequent lactation, based on grass-silage supplemented with concentrates during the winter, and grass pasture during the summer. The animals that received protein supplementation during the dry period produced significantly more milk protein (2-3 g/kg) during weeks 3-31 of the subsequent lactation than those which were fed silage alone. Milk yields were the same for the two groups whilst milk fat and lactose concentration were slightly higher from the supplemented animals. It is likely that the increase in milk protein concentration is due to a greater availability of labile body protein reserves for milk protein production.

5.2 INTRODUCTION

5.2.1 Dairy Cow Fat Reserves

A considerable amount of work has been undertaken to study the effects of dairy cow body fat reserves on the performance of the animal during lactation. At times when dietary energy supplies are insufficient, body fat can be mobilised to deliver large quantities of energy to the mammary gland to support the demands of lactation. During early lactation, the ability of the high yielding animal to consume sufficient quantities of feed to support milk production is limited. Increases in feed intake occur after calving but lag behind the increases in yield so that peak yield is reached before peak feed intake (see Garnsworthy, 1988). The result is generally a loss of live weight during early lactation as an appreciable amount of the fat reserve is used (Figure 5.1).

The ability of the dairy cow to mobilise fat and use the released energy to support lactation (i.e. 'milk off her back') led to the widespread use of this as a management tool. In order to get the animal ready for lactation and have plenty of fat to utilise for lactation, the practice of 'steaming up' was common until the mid-1980s. Leading up to calving, animals were fed increasing amounts of concentrates to about three quarters of their lactation allowances. In an extensive review, Broster (1971) discussed the effect on milk yield of feeding concentrates before calving, concluding that higher milk yields could be

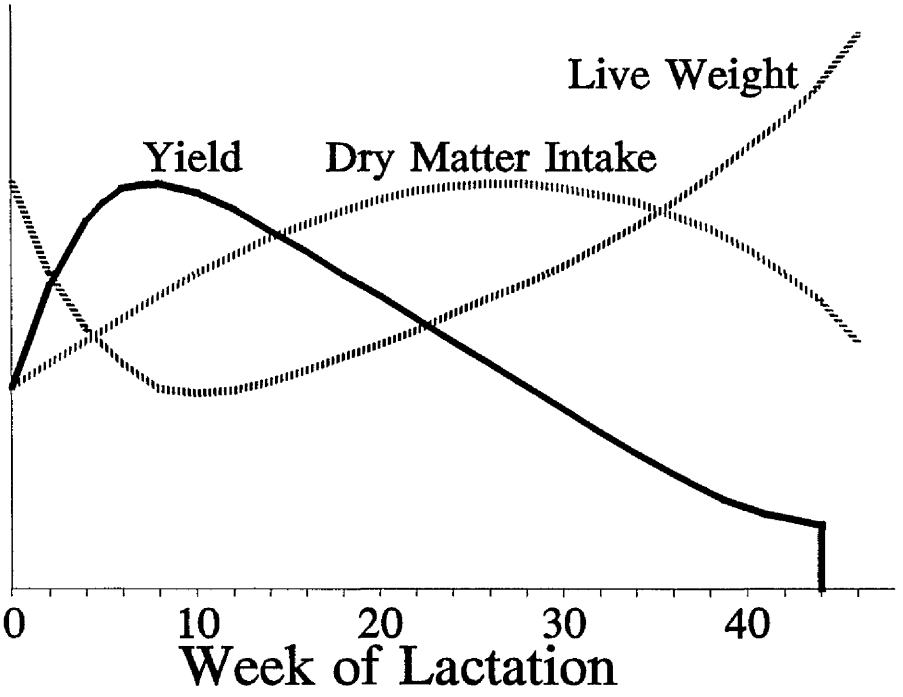


Figure 5.1 Typical changes in milk yield, dry matter intake and live weight of a dairy cow during lactation (after Garnsworthy, 1988).

achieved by additional feeding before calving. Frood and Croxton (1978) found that the condition score (CS) on a scale of 0-5 (0=emaciated, 5=obese; Lowman, Scott and Somerville, 1973) of an animal at calving was related to her ability to achieve potential milk yield, those scoring below 2 not reaching potential yield, and those above 2 yielding more, with maximal early lactation milk yields being achieved by those animals scoring 3.5. However, animals that are fat at calving are more prone to calving problems (due to physical restrictions) and are at greater risk of metabolic disorders such as ketosis and acidosis. The subsequent reproductive performance of the dairy cow was found to be unaffected by condition score at calving (Bouchier, Garnsworthy, Hutchinson and Benson, 1987), although the time to first service was greater for higher yielding animals (regardless of CS). Despite apparently significant effects of CS at calving on subsequent lactation performance in earlier studies, a more recent review of the literature by Garnsworthy (1988) found no significant effect of CS at calving (in the range 1-4+) on subsequent milk yields to week 12 of lactation. Recent evidence has therefore questioned the usefulness of steaming up and the calving of animals with a condition score much greater than 2.5, since any production advantages tend to be outweighed by health and reproductive disadvantages.

The effect of prepartum energy intake and condition at calving on milk protein concentration is somewhat unclear. Some authors have reported changes in protein concentration during early lactation after feeding cows on a higher plane of nutrition during the dry period, with both increases (Fronk, Schultz and Hardie, 1980; Cowan, Reid, Greenhalgh and Tait, 1981a) and decreases (Lodge, Fisher and Lessard, 1975) being reported. Several others, on the other hand, have found no effect of prepartum energy intake on milk protein concentration (Davenport and Rakes, 1969; Jaquette, Rakes and Croom, 1988; Holter, Slotnick, Hayes, Bozak, Urban and McGilliard, 1990). Accurate comparisons of the various results is difficult, because in order to test the effect of calving condition score on the subsequent lactation, several authors fed animals to obtain a specific CS at calving (Fronk *et al.* 1980; Treacher, Reid and Roberts, 1986; Garnsworthy and Jones, 1987; Jones and Garnsworthy, 1988; Jones and Garnsworthy, 1989; Holter *et al.*, 1990) while others classified animals as fat, medium or thin at calving after dry period feeding regimes with animals on each regime therefore not necessarily being classified into the same condition group (Bines and Morant, 1983; Davenport and Rakes, 1969). The problem with both these methods is that an animal in a particular CS group did not

necessarily receive the same dry period diet as the other animals, thereby introducing an unknown effect into the experimental design. Furthermore, none of these reports took into account the possibility of an effect of the animal's protein reserves.

In the reports of those experiments where a significant effect of prepartum diet and/or post partum condition score on milk protein concentration was given (Lodge *et al.*, 1975; Fronk *et al.*, 1980; Cowan *et al.*, 1981), the effects were not discussed in detail. Lodge *et al.* (1975) reported that thinner animals consumed more feed than fatter ones, an effect also reported by others (Garnsworthy and Topps, 1982; Bines and Morant, 1983; Treacher *et al.*, 1986; Garnsworthy and Jones, 1987; Jones and Garnsworthy, 1988) but without change to the milk protein concentration. Bines and Morant (1983) suggested that an increase in dry matter intake (DMI) by thin animals was due to a more rapid rate of fatty acid synthesis in adipose tissue from precursors which increases the flux of precursors absorbed from the rumen, allowing an increase in intake to occur. This was not observed by Reid, Roberts, Treacher and Williams (1986) who suggested that it was the more rapid oxidation of acetate to CO_2 in the adipose tissue of thin cows that increases the flux of precursors and hence DMI. Despite little experimental evidence in the above literature, an increase in DMI might be expected to increase milk protein concentration as energy intake is increased. Differences in milk protein concentration are only likely to occur if an increased supply of protein precursors to the mammary tissue is not associated with an increased energy intake with the greater DMI - otherwise increases in protein yield are likely to be associated with a concomitant increase in milk yield, with the result of a constant protein concentration (e.g. Treacher *et al.*, 1986). If increased DMI is achieved by increasing forage intake alone, the ratio of protein supply to energy supply to the tissues is unlikely to be increased significantly. Moreover, increasing the dietary forage intake may well produce a shift in the acetate:propionate ratio towards a condition less favourable for milk protein concentration as discussed in Chapter 3.

5.2.2 Dairy Cow Protein Reserves

Any energy from dietary components that is absorbed by the animal in excess of immediate requirements is stored largely as fat. Excess nitrogen, on the other hand, is excreted: there are no specific tissues devoted to nitrogen storage. During periods of nitrogen shortage, however, the body is able to mobilise protein from parts of the body where it is not immediately essential for use in other parts which take on greater

importance. These labile protein reserves not only exist in a different form to energy reserves, they are maintained differently.

Swick and Benevenga (1977) give an excellent discussion of labile protein reserves. The major point to note is that the relative rates of tissue protein anabolism and catabolism are sensitive to nutritional status, and when protein supply is adequate, anabolism exceeds catabolism, resulting in the net gain of protein, largely in skeletal muscle. However, under conditions of deprivation, catabolism exceeds anabolism, releasing amino acids from, for example, skeletal muscle for other purposes such as milk protein production. Lobley, Milne, Lovie, Reeds and Pennie (1980) estimated the total daily body protein synthesis rates in dairy cattle to be between 1.4 and 3.0 kg, with the contribution of the carcass (muscle, bone and brain) being some 32-33%, that of the gastrointestinal tract some 38-46%, and the rest due to the liver and skin. In a steady state situation the rate of degradation would match the rate of synthesis. If the intake of dietary crude protein fell short of requirements, a decrease in the rate of synthesis of some of the body components would allow continued rates of synthesis in others resulting in the channelling of not only dietary proteins but also tissue proteins to those areas that the body considers most important, which, in early lactation, includes the mammary gland. This situation was observed by Champredon, Debras, Patureau Mirand and Arnal (1990) in goats, where rates of protein synthesis were lower in certain parts of the body (in particular, certain skeletal muscles, skin and the head and feet), but were considerably higher in the mammary gland, when the animals were lactating compared to when they were dry. This is similar to the findings of Bryant and Smith (1982) who found that the rate of protein synthesis in skeletal muscle of lactating sheep was either unchanged or reduced compared to dry non-pregnant animals. This contrasts to the findings of Vincent and Lindsay (1985) where lactating sheep exhibited increased rates of muscle protein synthesis, but even greater rates of degradation, compared to dry non-pregnant animals. These results may be due to differences in the metabolism of various muscles during lactation (see Kelly, Summers, Park, Milligan and McBride, 1991). Riis (1988) suggested that the changes in net body protein synthesis were largely controlled by energy intake; body protein balance was found to be positive in early-lactation goats fed a high energy diet compared to animals fed a low energy diet, which were in negative energy balance and had a protein synthesis rate of approximately a quarter that of the high energy diet animals. The link between energy intake and protein synthesis rates was postulated to be the thyroid

hormones (Riis, 1988). A similar link between energy homeostasis and protein synthesis rates was reported by Champredon, Tesseraud, Debras, Tauveron, Bonnet, Larbaud, Balage, Makarski and Grizard (1993), who found that increased levels of insulin reduced body protein breakdown in goats, an effect that was enhanced during lactation. The mode of action remains to be elucidated (Champredon *et al.*, 1993), although if glucose (from the diet) leads to a reduction in body protein mobilisation (via insulin), one might expect to see more evidence of protein mobilisation (perhaps by higher milk protein yields) from animals using fat as an energy source since the insulin system would be bypassed, whereas the opposite is more usually seen.

Belyea, Frost, Martz, Clark and Forkner (1978) studied the body composition of dairy cows of different ages and production potentials throughout the gestation/lactation cycle using ^{40}K liquid scintillation detection. The results showed that multiparous cows had a greater protein mass (mean figures of 102 kg, 16.8% of live weight, LW) than heifers (97 kg, 17.1% LW), and that the protein mass decreased to a minimum at about week 6 of lactation (Figure 5.2). Fat mass also reached a minimum at this time. About half of the mean loss of 15 kg protein following parturition was accounted for by the foetus, the remainder was apparently lost due to the demands of lactation. It was noted that the range of protein loss (1-22 kg) was not correlated with milk production and it was suggested that some animals had a greater capacity to mobilise protein reserves than others.

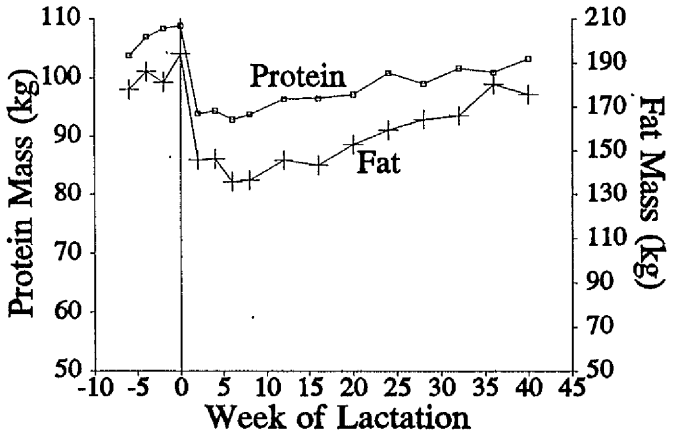


Figure 5.2 Mean dairy cow body protein and fat masses over the course of a lactation (data from Belyea *et al.*, 1978).

Similar changes in body protein mass over the gestation/lactation cycle have been reported by others in dairy cows (Gibb, Ivings, Dhanoa and Sutton, 1992) and ewes (Cowan, Robinson, Greenhalgh and McHattie, 1979); in both species the minimum protein content of the animal was seen to occur at approximately 6-8 weeks after parturition, with mean

losses of about 6 kg from calving in dairy cows. The advantage of the work done by Belyea *et al.* (1978), however, was that measurements could be made on the same animals over long periods of time, rather than on different animals in serial slaughter experiments (Cowan *et al.*, 19790; Gibb *et al.*, 1992). When ewes were fed at two planes of nutrition after parturition (Cowan, Robinson, McDonald and Smart, 1980), losses in body protein were found only in animals fed on the lower plane and which therefore had a lower protein intake. Cowan, Robinson, McHattie and Pennie (1981b) suggested that labile protein reserves were used to support milk protein production during early lactation when ewes were fed a low protein diet.

Whilst much work has investigated dairy cow fat reserves and their influence on lactational performance, relatively little has looked at the need for protein during early lactation and hence the utilisation of protein reserves. The data presented in the two previous chapters have indicated that both energy and protein supplies to the mammary tissue can limit milk and protein production respectively, both of which influence milk protein concentration. Paquay, De Baere and Lousse (1972) estimated the protein reserve of the nonlactating cow to be about 17 kg. Botts, Hemken and Bull (1979) measured protein reserves in early- to mid-lactation dairy cows and estimated the figure to be over 24 kg, about 27.1% of total body protein. Recent work with rats (Pine, Jessop and Oldham, 1992) has shown that they are able to utilise body protein reserves to support lactation (pup growth) for about 6 days if fed on a protein-free diet, having been fed a protein-rich diet prepartum. Similar results have been seen with dairy cows: when they were fed lower than recommended levels (80%) of crude protein for 60 days prior to calving (Chew, Murdock, Riley and Hillers, 1984b), they produced less milk and lower milk solids yields than animals fed 100% of recommended protein allowances. It should be remembered that whilst it is recognised that the energy requirements for lactation in the dairy cow must be buffered by her fat reserves, little regard is given to labile protein reserves in the consideration of current recommendations of protein requirements (TCORN, 1992). Deviations from the models put forward in that document may well in part reflect of differences in the status of protein reserves.

It remains unclear as to how much of the animal's protein reserve is used during the late stages of gestation to support the growth of the foetus. Leading up to parturition, the protein mass of the dairy cow was shown to increase relatively rapidly (Belyea *et al.*,

1978). There is no doubt as to the need for protein for the growth of the foetus, and the greatest need is during the last trimester of gestation. Prior and Laster (1979) showed that the maximal rate of foetal protein accretion (about 53 g/d) occurred at about day 243 of gestation, some 30-40 days before parturition. Traditionally, non-lactating dairy cows are rather neglected, often housed separately from lactating animals, and fed a relatively low quality diet. Because such a diet may be adequate to meet energy requirements, protein requirements are generally thought to be similarly met, but this is not necessarily true. Little is known about the source of the protein which flows from mother to foetus; it may either come from the mother's diet or from her body protein. Hook, Odde, Aguilar and Olson (1989) showed that calf weights were unaffected by level of protein fed to the mother for 70 days prior to calving, but that the milk yields of the cows fed a low protein diet were subsequently lower than those of animals fed a high protein diet, suggesting that the immediate needs of the foetus take priority over the potential needs of the calf later.

The reason that some authors have found relatively little body protein loss during lactation (Belyea *et al.*, 1978; Gibb *et al.*, 1992) whilst others have shown greater potential for loss (Botts *et al.*, 1979) may be because much is used to support foetal growth prior to experimental observations. There may also be limits to which animals may normally go, which may be less than their 'potential' limits. If protein reserves can be maintained and/or replenished during the dry period, they will be available for use during lactation. This hypothesis is supported by (and is the basis of) the work of Van Saun, Idleman and Sniffen (1993), who fed dairy heifers additional DUDP during the 3 weeks leading up to parturition, and found increased milk protein concentrations during the subsequent lactation. In a similar manner, but for different reasons, Chew, Eisenman and Tanaka (1984a) infused arginine intravenously into dairy cows during the 7 days leading up to calving, and found transient but dramatic increases in the serum concentrations of growth hormone, insulin and prolactin. During the following lactation, milk yields were significantly higher from those animals which received the arginine infusion. Chew *et al.* (1984a) speculated that the increased hormone concentrations stimulated mammary development to allow increased milk yields either by increasing the number of secretory cells or by increasing their activity (or both). A further possibility which they did not apparently consider was that the increased hormone concentrations conserved body stores of fat and protein and stimulated dry matter intake, although this was not reported. The significance of effects of treatments over relatively short periods of time leading up to

calving led to the conclusion that this period is particularly taxing on the animal's body reserves, especially since it is well known that the feed intake of the dairy cow is reduced for several days leading up to calving.

Knight and Wilde (1987) showed that the proliferation of mammary secretory cells leading up to lactation can greatly influence milk yield. It has been proposed that the effects of prepartum treatments - arginine infusion (Chew, Eisenman and Tanaka, 1984a) and bST injections (Stelwagen, Grieve, McBride and Rehman, 1992) - on subsequent differences in lactation performance may have mediated through an increase in the proliferation of mammary secretory cells. However, neither group could show this to have actually occurred. It may be possible that if an animal's protein status is high shortly before calving, the mammary gland may somehow become 'accustomed' to this and develop in a way which means that protein efficiency is increased during subsequent lactation.

5.2.3 Interaction of Fat and Protein Reserves

I am unaware of any work reported in the literature which has attempted to investigate the interaction between fat and protein reserves in the dairy cow and subsequent lactation performance. Most authors of work which was designed to investigate fat reserves did not consider labile protein reserves. Experiments designed to investigate protein reserves have usually attempted to keep animal fat condition constant. Although the underlying mechanisms that control and maintain fat and protein reserves are very different and therefore may not interact directly, the effects of one may have important consequences on the other. For instance, fat cows tend to eat less than thinner ones, making greater demands on labile protein reserves since crude protein intake will be reduced. If there is insufficient dietary protein to support lactation and labile protein reserves become fully depleted, lactation more or less stops in rats, regardless of energy supplies (Pine *et al.*, 1992). If protein supplies are limited but energy supplies are adequate or more than adequate, milk protein concentration may decrease as milk yield increases - this is frequently seen when fat is fed to dairy cows. If, however, dietary intake is limited, limiting both protein and energy intakes, but labile protein reserves are available for mobilization, milk protein concentration may increase. Cowan *et al.* (1981a) report increased milk protein concentrations from fatter cows, with no effect of condition score on DMI or milk yields. Those authors suggested that the increased protein output was made possible by the increased dietary intake in late pregnancy, i.e. conserving protein

stores for later release during lactation. On the other hand, Treacher *et al.* (1986) found no difference in milk composition between cows that were fed to be fat or thin at calving despite increased yields of protein, fat and lactose due to concomitant increases in milk yields, even though increased tissue protein mobilization was observed with the fatter animals (Reid *et al.*, 1986). Reid *et al.* (1986) suggested that the endogenous protein may not have been suitable for sustaining high milk yields, although it could have been that any potential effects were masked by the higher DMIs of the thinner animals (Treacher *et al.*, 1986). On the other hand, Rulquin and Vérité (1993) suggested that carnosine, a dipeptide of alanine and histidine that is associated with skeletal muscle, may act to reduce the requirement of dietary histidine and could have been a confounding factor in experiments that were designed to investigate limiting essential amino acids in milk production. Under current dry cow management regimes, it is possible that by feeding a relatively poor forage energy requirements are met but that protein requirements are not. Fat reserves are therefore being repleted but protein reserves may be being depleted.

5.2.4 Conclusions

The state of the body reserves of the dairy cow at calving has important consequences on her performance during the following lactation. Much work has been done to investigate the effect of fat reserves at calving, and while early work suggested that large amounts of fat were beneficial for supporting lactation, more recent work has suggested that the advantages of this are outweighed by the disadvantages in terms of reduced feed intake, and calving and reproductive problems. Very little work, however, has looked specifically at the protein reserves of the dairy cow and their effects on milk production, although there is some evidence that suggests that an increase in the quantities of body protein at the start of lactation can lead to increased milk protein production later. More work is, however, needed to see if the dairy cow protein reserve can be manipulated prior to calving, and, if this has a beneficial effect, how long that effect will last into the following lactation.

The experiment reported here was designed to test the hypothesis that if a small amount of high quality (rumen undegradable) protein is fed to dairy cows to cover their requirements and possibly replete protein reserves, in other words, separate the repletion of fat and protein reserves, milk production, and in particular milk protein production, may be increased during the subsequent lactation.

5.3 EXPERIMENTAL AIMS

A group feeding experiment was conducted to investigate the effect of restricting energy intake to predicted requirements and increasing undegradable protein (UDP) intake of dairy cows during the dry period on their performance during the subsequent lactation. The UDP supplement was intended to maintain body protein reserves as replete as possible for use during lactation. Animals were paired according to condition score and predicted calving date and within pairs were randomly assigned to one of two dry period treatments. After calving, all animals were given access to the same lactation diet. Blood samples were collected twice during the dry period and twice during lactation for metabolic profile analysis. Milk samples were collected every two weeks for the determination of fat, protein and lactose concentrations. Animals were weighed and scored for condition approximately every 4 weeks during lactation to assess the effect of condition score on lactation performance.

5.4 MATERIALS AND METHODS

5.4.1 Animals and Their Management

Twenty two pregnant multiparous Holstein-Friesian cows were drawn from the SAC Auchincruive herd for use in the experiment. Each was dried off at about 56 days prior to her predicted calving date. During the dry period, half of the animals were housed in the dry cow unit, a cubicle building with integral silage clamp, where they mixed with the remainder of the Auchincruive dry cows. The other half were kept in a cubicle house, separated from non-experimental animals. Both groups of dry animals had constant access to fresh water, a cubicle bedded with sawdust, and an outside yard.

Immediately prior to calving, all animals were handled without distinction between dry period treatments. The animals calved in a calving pen with straw bedding, and, when recovered, were housed in the main Auchincruive dairy unit together with other, non-experimental, animals. The lactating cows were housed in a cubicle unit during the winter, and grazed at pasture during the summer. They were milked using a 20/20 Herringbone milking parlour twice daily, at approximately 0500-0800 h and 1430-1700 h. Whilst housed, this was done in groups depending on stage of lactation, with the high-yielding group receiving the most even milking interval.

5.4.2 Experimental Design

The experiment was a group feeding design, with animals paired shortly before the end of lactation according to their most recent condition score (CS) and predicted calving date. Dry period treatments within each pair of animals were allocated randomly. The experiment was of continuous design with animals calving from 13th December, 1991 to 27th February, 1992 and from 17th March to 29th April, 1992.

Statistical analyses were carried using Genstat 5 (Lawes Agricultural Trust, 1990) and Minitab (Minitab, Inc., State College, Pennsylvania, USA). Effects were considered significant were $P<0.05$. Where $0.10>P>0.05$, the effect was considered interesting and worthy of further consideration.

The data obtained from blood samples were analyzed using analysis of variance using a blocking structure of Pair/Cow and treatment structure of Treatment*(State/Sample), where State was either dry or lactating, and Sample was one of two samples taken in each State.

Differences in group condition score were analyzed using the Mann-Whitney signed rank test, and differences between group live weights were analyzed using analysis of variance using a blocking structure of Pair/Cow and a treatment structure of dry period Treatment. Results of milk sampling were analyzed by REML (Patterson and Thompson, 1971) due to the unbalanced nature of the data. Several models were tested including the effects of calving date, lactation number, sample number (i.e. stage of lactation), pair and cow, with the effects of random components of variance tested using the deviance test (compared against the χ^2 distribution), and the effects of different fixed component models compared using the standard errors of the difference of the means. The final model that was chosen used a fixed component model of Calve+Sample*Treatment, where Calve was the month of calving, and a random component model of Pair. The significance of fixed effects was tested using the Wald statistic against the distribution of χ^2.

Due to the rapidly changing nature of milk produced in the first week of lactation and the fact that the first samples were taken anywhere between 0-13 days after calving, and at times included colostrum, the first milk sample was excluded from the analysis for all animals. Data were analyzed up to week 31 of lactation (up to 16 milk samples), although due to the spread of calving dates, not all animals had reached this stage of lactation.

Table 5.1 Composition of the silage fed to dry cows.

	Second Cut Grass-Silage
Dry Matter, g/kg	149
Organic Matter, g/kg DM	903
Crude Protein, g/kg DM	202
Metabolisable Energy, MJ/kg DM	10.5
Rumen Degradable Protein, g/kg DM	172
Undegradable Protein, g/kg DM	30
In vitro Organic Matter Digestibility, g/kg OM	725
D-Value, g/kg DM	655
NH_4-N, g/kg Total N	224
pH	4.5
Calcium, g/kg DM	6.7
Phosphorus, g/kg DM	3.4
Magnesium, g/kg DM	2.5

5.4.3 Diet Formulation and Production

5.4.3.1 Dry Period Diets

Two diets, control and supplemented, were fed to the experimental animals during the dry period. The control diet consisted of second-cut grass-silage (Table 5.1) fed *ad libitum*. Using SAC advisory software based on the recommendations of TCORN (1992; NW Offer, personal communication), and animal characteristics of a live weight of 575 kg (it was not possible to calculate a true mean live weight of the experimental animals), with a daily live weight gain of 0.5 kg, the predicted silage intake needed to meet metabolisable energy (ME) requirements of the control animals was 10.0 kg DM/d. This was predicted to supply an excess of 760 g effective rumen degradable protein (ERDP)/d, and an excess of 50 g digestible undegradable protein (DUDP)/d.

The supplemented dry period diet was formulated to meet ME and effective rumen degradable protein (ERDP) requirements. This was achieved by restricting silage intake to a target of 4.0 kg DM/d, supplemented with 0.5 kg/d prairie meal (0.46 kg DM/d) and barley straw fed at *ad libitum* rates, the predicted intake of which was 8.0 kg DM/d. This was predicted to supply an excess of approximately 300 g DUDP/d, some 250 g DUDP/d more than the control diet. Prairie meal was choosen in preference to another source of low degradable protein such as fishmeal due to its low calcium content. Both groups of animals had constant access to fresh water and a low calcium salt lick (declared content of 360 g Na/kg, 8.1 g Ca/kg, 2.3 g P/kg, 3 g Mg/kg).

5.4.3.2 Lactation Diets

During lactation, all animals had access to the same standard Auchincruive dairy cow diet. During the winter, this consisted of *ad libitum* access to first-cut grass-silage with concentrate supplements. The silage was fed as a mixture with Supergrains (a wheat-based distillery by-product; Borthwick, Glasgow, UK) on a 3:1 basis. Three kilograms per day of a milled barley/soya mix (2.7 kg barley with 0.3 kg high protein soya bean meal) was also fed. A standard dairy cow lactation concentrate was fed in parlour on a stepped flat-rate basis, with animals receiving 5.6 kg/d until day 100 of lactation, 3.2 kg/d from days 101-200 and 0.8 kg/d thereafter. After turnout, concentrate was then fed in parlour according to yield, with animals yielding over 30 kg/d receiving 3.2 kg/d and those below 30 kg/d receiving 0.8 kg/d. The concentrate fed to animals at pasture, and shortly before turnout, was the same as fed during the winter months, but with an increased Mg content.

5.4.4 Animal Feeding

5.4.4.1 Dry Period Feeding

Control dry cows were fed *ad libitum* directly from the silage face, using a steel cable barrier. Silage intake of the control animals was not measured. Supplemented animals were offered the same silage cut from the clamp, and fed in a ring feeder under shelter. Before it was fed, the silage was weighed, and the animals were allowed access to the silage for about 3 hours each morning to allow an estimated 4.0 kg DM per animal per day. If any silage remained after 3-4 days, it was removed, weighed, and replaced with fresh silage. Otherwise, the silage was fed as necessary.

Supplemented dry cows also had constant access to barley straw, fed indoors from a ring feeder. Bales were weighed as offered. Some wastage was produced, which was periodically removed and weighed.

Half a kilogram of prairie meal was offered in individual bins to each of the supplemented animals at approximately 1200 h each day. Once animals had become accustomed to the feeding regime the prairie meal was rapidly consumed which prevented stealing by other animals.

5.4.4.2 Lactation Feeding

During lactation, the silage mix was fed at approximately 0800 h and 1600 h. The barley/soya mix was fed on top of the silage at approximately 1200 h. None of the experimental animals reached the lowest level of concentrate allowance, since turnout occurred on the 5 May, 1992. After a short period of being out at pasture during the day and inside, fed silage, at night, the animals remained outside at all times. Concentrates fed automatically in-parlour were fed in equal portions at each of the two milkings.

5.4.5 Sample Collection and Analysis

Blood samples were taken from the coccygeal vessels by venepuncture at four times during the experiment for metabolic profile analysis (Payne, Dew, Manston and Faulks, 1970). Blood was analyzed for protein, albumin, urea, glucose, ß-hydroxybutyrate, non-esterified fatty acids (NEFA), magnesium and phosphorus by the Dairy Herd Health and Productivity Service (The Royal (Dick) School of Veterinary Studies, Veterinary Field Station, Easter Bush, Roslin). Target sampling dates were 14 days after drying off, 7 days

before predicted calving date, 10 days after actual calving date, and 42 days after actual calving date. In practice, samples were collected on a Monday to Thursday nearest the target date to allow immediate (i.e. next day) analysis where possible. Samples were taken between 0900 h and 1000 h, with animals either held in a crush or standing in a cubicle with as little stress and excitement to the animal as possible. Blood was collected into Vacutainer tubes (Becton, Dickinson and Co., Rutherford, New Jersey) that were packed and sent by post to arrive the following day for analysis. If immediate analysis was not possible, as, for example, during holiday periods, the blood was immediately centrifuged at 4°C for 20-30 minutes at approximately 1,700x*g*. Plasma was decanted into fresh tubes, frozen, and stored at -20°C until it was sent for analysis.

From the start of lactation of the first experimental animal, milk samples were collected every two weeks at 2 consecutive milkings, pm and am, until 2nd September, 1992. Milk samples were analyzed by the Department of Dairy Technology at Auchincruive for protein, fat and lactose concentrations using a Milko-Scan 203 analyzer (Foss Electric, Denmark). Milk constituent yields were calculated using the mean concentrations weighted for the yield at the time of collection multiplied by the total daily yield.

As part of the normal Auchincruive herd management programme, animals were weighed and scored for condition on a scale of 0-5 (to the nearest half point; Lowman *et al.*, 1973) approximately every four weeks.

5.5 RESULTS

5.5.1 Dry Period Feed Intake

On a group fed basis, the mean daily silage intake of the protein supplemented dry cows was 2.68 kg DM/d. The straw intake of the same animals was 1.96 kg DM/d. Both of these figures were below those predicted by the ration formulation software of 4.0 and 8.0 kg DM/d for silage and straw respectively. The silage intakes of the control group were not measured.

The feed analyses of the prairie meal fed to the supplemented dry cows and the parlour concentrate fed to all lactating animals are presented in Table 5.2. The composition of the silage fed to lactating animals is presented in Table 5.3. The analysis of Supergrains was typically 260 g DM/kg, 985 g OM/kg DM, 320 g CP/kg DM, 14.0 MJ ME/kg DM, 100 g oil/kg DM, 160 g crude fibre/kg DM, 0.8 g Ca/kg DM, 3.0 g P/kg DM and 0.1 g Na/kg DM (Borthwick Bulletin No. 4, Borthwick, Glasgow, Scotland).

Table 5.2 Composition of the prairie meal fed to dry cows on the protein supplemented diet and parlour concentrate fed to all lactating cows.

	Prairie Meal	Parlour Concentrate
Dry Matter, g/kg	913	882
Organic Matter, g/kg DM	983	894
Crude Protein, g/kg DM	577	216
Metabolisable Energy, MJ/kg DM	13.4	13.4
Neutral Detergent Fibre, g/kg DM	-	275
Acid Detergent Fibre, g/kg DM	28.0	158
Starch, g/kg DM	212	151
Water Soluble Carbohydrates, g/kg DM	1.4	68
Acid Hydrolysis Ether Extract, g/kg DM	85	61
In vitro Organic Matter Digestibility, g/kg OM	850	751
Potassium, g/kg DM	2.6	13.4
Calcium, g/kg DM	0.2	14.3
Phosphorus, g/kg DM	5.3	7.1
Magnesium, g/kg DM	0.6	8.8
Sodium, g/kg DM	0.1	4.9

Table 5.3 Composition of the silage fed to lactating cows (mean of 3 samples, each bulked over 10 days).

	First Cut Grass-Silage
Dry Matter, g/kg	254
Organic Matter, g/kg DM	932
Crude Protein, g/kg DM	182
Metabolisable Energy, MJ/kg DM	11.5
Rumen Degradable Protein, g/kg DM	155
Undegradable Protein, g/kg DM	27
Neutral Detergent Fibre, g/kg DM	435
Acid Detergent Fibre, g/kg DM	261
Water Soluble Carbohydrates, g/kg DM	40
Ether Extract, g/kg DM	45.7
Acid Hydrolysis Ether Extract, g/kg DM	56.3
In vitro Organic Matter Digestibility, g/kg OM	784
D-Value, g/kg DM	718
NH_4-N, g/kg Total N	89
pH	3.7
Calcium, g/kg DM	6.2
Phosphorus, g/kg DM	3.4
Magnesium, g/kg DM	2.5
Potassium, g/kg DM	20.8
Sodium, g/kg DM	4.0

The composition of the straw fed to the supplemented animals was not determined. However, barley straw analyzed at a later date had a dry matter (DM) content of 819 g/kg, a crude protein (CP) content of 44 g/kg DM with a rumen degradability of 0.5 g/g, and an ME density of 6.1 MJ/kg DM. On the assumption that the straw fed to the animals was of a similar nature, the mean total DM intake (DMI) of the animals was 5.1 kg/d, mean daily CP intake (CPI) was 0.890 kg/d, and the mean ME intake (MEI) was 46.2 MJ/d. On the further assumption that the rumen degradability of the prairie meal CP was 0.4 g/g, the mean daily rumen degradable protein (RDP) intake of the animals was 0.609 kg/d and the mean daily undegradable protein (UDP) intake was therefore 0.281 kg/d. The overall CP, UDP and ME densities of the supplemented dry cow diet were

approximately 174 g CP/kg DM, 55 g UDP/kg DM and 9.1 MJ ME/kg DM respectively, assuming additivity, compared with 202 g CP/kg DM, 30 g UDP/kg DM and 10.5 MJ ME/kg DM for the silage alone.

5.5.2 Health

During the experiment, several animals did not complete the dry period treatment or were ill during lactation. Some of the blood samples shortly before calving were missed since the animals calved earlier than expected. Missing values due to illness were coded as such in the final statistical analyses.

Two animals did not complete the dry period treatment due to factors unrelated to the treatment; one was not dried off as specified and received only a few days of her allocated treatment and another injured herself after getting stuck under a cubicle division. Of the 11 original pairs of animals, only 9 pairs were used for the analysis of milk yield and composition data, and for the analysis of live weight and condition score data. The data from the two half pairs which received the correct dry period treatment were used in the analyses of blood samples.

A summary of the health problems encountered and treated during lactation is given in Table 5.4. The greatest problem was lameness due to interdigital dermatitis, a problem that was prevalent in the whole Auchincruive herd and was not confined to the experimental animals. No statistical analysis was attempted on the health data.

Table 5.4 Frequency of health problems treated during lactation (numbers excluding repeats by the same individual in parenthesis).

	Dry Period Treatment	
	Control	Supplemented
Retained Foetal Membrane	2	1
Infection of the Uterus	1	0
Lameness	8 (5)	2
Mastitis	1	3 (2)

Table 5.5 Summary of blood metabolic profile analyses. Blood samples were taken from control and supplemented animals twice during the dry period and during lactation (BOHB=ß-hydroxybutyrate; NEFA=non-esterified fatty acids).

Treatment:	Control				Supplemented						
State:	Dry		Lactating		Dry		Lactating			F Probability	
Sample:	1	2	1	2	1	2	1	2	SED	Tr't	State
Protein, g/l	83.6	74.9	77.5	85.2	85.4	72.7	77.5	85.4	3.83	0.985	0.062
Albumin, g/l	37.5	33.9	34.5	36.7	39.2	36.7	37.9	39.7	1.15	0.010	0.452
Globulin*, g/l	46.2	41.0	43.1	48.5	46.2	36.1	39.7	45.8	3.39	0.385	0.042
Urea, mM	2.68	2.79	2.46	2.86	2.58	2.68	2.23	2.87	0.273	0.595	0.498
Glucose, mM	4.09	3.83	3.43	4.08	4.01	3.49	3.58	4.07	0.196	0.535	0.474
BOHB, mM	0.85	0.73	0.94	0.84	0.63	1.30	0.72	1.07	0.175	0.396	0.906
NEFA, mM	0.40	0.68	0.53	0.36	0.66	0.50	0.63	0.39	0.109	0.276	0.155
Mg, mM	1.09	1.00	1.04	1.07	1.08	1.03	1.06	1.08	0.035	0.401	0.387
P, mM	1.97	1.92	1.60	1.81	2.43	2.22	1.80	1.86	0.109	0.004	<.001

* Globulin calculated as Protein-Albumin

5.5.3 Metabolic Profiles

A summary of the metabolic profile data is presented in Table 5.5. From this it can be seen that blood albumin levels were consistently higher for the supplemented animals ($P<0.05$). Similarly, phosphorus levels were significantly higher for these animals, particularly during the dry period ($P<0.01$).

For both dry period groups, blood albumin concentrations were lowest around calving, dropping from the first to the second samples during the dry period, although the decrease of 3.6 g/l ($P<0.001$) of the control animals was greater than that of the supplemented animals (2.5 g/l, $P<0.05$). Similar effects were seen for total protein (and hence globulin) concentrations. During lactation, the reverse was seen, with increases in the protein fractions of blood from week 1 to week 6 of lactation for both control ($P<0.05$) and supplemented ($P=0.063$) groups.

Blood glucose concentrations exhibited similar changes to those of blood protein, being significantly lower closer to calving than either 7 weeks before or 6 weeks after ($P<0.01$), apart from during the dry period in the control animals ($P=0.085$).

During the dry period, NEFA concentrations differed in animals between being dried off and shortly before calving. The direction in which the changes occurred, however, was the opposite for each dry cow treatment: a significant increase in blood NEFA concentration towards calving was observed in dry control cows ($P<0.05$) whereas there was a trend ($P=0.083$) towards a decline in NEFA concentration in the supplemented animals. There was a decrease in NEFA concentrations from early to peak lactation to similar levels for both groups, although this was only significant for the supplemented animals ($P<0.05$).

Blood ß-hydroxybutyrate concentrations were particularly high shortly before calving for the supplemented animals, and were significantly higher than the first dry period blood sample for these animals ($P<0.001$). The situation was reversed, like NEFA concentrations, but in opposite directions, for the dry control cows, although the difference for these animals was not significant.

5.5.4 Live Weights and Condition Scores

The mean condition scores (CS) and live weights that were recorded shortly before the animals were dried off, shortly after calving, and at about peak lactation (weeks 8-11) are presented in Table 5.6. It can be seen that there were no significant differences in CS or live weights between groups at any time. The CS for the supplemented group were consistently slightly higher than those of the control group. Changes over time in both CS and live weight were also analyzed and although there were large declines in live weight over the dry period, there were no significant differences between groups.

Table 5.6 Mean condition scores and live weights (kg) at three points during the experiment - shortly before drying off, shortly after calving, and at about peak lactation. Effect of foetal weight not taken into account.

	Condition Score			Live Weight			
	Control	Suppl'd	MW Sig*	Control	Suppl'd	SED	F Prob
Before Drying Off	2.8	3.0	ns	648	619	20.0	0.219
Post Calving	1.8	2.1	ns	534	565	29.3	0.320
Peak Lactation	1.7	1.9	ns	556	581	36.6	0.539

* Significance of differences between condition scores of groups as tested by Mann-Whitney signed rank test, ns=not significant.

5.5.5 Lactation Performance

Model means of milk yields and milk protein, fat and lactose concentrations and yields, and the significance of effects of each dry period treatment are presented in Table 5.7. Milk yields were not affected by dry period treatment, but the concentrations of protein, fat and lactose were all higher (protein and lactose significantly so) from animals given the protein supplemented dry period treatment.

Model means for each of the two dry period treatments for the lactation up to week 31 are presented graphically in Figures 5.3-5.9. The effects of dry period treatment on milk protein and lactose concentrations appeared to last until about week 25 of lactation, and to about week 20 on milk protein and lactose yields. The concentration of milk fat was very variable compared to the other two major milk solids, although it was consistently higher from the supplemented animals until about week 15 of lactation.

The effect of sample number (i.e. stage of lactation) was highly significant ($P<0.001$) for all the variables presented except fat concentration ($P=0.067$). There were no significant effects of interaction between sample number and dry period treatment. The effect of month of calving was significant for milk fat yield ($P<0.05$), for milk protein concentration and yield ($P<0.01$), and for lactose concentration ($P<0.001$). The mean effects of month of calving on these variables are presented in Figures 5.10 and 5.11.

Table 5.7 Summary of results for the lactation (weeks 3-31) following different treatments during the dry period.

	Dry Period Treatment			
	Control	Supplemented	SED	χ^2 Probability
Milk Yield, kg/d	27.0	26.9	0.86	0.752
Protein, g/kg	29.4	32.0	0.40	<.001
Fat, g/kg	40.4	42.3	1.50	0.083
Lactose, g/kg	45.2	46.0	0.37	0.007
Protein Yield, g/d	790	860	28.1	<.001
Fat Yield, g/d	1095	1162	52.6	0.061
Lactose Yield, g/d	1232	1257	42.0	0.273

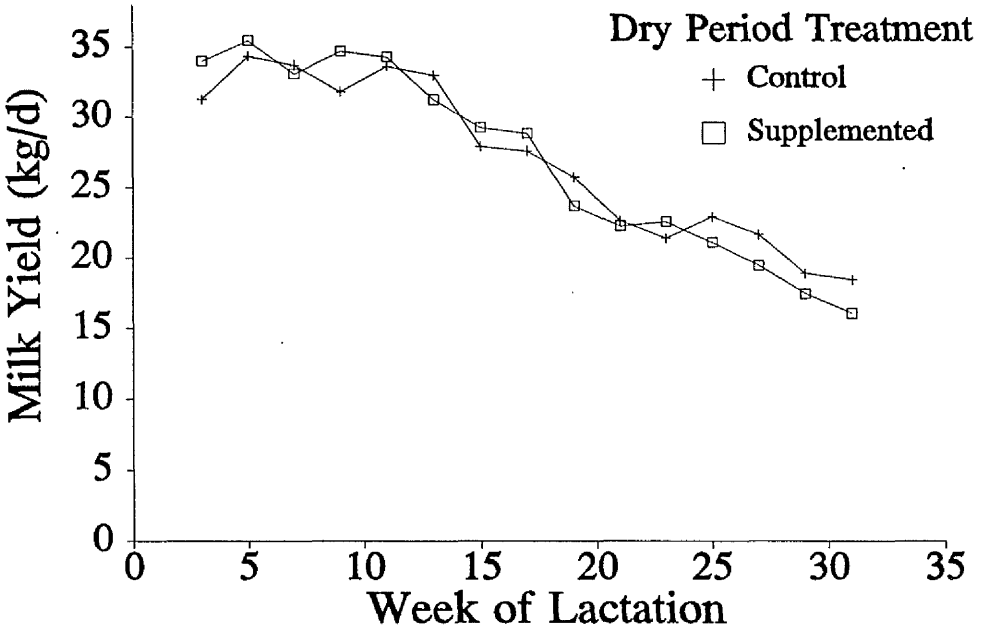


Figure 5.3 Model mean daily milk yields for each dry period treatment.

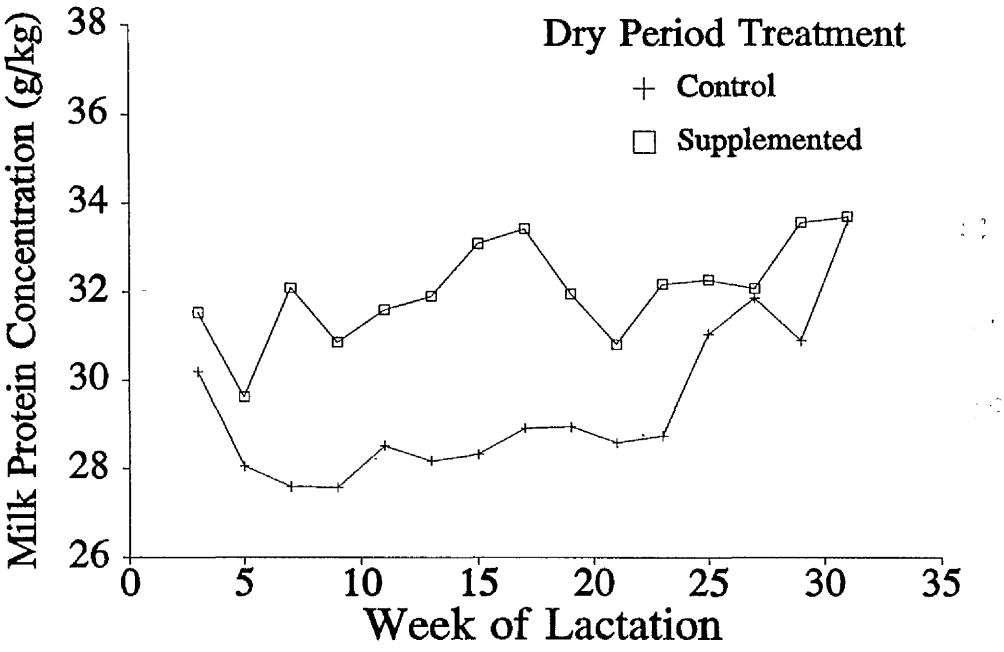


Figure 5.4 Model mean milk protein concentrations for each dry period treatment.

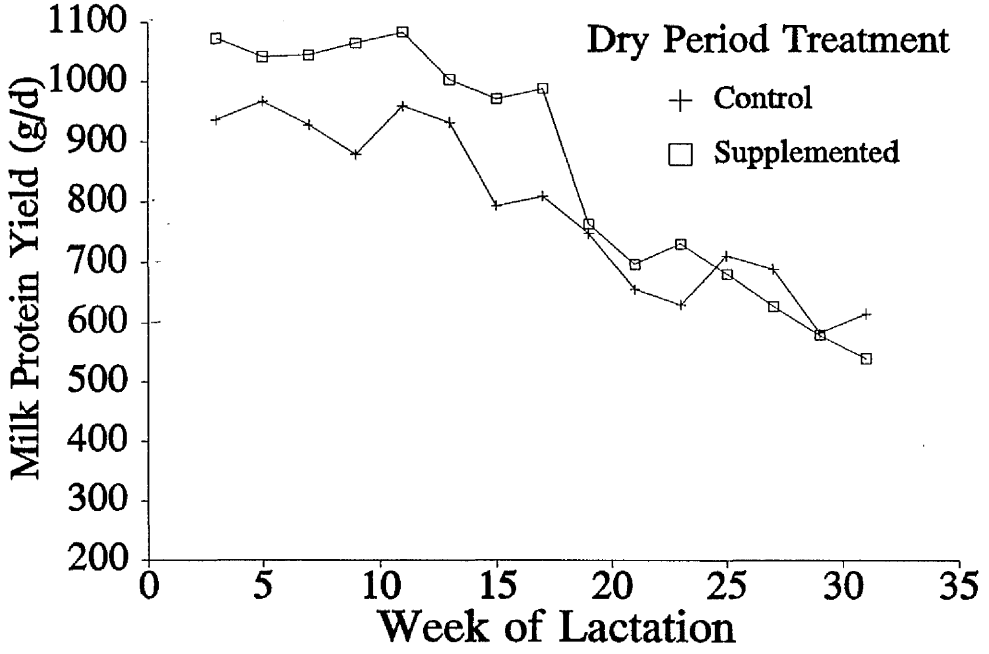


Figure 5.5 Model mean daily milk protein yields for each dry period treatment.

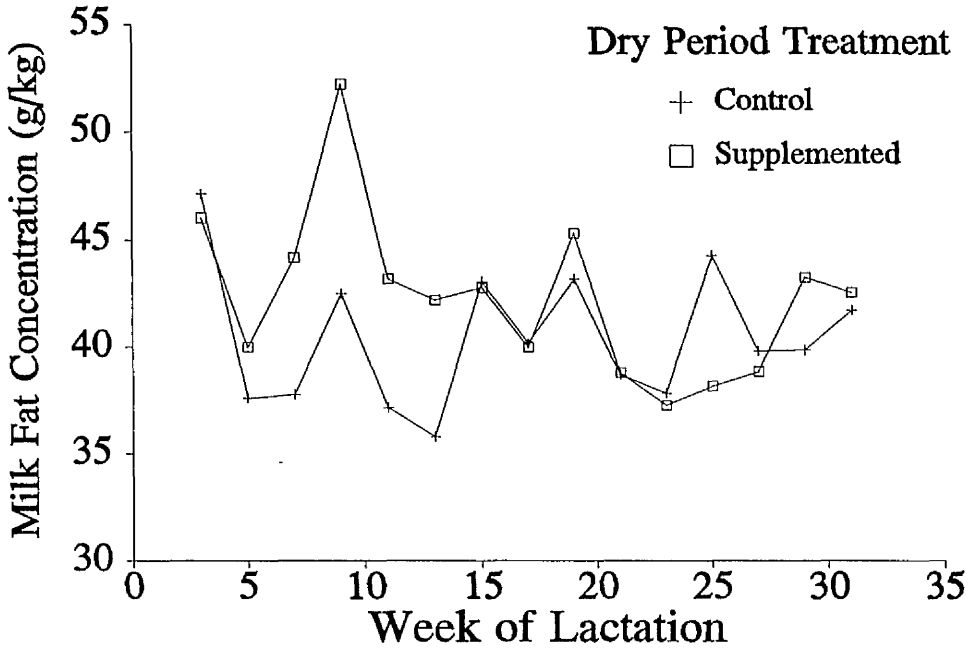


Figure 5.6 Model mean milk fat concentrations for each dry period treatment.

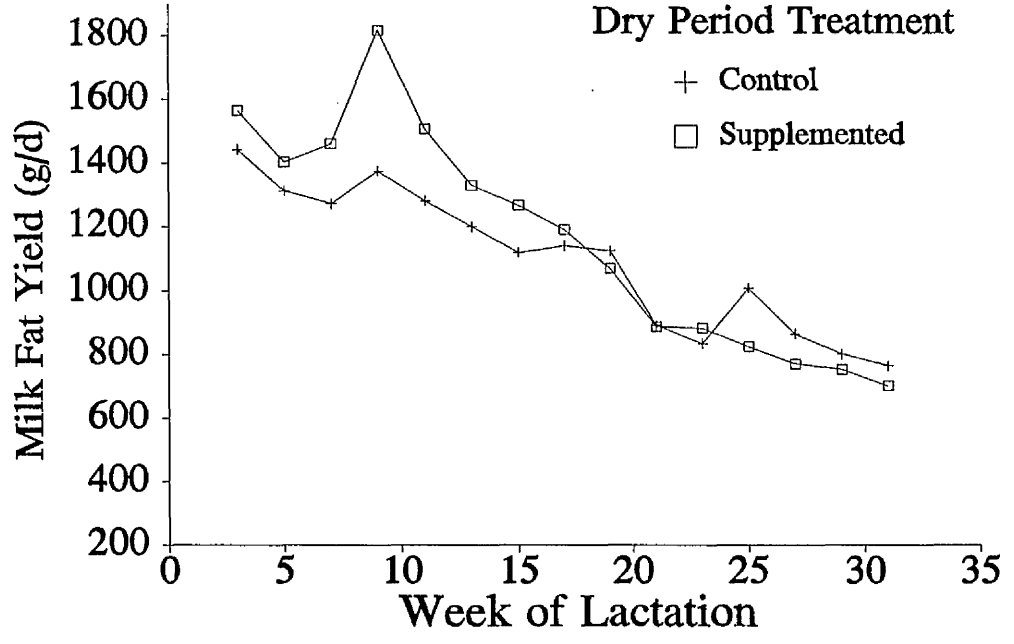


Figure 5.7 Model mean daily milk fat yields for each dry period treatment.

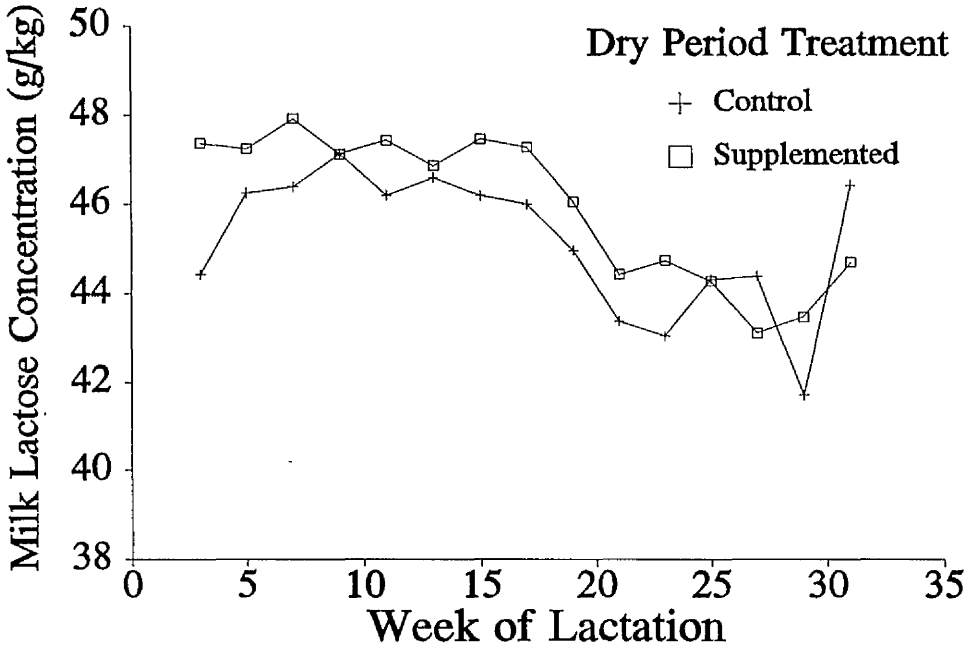


Figure 5.8 Model mean milk lactose concentrations for each dry period treatment.

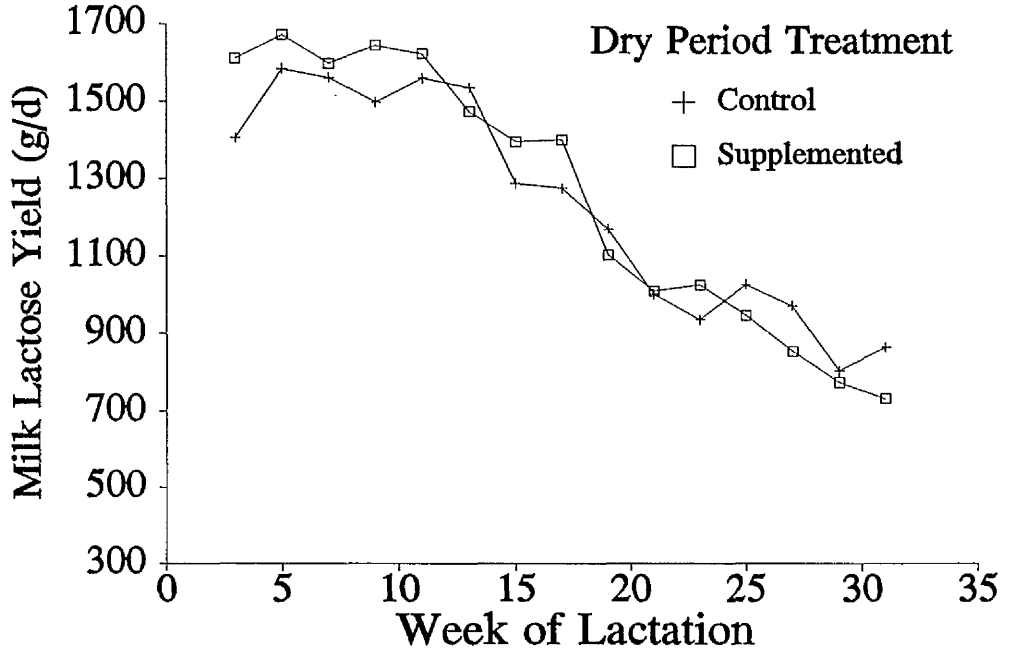


Figure 5.9 Model mean daily milk lactose yields for each dry period treatment.

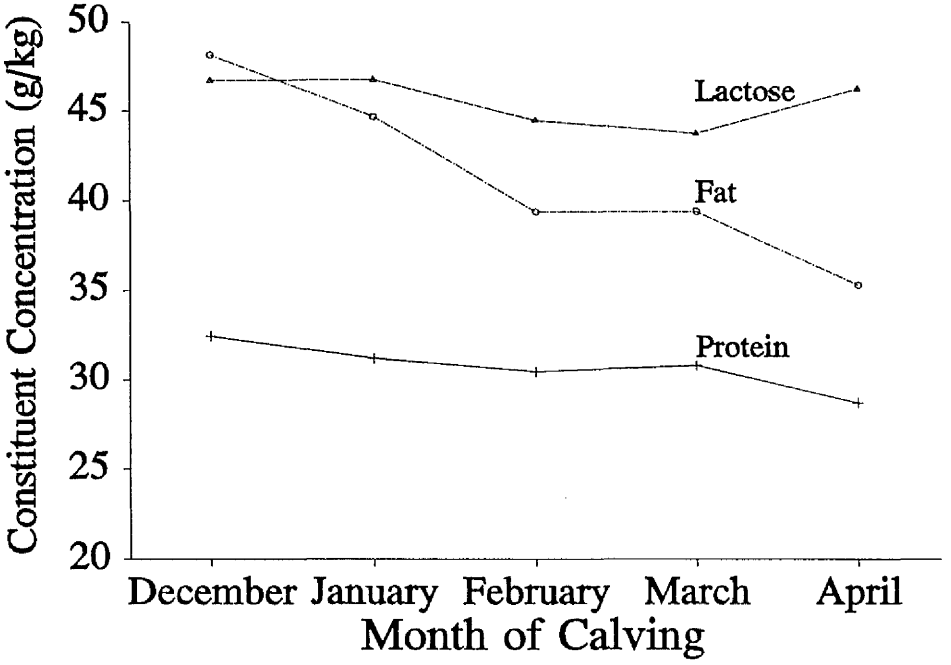


Figure 5.10 Model mean effects of month of calving on milk constituent concentrations.

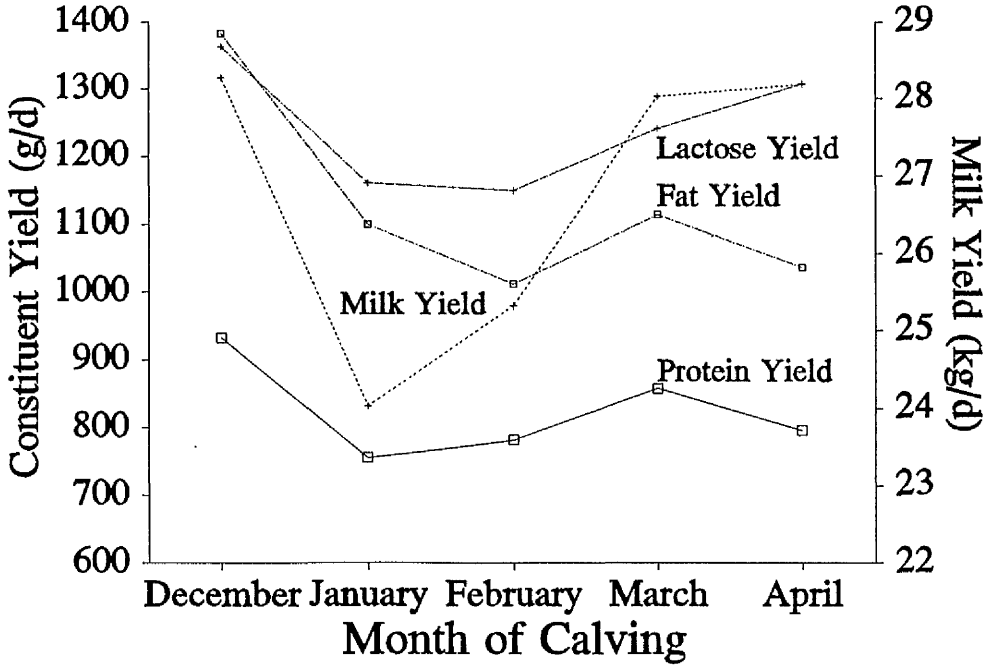


Figure 5.11 Model mean effects of month of calving on daily milk yields and daily yields of milk constituents.

5.6 DISCUSSION

This experiment was conducted to examine the influence of dry period protein supplementation on milk protein concentration by feeding a small amount of additional high-quality protein during the dry period. This objective was achieved, with a striking difference in milk protein (and milk solids in general) between those animals that were fed the supplemented dry cow diet compared to those that went through the usual (control) dry cow management regime.

During the dry period, the mean daily intakes of silage of the supplemented animals were lower than formulated/predicted by approximately 30%. This was partly as a result of the way in which the silage was fed, with access to the silage granted for only a short time each day, and partly because of the poor quality of the silage. However, dairy cows are capable of eating more than 2.7 kg of silage DM in 3 hours, and had they needed to, it is likely that they may have eaten more than this in the access time they were given. There is conflicting evidence to the extent that the feeding system may have restricted them in comparison with control animals since milk production between groups was the same, but blood ß-hydroxybutyrate levels were considerably higher at the end of the dry period (see later) in comparison to the control animals, indicating a greater degree of fat mobilization (Kunz, Blum, Hart, Bickel and Landis, 1985).

Dairy cows frequently eat more than is necessary during the dry period if given the option of *ad libitum* intake, with the result of being over-fat at calving and more prone to calving, metabolic and reproductive problems (Fronk *et al.*, 1980; Johnson and Otterby, 1981). There is little evidence of the control animals eating more than necessary in this study, in terms of differences in condition score between the two groups, probably because of the low dry matter content of the silage. Similarly, although the DM intakes of the supplemented animals were lower than predicted to meet their ME requirements, they calved at a similar CS to the control animals, suggesting that the ME intakes achieved were similar for both groups. The condition scores of both dry period groups dropped by one point over the dry period, rather a large amount for a dairy cow in the last 8 weeks of pregnancy. The prairie meal fed to the supplemented animals may have affected their dry matter intakes, although considering the very poor quality of the silage fed, more time would have been needed to allow greater silage consumption. The metabolisable protein content of the silage was low due to its high protein degradability and low efficiency of

nitrogen capture, and therefore, even though the supplemented dry cow diet had a lower concentration of crude protein, the metabolisable protein supply to the animals was higher. It is possible that in general dry cows increase their intakes of silage to increase crude protein intake in order to maintain foetal growth (see Oldham and Emmans, 1988) without resorting to the mobilisation of body protein, the by-product being an increase in the fat reserves of the animal as an excess of energy intake is stored. Kyriazakis and Oldham (1993) showed that growing lambs were able to select diets to meet their CP requirements and perhaps avoid excess CP intake. Cooper, Kyriazakis, Anderson and Oldham (1993) found that pregnant ewes given a free choice of two foods selected a food with a high CP content when that food also had a high ME density, but not when the food had a low ME density. This was presumably because the animal's requirements for protein and energy are closely linked with the relative benefits and costs associated with the utilisation of nutrients from the diet or body stores (Riis, 1988; Tesseraud *et al.*, 1991). In this study, much of the animals' CP requirements could have been met by the prairie meal supplement, and silage intake therefore may have been reduced to reduce the intake of excess RDP. This area of possible diet selection deserves further research consideration.

The lower-than-predicted intakes of straw by the supplemented animals were not wholly unexpected; that such high levels were predicted was due to a shortcoming of the formulation software which formulates on ME requirements and does not take factors such as palatability into account. Straw was provided both as a low quality bulk fodder, and as something to keep the cows occupied when they were not able to access the silage. Ruminants forage almost constantly and it was therefore considered important to provide the supplemented cows with something to chew at all times.

Although the silage intake of the control group could not be measured, it is possible to make a very rough estimate of this in view of the similar changes in condition score and liveweight to the supplemented group, assuming a similar ME intake. The mean daily MEI of the supplemented group was estimated to be 46.2 MJ/d - less than maintenance requirements, and hence the large drop in live weights over the dry period. This may also be a slight underestimate due to changes in the dry matter content of the refusals; however, even if refusals are assumed to have been nil, then the increases in DMI and MEI would only have been 0.120 kg DM/d and 1.4 MJ ME/d respectively. If the control group managed a similar MEI as the supplemented group, then the dry matter intake would have

been approximately 4.4 kg/d, or approximately 30 kg silage fresh weight per day. This would have provided approximately 0.890 kg CP/d, with 0.757 kg RDP/d and 0.133 kg UDP/d. If this were the case, then the mean daily CPIs of the two groups would have been very similar, but the quality of the dietary protein was rather different, and the supplemented animals would have received about 150 g UDP/d more than the control animals. The extra RDP received by the control animals would have been lost as ammonia and hence urea, some of which would have been recycled in saliva, but much of which would have been excreted; this is reflected in the slightly higher blood urea concentrations of these animals when dry.

During the lactation following the dry period treatments, animals had equal access to the same winter diet of the silage/Supergrains mix, barley/soya mix and in-parlour concentrates and the summer diet of grass and more a limited in-parlour concentrate ration. On this diet, the mean milk yields obtained from the two groups of animals up to week 31 of lactation were very similar. The mean milk composition, however, was very different, with milk protein concentration in particular being increased by some 2-3 g/kg from animals which were fed the supplemented dry cow diet. The mean milk protein concentrations from the control group were rather lower than expected for the first 25 weeks or so of lactation, whereas those of the supplemented group were rather higher. It is, however, difficult to determine which group's data were closer to 'normality', or whether 'normality' is somewhere between the two. In the experiments described in Chapters 3 and 4, milk protein concentrations were higher, although these were from animals at a later stage of lactation and on diets which were specifically designed to influence the concentration of milk protein. Fat and lactose concentrations were also increased in milk produced by the same animals; the high variability associated with milk fat concentrations (as seen in Figure 5.6) led to a high SED and hence a relatively low level of significance (P=0.083) despite an increase of approximately the same magnitude as that seen for milk protein. The situation was the opposite for lactose concentrations (i.e. low variability and hence high degree of significance with small differences in mean values), which is to be expected considering lactose's osmotic role in milk.

Since milk yields were the same for both groups, the increases in constituent concentrations were brought about by increased levels of production of those constituents. In order to be able to produce more milk solids, there must have been either an increase

in the supplies of precursors to the mammary tissue (i.e. increased blood concentrations, increased mammary blood flow, or increased efficiency of extraction, or a combination of all three), with two potential sources - the diet, or the animals' body tissues - or an increase in the efficiency of utilisation of those nutrients within the mammary tissue. Although the lactating animals were given equal access to the same diet, the supplemented animals may have eaten more of the silage/Supergrains mix or grass; an increase in metabolisable energy intake tends to increase the concentration of milk protein. It is not known what the mean feed intakes were for the two groups during lactation but there are, however, certain factors which indicate that the supplemented animals would not have eaten significantly more than the control animals. Thinner animals tend to eat more than fatter ones, although the increase is small, and is generally only apparent when the condition scores differ by more than 1-2 points (Garnsworthy, 1988). In this study, the condition scores were not significantly different and are unlikely to have caused the differences seen. In fact the mean CS of the supplemented animals was slightly higher than that of the control group. Any increase in forage intake, thereby increasing the forage:concentrate (F:C) ratio, would also have tended to reduce milk protein concentration (Section 3.2.3). In this study milk protein concentration was increased, but so was milk fat concentration, which tends to increase with an increase in forage intake (Thomas, C, 1984; Sutton and Morant, 1989). Therefore, if the an increase in the F:C ratio was the cause of the increased fat concentrations, some other factor (e.g. the increase in energy intake) must have counteracted the tendency to reduce the milk protein concentration. Alternatively, an increase in the fat concentration may have resulted in an increase in the efficiency of utilisation of energy yielding nutrients reaching the mammary tissue and a change in the partitioning of these nutrients (i.e. glucose, NEFA, acetate) within the mammary gland.

Blood albumin levels provide further evidence that the milk protein levels of supplemented cows were aided by the use of protein reserves. Blood albumin levels have been shown to correlate very well with body nitrogen stores in sheep (Sykes, 1976). Van Saun *et al* (1993) observed increases in both milk protein and fat concentrations over the first 50 days of lactation, although, similar to this study, only the response of the former was significant. However, part of the effect was caused by a (small) decrease in milk yield, with relatively small increases in daily milk protein and fat yields. Van Saun *et al* (1993) suggest that increases in the milk protein concentration were brought about due to the

ability of the animals fed higher levels of undegradable intake protein during the last 3 weeks of pregnancy to utilize body protein reserves, which were higher owing to decreased utilisation to support foetal growth. The results of the present study support this hypothesis, and differences between the two studies may be in part due to the maturity of the animals used - heifers by Van Saun *et al* (1993) and multiparous cows in this study.

Blood ß-hydroxybutyrate levels provide an index of the level of gluconeogenesis from fat and was found to be a sensitive indication of energy intake relative to requirements in pregnant, non-lactating cattle (Russell and Wright, 1983). Similarly, NEFA concentrations provide an index of the extent of fat reserve mobilisation (Russell and Wright, 1983) when energy intake is limited. Blood glucose concentrations were lowest around parturition, with the lowest glucose levels at this time being associated with highest BOHB levels in the supplemented animals before calving. Elevated BOHB levels shortly before calving for the supplemented animals indicate a shortage of normal gluconeogenic precursors (i.e. propionate) at this time, and may point to a difference in the utilisation of fat reserves between the two groups. Miller *et al.* (1991a) found that the blood concentrations of NEFA and BOHB were the factors which most influenced the availability of these precursors of milk fat synthesis - although the blood concentrations of these were not significantly different between the two dry period groups in the present study. Therefore, either the slight increase in the blood concentrations of these in the supplemented animals had a biologically significant effect on increasing the concentration of fat in milk, or some other unknown factor was involved - perhaps increased mammary blood flow. The milk lactose concentration of the supplemented animals was significantly higher than that of the control animals. This indicates that there was a more than adequate supply of glucose to the mammary tissue (Kuhn *et al.*, 1980), some of which may therefore have been available as an energy source for fatty acid synthesis from acetate and BOHB (Figure 4.1). The evidence from the results of this trial is not clear, however, and further study would be useful. It is a little strange that the concentrations of all three major constituents increased in the milk of the supplemented animals, without an increase in milk yield. One would expect an increase in milk yield if the precursors of the milk constituents are available, as was obviously the case in this study, and yet milk yields were not affected. In Chapter 4, the concentrations of K^+, Na^+, and Cl^- balanced the concentration of lactose, and it is assumed that this is what happened in the milk of the cows of the present study. The milk concentration of K^+ in particular probably decreased to allow the increase in the

concentration of lactose; why this should have occurred is unclear.

By pairing animals on calving date, differences in the nutrition of animals throughout the experiment were taken into account in the analysis of the data. The effect of calving date had a similar role in removing some of the variation between pairs at different stages of lactation - the effect of turnout for example. Animals calving in January, for instance, were seen to have a much lower mean milk yield than those calving in December, March or April. Such seasonal variation is well known (Wood, 1976; Ng-Kwai-Hang, Hayes, Moxley and Monardes, 1985), and was probably due to the variation in feed quality at different times during the experiment.

Health problems associated with the two dry cow diets are difficult to assess with such small numbers of animals. The most obvious problem was that of interdigital dermatitis, causing lameness, of which there was a very great incidence in the Auchincruive herd as a whole. The supplemented animals perhaps had a lower incidence of this than the control group during lactation, and the reason for this could have been their being housed away from the other animals during the dry period.

5.7 CONCLUSIONS

It is difficult to determine the exact cause of the increases in milk solids concentrations, particularly that of milk protein, since the intakes of the two groups of animals during lactation are unknown. Several factors lead to the conclusion that intakes did not differ between the two groups. It is clear is that a small change in the management of the animals during the dry period had a profound beneficial effect on the composition of the milk from those animals during the subsequent lactation, the basis of which was probably the maintenance of labile protein reserves during the last stage of pregnancy (as indicated by blood albumin concentrations), making them available for use later during lactation. Further investigation would clearly be very interesting, with particular attention being paid to feed intakes throughout the gestation/lactation cycle, more detailed differences in milk composition, and perhaps nitrogen balance studies.

Chapter 6

Effects of Increased Dietary Undegradable Protein During the Dry Period: Milk Yield and Composition During the Subsequent Lactation and Response to a Challenge by a 'Milk Protein Enhancing' Lactation Diet

6.1 SUMMARY

In the previous chapter, an experiment was described in which dairy cows were fed a small amount of a high protein supplement during the dry period. Milk yields were unaffected by prepartum diet, but the concentrations of milk protein and of fat and lactose were increased as the daily production of these constituents was increased. The major effect of feeding a protein supplement during the dry period was probably to conserve or replenish labile protein reserves. In order to answer some of the questions which were left unanswered by that experiment, a similar experiment was carried out with three periods of intensive study, one during the dry period, and one each during early- and mid-lactation. Thirty-six multiparous dairy cows were paired on condition score, predicted calving date and genetic index for milk protein concentration shortly before drying off. One animal of each pair was fed a control diet of a mix of grass silage and a distillery by-product fed at *ad libitum* quantities. The other animal of each pair was fed a restricted quantity of silage, had *ad libitum* access to barley straw, and was supplemented with 0.5 kg/d prairie meal. During the subsequent lactation, all animals had access to the same lactation diet, based on grass silage and concentrates fed at a flat rate. The effect of supplementing the animals during the dry period was to raise mean milk yields (32.7 vs 36.3 kg milk/d, SED 0.62; control vs supplemented), and milk protein concentration (30.7 vs 31.5 g milk protein/kg, SED 0.28), without apparent differences in dry matter intakes. In order to study the interaction of dry period treatment and lactation diet, during mid-lactation, 12 animals (6 from each dry period treatment) were fed one of two contrasting experimental lactation diets, one designed to enhance milk protein concentration (high protein, low fat; HPLF) and the other to depress milk protein concentration (low protein, high fat; LPHF). Milk yields of the animals on these two diets were similar, with milk protein concentration being increased by approximately 2 g/kg by the 'enhancing' diet compared to the 'depressing' diet, with control animals producing milk with a higher milk protein concentration than the supplemented animals (33.1, 31.2, 31.4 and 29.5 g milk protein/kg, SED 1.50; control HPLF, LPHF and supplemented HPLF, LPHF). Increases in milk protein reflected increases in casein concentration.

6.2 INTRODUCTION

The experiment described in Chapter 5 showed that relatively large effects on lactation performance - in terms of milk solids production - could be brought about by simple changes in the management of the dairy cows during the dry period, in particular that of feeding a small amount of a high quality protein supplement. The previous experiment was, however, designed as a initial study with a relatively small number of animals and few observations. The effects on milk protein concentration were very clear, although the causes were not.

One of the unknown factors from the initial study was the feed intake of the control animals during the dry period. It is not clear if the feed intakes of the animals were affected by dry period treatment, although if they were the effect was not great. A further question is how would the animals react in terms of milk production (volume and solids) if challenged by a diet that was designed to either increase or decrease the concentration of milk protein when the 'plane' of protein production had already been determined by the animal's nutrition during the dry period. An understanding of this interaction may elucidate further the way in which the dry period treatments effect milk production.

It was decided to repeat the experiment with a greater number of animals and with more detailed observations in order to answer some of the questions raised by the previous experiment; most of the introductory material for this chapter was presented in Sections 3.2, 4.2 and 5.2, and is not repeated here.

6.3 EXPERIMENTAL AIMS

The aim of this experiment was to investigate the effect of restricting energy intake to predicted current AFRC requirements and increasing undegradable protein intake of dairy cows during the dry period on performance during the subsequent lactation. Animals were paired according to condition score, predicted calving date and genetic potential for milk protein production and were randomly assigned to one of two dry period treatments. Individual feed intake measurements were made on a subsample of animals during the dry period. After calving, all animals were given equal access to the same diet. Detailed feed intake and lactation performance studies were carried out on a subsample of animals during early lactation. Metabolic profile analyses were performed on all the animals twice during the dry period and twice during lactation. During mid-lactation, a subsample of the animals were used to investigate the interaction of short term lactation diet on the long term dry period treatment by feeding two lactation diets designed to increase and decrease the concentration of protein in milk in a changeover experiment.

6.4 MATERIALS AND METHODS

6.4.1 Animals and Their Management

Thirty-six pregnant multiparous Holstein-Friesian cows were drawn from the SAC Auchincruive herd for use in the experiment. Animals were dried off approximately 56 days prior to predicted calving date. During the dry period, animals were housed in the main Auchincruive dairy cubicle unit in two separate groups gated off from lactating animals. When the first animals of each group were dried off, each was given a non-experimental dry cow for company that was treated in the same way as an experimental animal. All animals had access to fresh water and an individual cubicle bedded with sawdust.

During the dry period, 6 pairs of animals with closely coinciding predicted calving dates were chosen for intensive feed intake study (Figure 6.1). However, one of these animal aborted after 3 days on the experimental diet, and her pair injured a leg. Consequently, 10 animals were housed in a metabolism unit for a period of 2 weeks in individual stalls fitted with de Boer yokes, with stall length adjusted to each animal. Each animal had constant access to fresh water. The housing of the 10 animals was staggered over 3 weeks in two groups of 4 and 6 animals respectively; the 4 animals with the earliest calving dates were housed a week before the remaining 6 so that all animals were studied approximately during the middle of their dry periods.

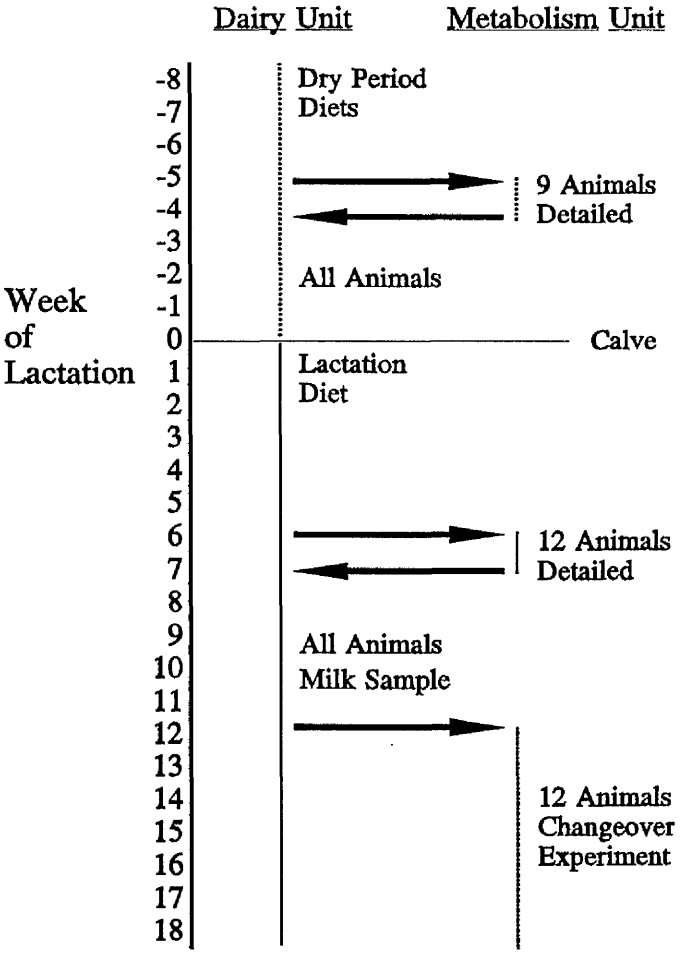


Figure 6.1 Schematic diagram of timing of parts of the experiment in relation to the stage of lactation. Actual timings for individual animals in detailed studies depended on calving date.

Immediately prior to calving animals were withdrawn from the dry period treatment at the herdman's discretion and without reference to treatment. They were moved to a calving pen with straw bedding to calve, and when recovered were housed in the main Auchincruive dairy unit together with other, non-experimental, animals. The lactating cows were housed in a cubicle unit during the winter and on pasture during the summer. They were milked using a 20/20 Herringbone milking parlour twice daily, at 0500-0800 h and 1430-1700 h. Whilst housed, the most recently calved animals were milked first in the morning and last in the afternoon to achieve the most even milking interval.

During early lactation (weeks 5-12), the same 10 animals as previously used together with two additional cows were housed in the same metabolism unit for 2 weeks for further intensive study work. They were milked *in situ* using a vacuum line and bucket units starting at approximately 0530 h and 1530 h. Milk yields were recorded at each milking by weighing.

Four weeks after the end of the second metabolism unit study period, at about weeks 11-18 of lactation ("mid-lactation"), the same 12 animals were again housed in the metabolism unit for more detailed work. They were milked *in situ* using a milk line and clusters starting at approximately 0630 h and 1530 h. Milk yields were recorded at each milking by volume using milk meters.

6.4.2 Experimental Design

The main part of the experiment was a continuous group feeding design, with animals paired shortly before the end of lactation according to their most recent condition score (CS), predicted calving date, and where available, their genetic potential for milk protein concentration (ICC83 for P%, Scottish Milk Marketing Board, Underwood Road, Paisley). Animals for which no index had been calculated were paired together on condition score and predicted calving date alone. Dry period treatments within each pair of animals were allocated randomly. Each of the first two periods in the metabolism unit consisted of 2 weeks only since it was considered that animals needed little adjustment to the diet because the diets were the same whether fed in the metabolism unit or in the main cubicle unit. Minimal time was needed for animals to adjust to the metabolism stalls since some of the animals had previously been housed in the metabolism unit.

The second part of the experiment was an investigation of two contrasting lactation diets fed in a 2x2 complete changeover design based on six 2x2 Latin Squares. This part of the experiment was a split plot design with 3 2x2 complete Latin Squares in each plot, each plot consisting of animals from one of the two dry period groups. The mean milk yields of each animal recorded during the period in the metabolism unit during early lactation were used to allocate animals to Latin Squares, with the 2 lowest yielding animals from each dry period group being assigned to square 1, the next 2 to square 2 and the 2 top yielders to square 3 of each plot. Dietary treatments were allocated at random within each square. Each experimental period of 3 weeks consisted of 2 weeks adjustment and 1 week collection periods.

The statistical analysis of the data collected was achieved using Genstat 5 (Lawes Agricultural Trust, 1990) and Minitab (Minitab Inc., State College, Pennsylvania, USA). For data that was balanced (metabolic profile data, and data from the three periods in the metabolism unit), analysis of variance was employed. However, where the data was inherently unbalanced (lactation performance data), it was analyzed using residual maximum likelihood (REML; Patterson and Thompson, 1978). For condition score data, Mann-Whitney signed rank tests were used.

The model used to analyze metabolic profile data collected from dry animals in the metabolism unit had a treatment structure of Treatment and a blocking structure of Pair/Cow/Sample. Purine derivative excretion data collected at this time used a treatment structure of Treatment*Day*Time and a blocking structure of Pair/Cow.

The model used to analyze milk and feed data collected from the animals in the metabolism unit during early lactation had a treatment structure of Treatment and a blocking structure of Pair/Cow. Metabolic profile and purine derivative excretion data were analyzed using the models described for the dry cows.

The model used to analyze milk, feed and metabolic profile data collected from the animals on the change-over experiment had a treatment structure of Treatment*Diet, where treatment, as above, refers to the dry period treatment, and a blocking structure of Pair/Cow/Period/Square. The treatment structure for the purine derivative data was Treatment*Diet*Day*Time, with the blocking structure Pair/Cow/Period/Square.

To analyze the lactation data collected from all animals in the dairy unit, the same model was used as previously (Chapter 5). This had a fixed component model of Calve+Sample*Treatment, where Calve was month of calving and Sample is synonymous with week of lactation. The random component model was Pair. The metabolic profile data collected from all animals was analyzed by analysis of variance with a treatment structure of Treatment*(State/Sample), where State refers to either dry or lactating, and Sample was one of the two samples taken at each State. The blocking structure was Pair/Cow.

For all statistical analyses where data were missing, because either an animal was ill or an animal was on a different part of the experiment, missing value codes were used in their place.

6.4.3 Diet Formulation and Production

6.4.3.1 Dry Period Diets

Two diets were fed to the dry cows: control and supplemented. The control diet consisted of *ad libitum* access to a first cut grass-silage mixed with either Supergrains (a wheat-based distillery by-product; Borthwick, Glasgow, UK) or pressed sugar beet pulp in the ratio of 3:1, silage:Supergrains/sugar beet pulp ("silage mix").

The experimental (supplemented) dry cow diet was formulated based on the previous year's experimental dry cow diet using SAC advisory software based on the findings of TCORN (1992; NW Offer, personal communication). Using the results of pre-trial analyses of the silage and literature values of Supergrains composition (Borthwick, Glasgow, UK) (Table 6.1), the diet was formulated to provide predicted metabolisable energy (ME) and rumen degradable protein (RDP) requirements for a 600 kg animal in the 8th month of pregnancy with no weight gain.

Table 6.1 Pre-trial analysis of silage (mean of 4 core samples) and literature analysis of Supergrains (Borthwick, Glasgow, UK) for dry cow diet formulation.

	First Cut Grass-Silage	Supergrains
Dry Matter, g/kg	243	260
Organic Matter, g/kg DM	933	985
Crude Protein, g/kg DM	191	320
Metabolisable Energy, MJ/kg DM	11.4	14.0
Rumen Degradable Protein, g/kg DM	163	-
Undegradable Protein, g/kg DM	28	-
In vitro Organic Matter Digestibility, g/kg OM	770	-
D-Value, g/kg DM	708	-
NH_4-N, g/kg Total N	116	-
pH	3.8	-
Calcium, g/kg DM	5.7	0.8
Phosphorus, g/kg DM	3.6	3.0
Magnesium, g/kg DM	2.6	-
Sodium, g/kg DM	-	0.1

The predicted daily *ad libitum* silage mix intake of the control dry cow group was 7.1 kg DM/d, according to predicted ME requirements. The supplemented dry cow diet was formulated using a restricted silage mix with a target intake of 4.1 kg DM/d, with 0.5 kg/d prairie meal and a predicted intake of 5.8 kg DM/d barley straw. Following the initial study's results (see Chapter 5), the predicted straw intake was considered to be rather high, but this was accepted as part of the treatment and no attempt was made to formulate a diet with a lower predicted straw intake.

Both groups of dry animals had constant access to a low calcium salt lick (Frank Wright Ltd, Ashbourne, Derbyshire, UK; declared content of 360 g Na/kg, 13 g Ca/kg, 2.3 g P/kg, 3.0 g Mg/kg) whilst in the dairy cubicle unit.

6.4.3.2 Lactation Diets

During lactation, all animals (apart from those 12 on the changeover experiment) were given access to the same diet together with non-experimental animals. During the winter this consisted of a silage-based mix supplemented with concentrates. The silage mix

consisted of the same silage mix of Supergrains or pressed beet pulp as fed to the dry animals. The morning feed also contained a mix of ground barley and white fish meal (2.7 barley:0.3 fish meal) mixed with the silage to provide approximately 3.0 kg/d per animal. A standard lactation concentrate was fed at the rate of 3.2 kg/d. In addition to this, a beet-blend concentrate was fed on top of the morning silage to the formulated quantity of 3.0 kg per head. Turnout occurred on the 30th April, 1993, and after a short period during which animals were out at pasture during the day and inside at night with *ad libitum* access to silage, they were fed the summer diet of grass pasture with a small amount of concentrate supplement. Animals yielding above 30 kg/d received 3.2 kg of concentrate fed in-parlour, animals yielding below 30 kg/d received a token 0.8 kg/d.

6.4.3.3 Mid-Lactation Changeover Diets

For the mid-lactation changeover experiment, two experimental lactation diets were formulated according to the experience gained from the experiments described in Chapters 2 and 3. One was formulated to increase milk protein concentration and the other was formulated to reduce milk protein concentration. Two experimental lactation concentrates were formulated and produced by Dalgety Agriculture Ltd (Aztec West, Bristol, UK) to guideline specifications. The two concentrates were formulated to provide similar (high) levels of ME, although the quality of the ME was formulated to be different for each. The 'milk protein enhancing' concentrate (designated HPLF; high protein, low fat) was formulated to contain a high fermentable ME (FME) content, with high levels of starch and sugars and low levels of fat. The crude protein content of this concentrate was formulated to be relatively high. In contrast, the 'milk protein depressing' concentrate (designated LPHF; low protein, high fat) was formulated to contain lower levels of FME, with a high fat, low starch/sugar content, and a lower crude protein content. The predicted effective rumen degradable protein (ERDP):FME ratio of the two concentrates was formulated to be the same, at about 11.6 g/MJ. The composition of the two concentrates is presented in Table 6.2. The diets were designed to be closer to a practicable dairy cow diet than previous experimental diets (described in Chapters 3 and 4), and animals were given *ad libitum* access to a good quality second cut grass-silage with a daily allowance of 8 kg concentrate supplement.

The concentrates were produced as a single batch shortly before the start of the changeover experiment, and were stored under cover until used.

Table 6.2 Composition of the two contrasting experimental lactation concentrates.

	Concentrate	
Raw Material (%)	HPLF	LPHF
Wheat	27.8	-
Wheat Feed	-	4.6
Maize Gluten	10.0	7.6
Maize Germ	-	4.8
Molassed Sugar Beet Pulp	17.1	27.3
Extruded 00-Rapeseed Meal	1.3	13.5
Peas	18.1	-
Sunflower Seed Meal	-	12.0
High Protein Soya Bean Meal	19.2	-
Palm Kernel Expeller	-	10.0
Molaferm	4.0	12.0
Palm Acid Oil	0.5	4.0
Tallow/Soya Fat	-	1.0
Minerals and Vitamins	2.0	3.2

6.4.4 Animal Feeding

6.4.4.1 Dry Period Feeding

In the main dairy cubicle building, the animals were fed at a barrier using a mixer/feeder wagon during the dry period and lactation. The silage mix was fed to the dry cows at approximate 1600 h. The control animals received a sufficient quantity each day to allow *ad libitum* intakes. The supplemented animals received a weighed quantity of approximately 4.1 kg DM per head (based on the pre-trial analysis) spread as evenly as possible along the barrier. The amount of silage offered to each group was recorded as it was fed. Refusals from the control group were collected weekly.

Supplemented animals had constant access to barley straw, fed from a specially constructed box to minimize wastage. Prairie meal was fed in 0.5 kg rations to each of the supplemented animals on top of the fresh silage as each animal arrived at the feed barrier. Animals were encouraged to consume their ration quickly, which reduced stealing by other cows.

In the metabolism unit, the 10 dry animals were fed individually with the same silage mix fed in the dairy cubicle unit. This was done at the same time as for the main groups of animals, at about 1600 h. The control animals received approximately 10% more than required for *ad libitum* intake. Supplemented animals received 4.1 kg DM silage mix per day, with their 0.5 kg allowance of prairie meal fed on top. These animals also had *ad libitum* access to barley straw which had been re-baled into small bales, and was fed from specially constructed wooden boxes to reduce wastage. During the 14 days in the metabolism unit, animals were not given access to the mineral blocks.

6.4.4.2 Lactation Feeding

During lactation, the animals were fed by Auchincruive staff twice daily at approximately 0800 h and 1600 h. The parlour concentrate was fed automatically in-parlour in 2 equal portions of 1.6 kg per milking.

The early lactation metabolism unit study occurred when all animals were housed inside and the cows were fed the same silage mixes as fed to the main herd at the same times. Bins of silage mix were filled and fed twice daily with sufficient quantities for *ad libitum* intake. Refusals were removed before each new bin was offered; in this respect the feeding differed slightly to that of the main herd, where new silage was fed on top of old. The beet blend concentrate ration was fed on top of the morning silage, and the parlour concentrate rations were fed at each milking.

6.4.4.3 Mid-Lactation Changeover Feeding

During mid-lactation the same 12 animals housed in the metabolism unit during early lactation were again housed in the metabolism unit and fed two different experimental lactation concentrates. Silage was fed individually in bins filled from a feeder wagon daily at approximately 0830 h. The previous day's refusals were cleared away before offering new silage at about 10% above *ad libitum* requirements. If an animal consumed her full ration of silage one day, extra silage was provided the following day. Concentrates were fed in equal portions of 4 kg at each milking.

6.4.5 Sample Collection and Analysis

Blood samples were taken from the coccygeal vessels by venepuncture from all experimental animals at four times during the main experiment for metabolic profile

analysis (Payne, Dew, Manston and Faulks, 1970). These were analyzed for protein, albumin, urea, glucose, ß-hydroxybutyrate (BOHB), non-esterified fatty acids (NEFA), magnesium and phosphorus by the Dairy Herd Health and Productivity Service (The Royal (Dick) School of Veterinary Studies, Veterinary Field Station, Easter Bush, Roslin, Midlothian, UK). Target sampling dates were 7 days after drying off, 10 days before predicted calving date, 42 days after calving and 60 days after calving. In practice the samples were collected on a Monday to Thursday nearest the target date to allow analysis the following day. In addition to these, samples were collected from animals in the metabolism unit at the start and end of each collection week. Samples were taken starting at approximately 0845 h with animals standing in a cubicle or stall, with as little stress and excitement to the animals as possible. Blood was collected into Vacutainer tubes (Becton, Dickinson and Co., Rutherford, New Jersey, USA), one each containing lithium heparin and potassium oxalate/sodium fluoride. If next day analysis was not possible, the blood was immediately centrifuged at 4°C to 20-30 minutes at approximately 1,700x*g*. Plasma was decanted into fresh tubes, frozen and stored at -20°C until it could be sent for analysis.

From the start of lactation of the first calved experimental animal, milk samples were collected weekly at 2 consecutive milkings, pm and am, until the 1st June, 1993. Milk samples were preserved with a Lactab milk preservative tablet (Thompson and Capper Ltd, Runcorn, Cheshire, UK) and stored at 4°C until analyzed. They were analyzed by the Dairy Technology Department at Auchincruive using a Milko-Scan 203 analyzer for protein, fat and lactose concentrations. Milk constituent yields were calculated from the concentrations weighted by the milk yield at the time of collection multiplied by the total daily milk yield.

Milk samples were collected from lactating animals housed in the metabolism unit over 4 consecutive milkings at the end of each experimental collection period. Milk samples were collected, preserved and analyzed for protein, fat and lactose as described above. Additional unpreserved milk samples were collected and stored at 4°C until bulked according to yield at the time of collection. Sub-samples of these bulked milk samples were frozen and stored at -20°C until later analysis of milk minerals (Ca, K, Na, Cl, Mg and P) by the Analytical Services Unit at Auchincruive. Fresh samples were analyzed immediately by the Dairy Technology Department at Auchincruive for crude protein,

casein, non-protein nitrogen, urea and organic acids. The milk from the early lactation metabolism unit animals was also analyzed for whey protein fractions (α-lactalbumin, ß-lactoglobulin, bovine serum albumin and immunoglobulins) using the procedure set out in International Dairy Federation (IDF) standard 162:1992. At the time of measurement, the technique was still under development and standards for the whey protein fractions were not available. Therefore, absolute concentrations could not be calculated, but peak heights allowed comparisons to be made in relative terms.

During the last 7 days of each experimental collection period in the metabolism unit the intakes of silage fed on an *ad libitum* basis were calculating by recording the quantities offered and refused daily. Straw intakes of the supplemented dry cows were measured over the whole 7 day period. Samples of the different silages and mixes offered were collected and bulked over the 7 day periods. To allow metabolisable energy prediction of the silage mixes, samples of the different constituents were collected for separate analyses to prevent the high oil and fat contents of some of the constituents interfering with the *in vitro* technique used for forage analysis. Samples of the straw and concentrates fed were collected and bulked over the 7 day collection periods. Feed samples were frozen and stored at -20°C for later analysis.

Spot urine samples were collected from the dry and lactating animals housed in the metabolism unit starting at 1030 h and 1430 h over 2 consecutive days at the end of each experimental collection period. Subsamples of urine collected from dry and early lactation animals were immediately diluted 1 in 20 (75 μl urine in 1.5 ml) with 0.1 M ammonium dihydrogen orthophosphate solution containing 0.1 M allopurinol as an internal standard. This was done directly into autosampler vials that were sealed with a crimped cap. These samples were either analyzed immediately using a high-performance liquid-chromatography (HPLC) technique for measuring the urinary purine derivatives (PD) or were frozen upright and stored at -20°C until analyzed.

Condition scores of all animals were assessed shortly after being dried off and shortly after calving. Animals that were housed in the metabolism unit for the mid-lactation changeover experiment were scored for condition at the start and end of each experimental period.

6.5 RESULTS

6.5.1 Health

During the course of the experiment, several animals became ill. Two animals failed to successfully complete the dry period treatments: one aborted and led to the use of only 10 cows for the dry period metabolism unit intake study, another calved 6 weeks earlier than expected due to an inaccurate pregnancy diagnosis. All other animals completed the dry period treatments without serious problems. One of the 10 animals that were housed in the metabolism unit during the dry period was too large and was removed. She was transferred to a straw box where she continued to receive silage and a daily ration of prairie meal. During the mid-lactation changeover experiment, one animal developed a solar ulcer during the second experimental period and was moved to a straw bedded pen to recover. For statistical analyses, missing value codes were used in place of data that were not available for these two animals. Also during the mid-lactation changeover experiment, 2 animals developed mild mastitis but were treated and recovered. One of the animals showed the first signs of mastitis at the very end of the experiment, and the milk collected for mineral, nitrogen fraction and organic acid determination for this animal was discarded for the last two milk samples - the bulked sample for this animal was therefore composed of only the first two samples' milk.

6.5.2 The Dry Period

6.5.2.1 Feed Intakes

The analyses of the silage mix, straw and prairie meal fed to the cows during the dry period are given in Table 6.3. On a group fed basis, the mean daily intake of silage mix was 10.0 kg DM/d. The mean of individually measured daily silage intakes was 10.1 kg DM/d. The supplemented group mean daily intake of silage mix was 5.0 kg DM/d compared to the target figure of 4.1 kg/d - the discrepancy being due to the method by which the silage intakes were controlled and differences in the DM contents of pretrial silage samples and silage actually fed. The group mean daily straw intake was 2.2 kg DM/d compared to the mean of individually measured daily straw intakes of 3.0 kg DM/d. This, together with the 0.5 kg/d ration of prairie meal, gave total mean DM intakes (DMI) for the supplemented animals of 7.6 kg DM/d and 8.0 kg DM/d for group fed and individually measured intakes respectively.

Table 6.3 Composition of the silage mix fed to all dry cows (mean of 3 samples) and the prairie meal (mean of 3 samples) and straw to dry cows on the protein supplemented diet.

	Silage mix	Prairie Meal	Barley Straw
Dry Matter, g/kg	252	880	819
Organic Matter, g/kg DM	928	988	963
Crude Protein, g/kg DM	178	705	44
Metabolisable Energy, MJ/kg DM	11.3	14.2	6.1
Neutral Detergent Fibre, g/kg DM	489	41.0	823
Acid Detergent Fibre, g/kg DM	289	32.0	508
Starch, g/kg DM	18.6	118	13.9
Water Soluble Carbohydrates, g/kg DM	10.1	1.4	0.1
Acid Hydrolysis Ether Extract, g/kg DM	63.4	88.0	14.6
In vitro Organic Matter Digestibility, g/kg OM	761*	853	437
Potassium, g/kg DM	13.0	1.0	8.1
Calcium, g/kg DM	4.8	0.4	4.1
Phosphorus, g/kg DM	3.1	2.5	0.6
Magnesium, g/kg DM	2.1	0.6	0.6
Sodium, g/kg DM	3.5	2.2	2.1
pH†	4.2	-	-
D-Value†, g/kg DM	690	-	-

* Silage alone and silage mix differed by very little.
† Silage alone.

The mean daily intakes of crude protein and metabolisable energy for the group fed animals are presented in Table 6.4. From this it can be seen that the supplemented group, despite having a lower intake of DM and a diet of a lower crude protein content, consumed approximately 101 g UDP/d more than the control group - a potential benefit of more than 5.6 kg extra UDP over the course of the dry period. The supplemented animals also had a daily MEI of only two thirds of that of the control group.

Table 6.4 Mean diet densities and daily intakes of crude protein, rumen degradable protein (RDP) and undegradable protein (UDP), and metabolisable energy of group fed dry dairy cows.

	Dry Period Treatment	
	Control	Supplemented
Diet Crude Protein, g/kg DM	178	171
Diet Metabolisable Energy, MJ/kg DM	11.3	10.0
Dry Matter Intake, kg/d	10.0	7.6
Crude Protein Intake, kg/d	1.78	1.30
Rumen Degradable Protein*, kg/d	1.51	0.93
Undegradable Protein, kg/d	0.27	0.37
Metabolisable Energy Intake, MJ/d	113	76

* Assuming rumen protein degradabilities of 0.85, 0.50 and 0.40 g/g for silage mix, straw and prairie meal respectively (NW Offer, personal communication).

6.5.2.2 Metabolic Profiles

A summary of the metabolic profile data from the 9 healthy animals in the metabolism unit during the dry period is presented in Table 6.5. These data represent mean values for animals approximately in the middle of the dry period. The only effect of dry period treatment was a trend towards an increase in blood protein concentration brought about by an increase in the globulin concentration. Metabolic profile data for all animals shortly after being dried off and shortly before calving are presented in Table 6.9.

6.5.2.3 Purine Derivative Excretion

Urinary purine derivative excretion was used as an index of microbial protein yield from the rumen. There were no significant differences between the excretion of allantoin, uric acid, hypoxanthine or xanthine, nor, therefore, of allantoin plus uric acid or all PDs, when expressed as a ratio to urinary creatinine. Mean allantoin/creatinine (A/C) ratios were 1.04 and 0.99 (SED 0.068), and mean uric acid/creatinine (U/C) ratios were 0.12 and 0.10 (SED 0.016) for control and supplemented treatments respectively. The effect of interaction of time of sampling and treatment was significant for U/C ($P<0.01$), with lower ratios in the morning than in the afternoon for the supplemented animals but not for controls (mean values, am vs pm, of 0.12 vs 0.12 and 0.08 vs 0.12 (s.e.d 0.017) for control and supplemented animals respectively). The interaction of time and treatment was not significant for A/C, but it was for AU/C ($P<0.05$) due to the influence of uric acid.

Table 6.5 Summary of metabolic profile data of 9 dry animals housed in the Auchincruive metabolism unit at approximately the middle of the dry period (BOHB=ß-hydroxybutyrate; NEFA=non-esterified fatty acids).

	Dry Period Treatment			
	Control	Supplemented	SED	F Probability
Protein, g/l	79.3	83.3	1.48	0.074
Albumin, g/l	38.9	39.4	0.32	0.212
Globulin*, g/l	40.4	43.9	1.25	0.068
Urea, mM	1.87	2.26	0.172	0.108
Glucose, mM	4.69	4.31	0.290	0.286
BOHB, mM	0.67	0.59	0.092	0.495
NEFA, mM	0.24	0.27	0.049	0.638
Mg, mM	1.08	1.13	0.037	0.334
P, mM	1.88	1.77	0.101	0.389

* Globulin=Protein-Albumin.

6.5.2.4 Condition Scores

The condition scores of all animals recorded shortly after they were dried off and shortly after calving are presented in Table 6.6. It can be seen from this that at calving the mean CS of control animals was significantly higher ($P<0.05$) than that of the supplemented animals, although the numerical difference was small. Despite randomisation the control animals had a slightly higher mean condition score as they were dried off, and the dry period treatment caused them to lose slightly less condition over the dry period than the supplemented animals. The difference between the two groups in change over the dry period was not significantly different.

Table 6.6 Mean condition scores of dry period groups after being dried off and at calving, the change in CS between the two times, and the t probability as determined by Mann-Whitney signed rank test.

	Dry Period Treatment				
	Control		Supplemented		
	Mean	SE	Mean	SE	t Probability
At Drying Off	2.94	0.616	2.59	0.417	0.080
At Calving	2.86	0.509	2.47	0.386	0.027
Change	-0.08	0.462	-0.13	0.342	0.588

6.5.3 Main Lactation

6.5.3.1 Feed Intakes

The composition of the silage mixes and concentrates fed to lactating animals is given in Table 6.7. Feed intakes of animals during lactation are presented later

Table 6.7 Composition of the silage mixes and concentrates fed to lactating animals (PC=Parlour Concentrate; BB=Beet Blend). Note: the PM silage mix is the same as that presented in Table 6.3 and is given again here for comparative purposes.

	Silage mixes		Concentrates	
	AM	PM	PC	BB
Dry Matter, g/kg	312	252	859	874
Organic Matter, g/kg DM	911	928	899	864
Crude Protein, g/kg DM	161	178	204	207
Metabolisable Energy, MJ/kg DM	11.4	11.3	13.4	9.1
Neutral Detergent Fibre, g/kg DM	369	489	273	312
Acid Detergent Fibre, g/kg DM	196	289	162	223
Starch, g/kg DM	133	18.6	67.0	28.0
Water Soluble Carbohydrates, g/kg DM	20.8	19.0	82.0	79.4
Acid Hydrolysis Ether Extract, g/kg DM	46.8	63.4	99.8	73.6
In vitro Organic Matter Digestibility, g/kg OM	810	761	-	657
Potassium, g/kg DM	11.5	13.0	11.7	15.8
Calcium, g/kg DM	8.9	4.8	13.9	19.3
Phosphorus, g/kg DM	4.7	3.1	6.3	6.0
Magnesium, g/kg DM	2.4	2.1	4.2	4.2
Sodium, g/kg DM	4.0	3.5	5.0	8.5
pH*	4.2	4.2	-	-
D-Value*, g/kg DM	690	690	-	-

* Silage alone.

Table 6.8 Frequency of health problems treated during lactation (numbers excluding repeats by the same individual in parenthesis).

	Dry Period Treatment	
	Control	Supplemented
Retained Foetal Membrane	3	3
Milk Fever	2	1
Acidosis	1	0
Lameness	4 (2)	2
Mastitis	16 (3)	2 (1)

6.5.3.2 Health

Health problems encountered during lactation in the main dairy unit are tabulated in Table 6.8. Animals which developed mild cases of mastitis were not sampled until they had recovered. One of the control animals became seriously ill with acidosis, and subsequently failed to produce more than a few litres of milk each day. She was therefore discarded from the experiment, but was fortunately (from an experimental view) the pair of another discarded animal (early calver). The final statistical analysis of the lactation performance data was done using data from 32 animals, i.e. 16 complete pairs. Any carryover effects from the 2 lactation diets fed to the 12 animals housed in the metabolism unit were not considered since the sampling of all animals stopped when these were released back into the main herd.

6.5.3.3 Metabolic Profiles

A summary of the metabolic profile data collected from all animals is given in Table 6.9. The interaction between treatment and state was significant for blood urea ($P<0.05$) and blood NEFA ($P<0.001$) concentrations. Control animals had a higher mean blood urea concentration during the dry period than supplemented animals and a lower mean blood urea concentration during lactation. NEFA concentrations exhibited the opposite trends, with supplemented animals having a higher mean blood NEFA concentration during the dry period than control animals and a lower NEFA concentration during lactation.

Table 6.9 Summary of blood metabolic profile analyses of all experimental animals. Blood samples were taken from control and supplemented animals twice during the dry period and twice during lactation (BOHB=ß-hydroxybutyrate; NEFA=non-esterified fatty acids).

Treatment:	Control				Supplemented						
State:	Dry		Lactating		Dry		Lactating			F Probability	
Sample:	1	2	1	2	1	2	1	2	SED	Tr't	State
Protein, g/l	83.6	79.7	85.8	87.1	83.6	76.9	83.5	83.7	2.30	0.248	<.001
Albumin, g/l	38.1	38.3	38.3	38.8	38.8	38.1	38.3	38.9	0.53	0.591	0.316
Globulin*, g/l	45.5	41.4	47.5	48.3	44.8	38.8	45.2	44.8	2.04	0.173	<.001
Urea, mM	2.61	2.31	2.80	2.53	2.13	2.42	3.05	2.67	0.180	0.929	<.001
Glucose, mM	4.13	4.21	3.53	3.75	4.17	3.92	3.57	3.93	0.122	0.928	<.001
BOHB, mM	0.48	0.49	1.02	0.78	0.34	0.42	0.89	0.81	0.119	0.247	<.001
NEFA, mM	0.22	0.25	0.36	0.30	0.27	0.33	0.28	0.29	0.034	0.624	0.003
Mg, mM	1.04	1.00	1.10	1.10	1.04	0.94	1.08	1.09	0.037	0.371	<.001
P, mM	2.05	1.82	1.95	1.86	1.93	1.80	1.87	1.97	0.138	0.671	0.819

* Globulin=Protein-Albumin.

6.5.3.4 Milk Production

The results of lactation performance for the first 18 weeks of lactation are presented in Table 6.10. Milk yield was very significantly increased by the supplemented dry period treatment, whereas protein concentration was only slightly increased, although the difference was statistically significant. Mean milk fat concentrations are very high, but this is partly a result of the very high milk fat concentrations observed in early lactation. Model means for each of the two dry period treatments are presented in Figures 6.2-6.7. All the significant effects that were seen lasted until at least week 18 of lactation, when sampling was terminated.

The effect of stage of lactation (i.e. Sample) was highly significant ($P<0.001$) for all variables presented, and the interaction between stage of lactation and dry period treatment was significant for fat concentration only ($P<0.05$). The effect of month of calving was significant for lactose yield ($P<0.05$) and there were trends for protein concentration ($P=0.063$) and protein yield ($P=0.069$) to be affected by month of calving.

Table 6.10 Summary of results for early lactation of all animals (weeks 1-18) following different treatments during the dry period.

	Dry Period Treatment			
	Control	Supplemented	SED	χ^2 Probability
Milk Yield, kg/d	32.7	36.3	0.62	<.001
Protein, g/kg	30.7	31.5	0.28	0.020
Fat, g/kg	45.3	44.1	1.09	0.237
Lactose, g/kg	45.6	45.8	0.27	0.147
Protein Yield, g/d	1006	1106	21.0	<.001
Fat Yield, g/d	1486	1552	49.5	0.089
Lactose Yield, g/d	1499	1616	31.0	<.001

Even though animals were randomly assigned to dry period treatments within each pair, the control group of animals started the dry period in slightly better condition than the supplemented cows. Since the major effect of the supplemented dry period treatment was to increase the milk yields of cows which had been subjected to it, the effect of animal potential was tested by using the previous lactation's 305 day milk yield of each animal as a covariate. There was no significant difference in the results when this was done, nor were there significant differences in the results of milk protein and fat concentrations when the previous lactation's mean protein and fat concentrations were used as covariates.

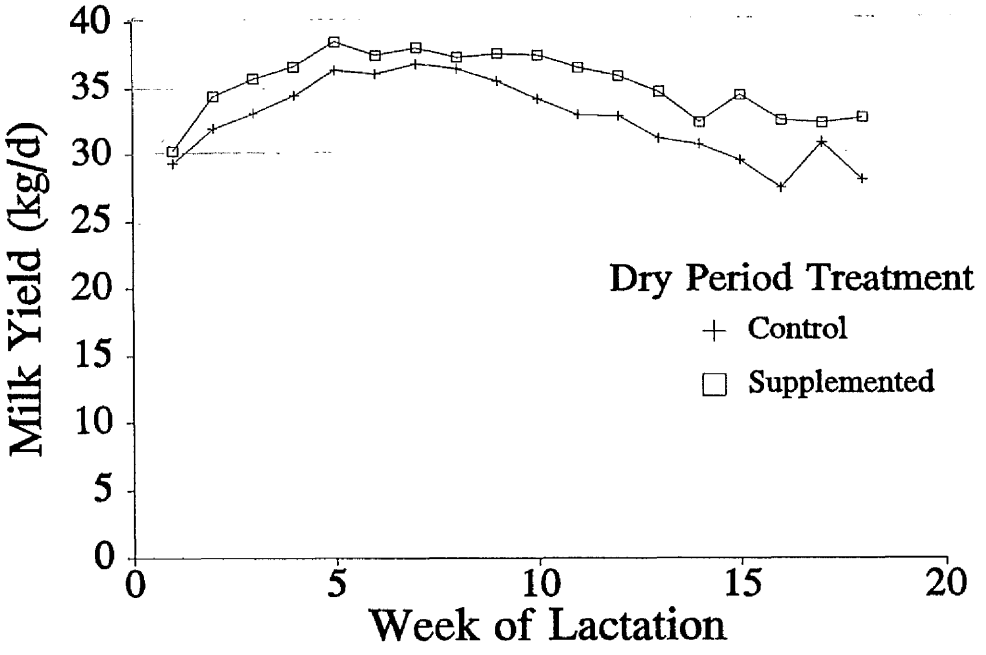


Figure 6.2 Model mean daily milk yields for each dry period treatment.

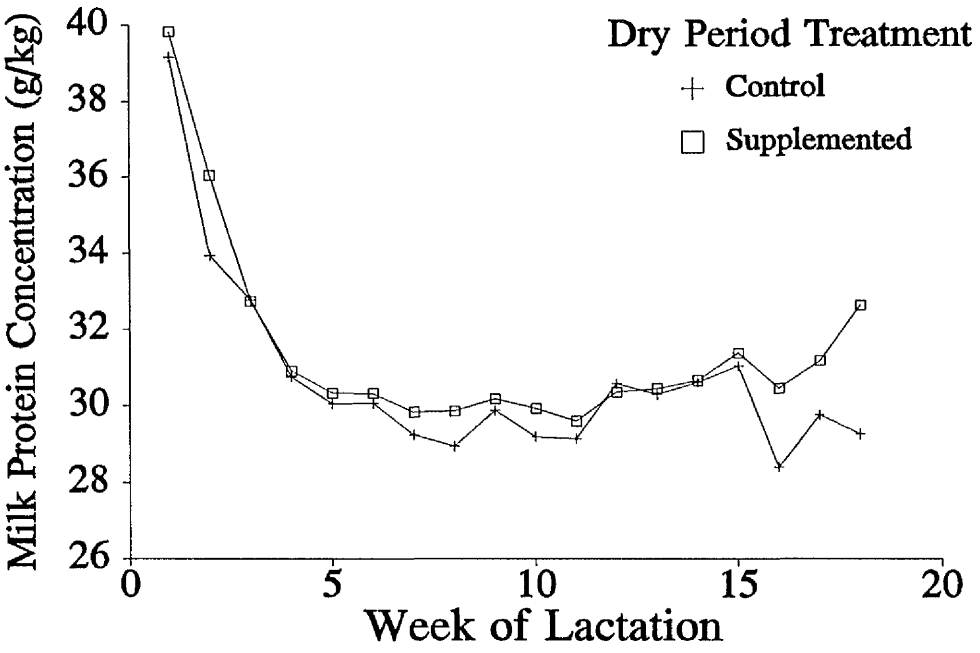


Figure 6.3 Model mean milk protein concentrations for each dry period treatment.

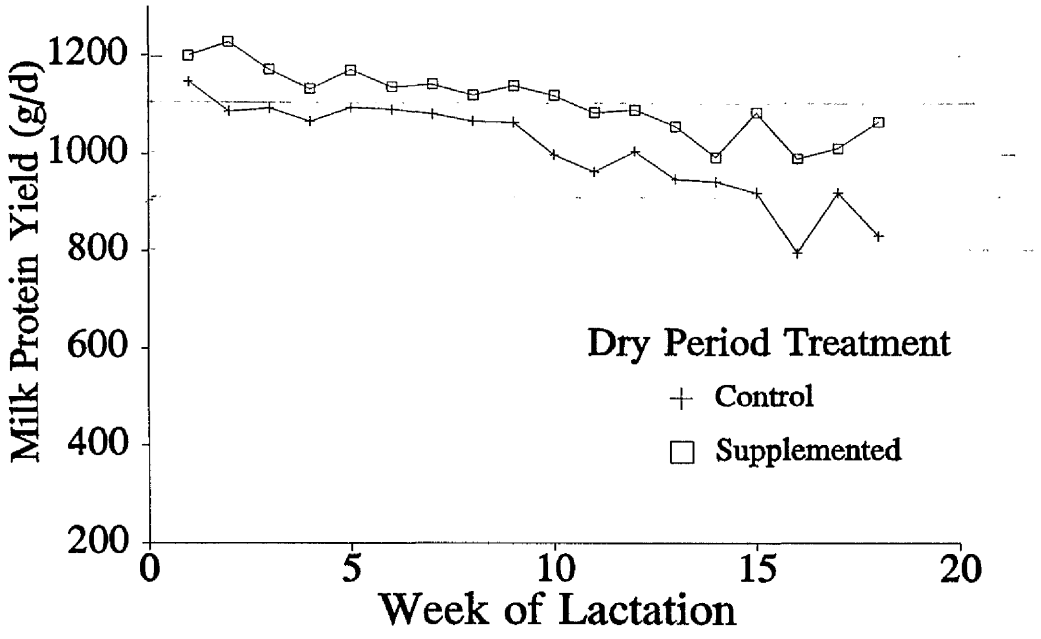


Figure 6.4 Model mean daily milk protein yields for each dry period treatment.

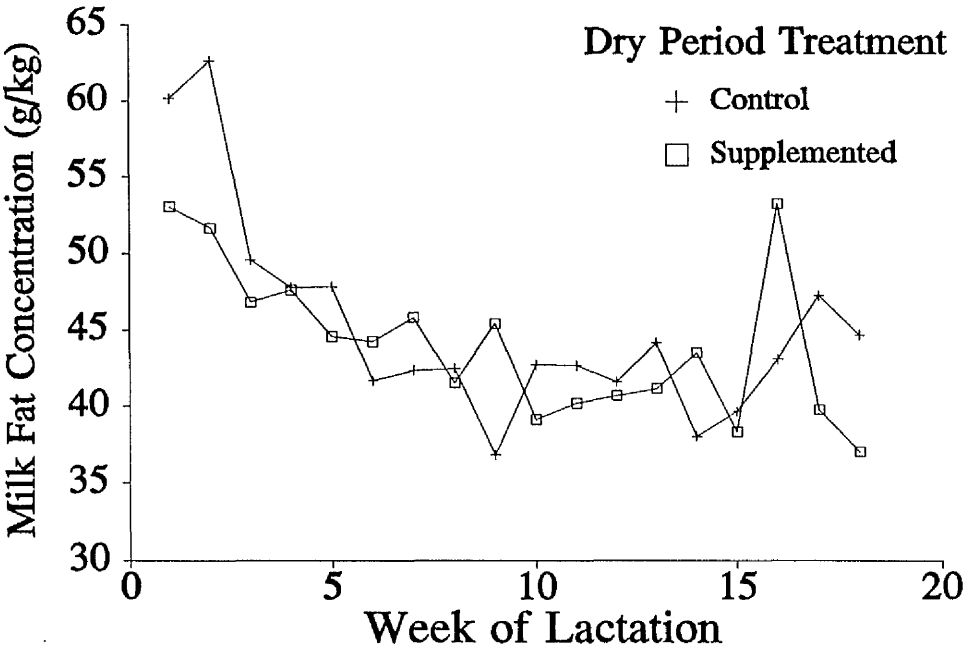


Figure 6.5 Model mean milk fat concentrations for each dry period treatment.

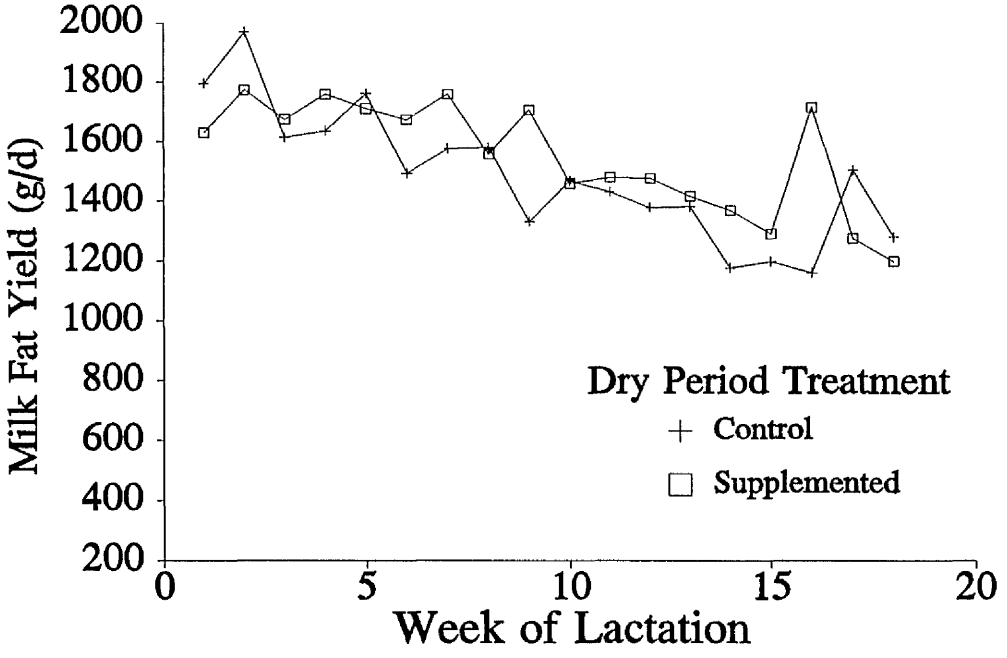


Figure 6.6 Model mean daily milk fat yields for each dry period treatment.

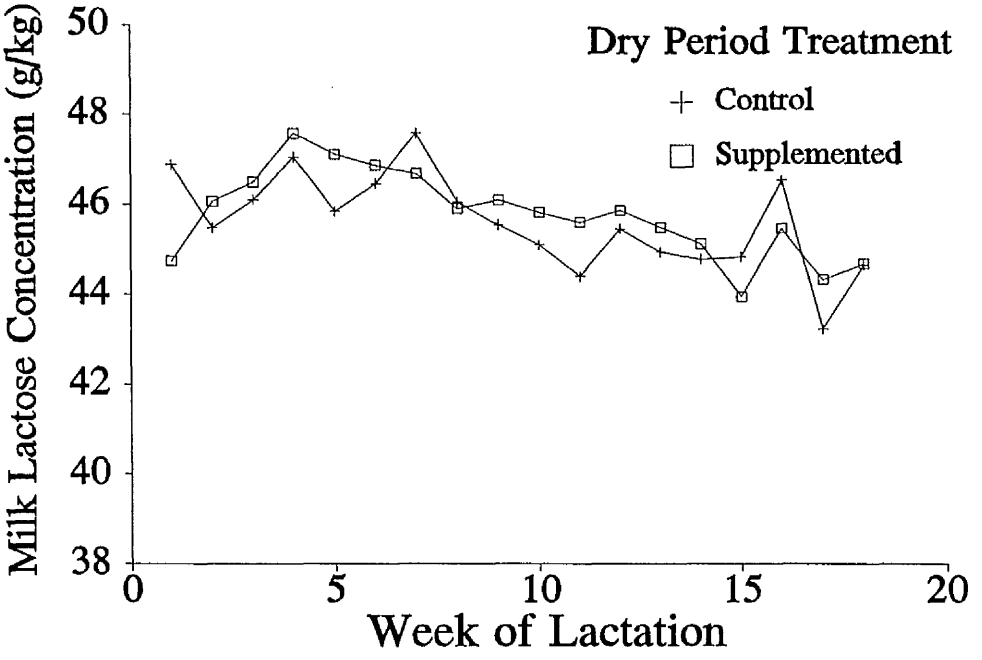


Figure 6.7 Model mean milk lactose concentrations for each dry period treatment.

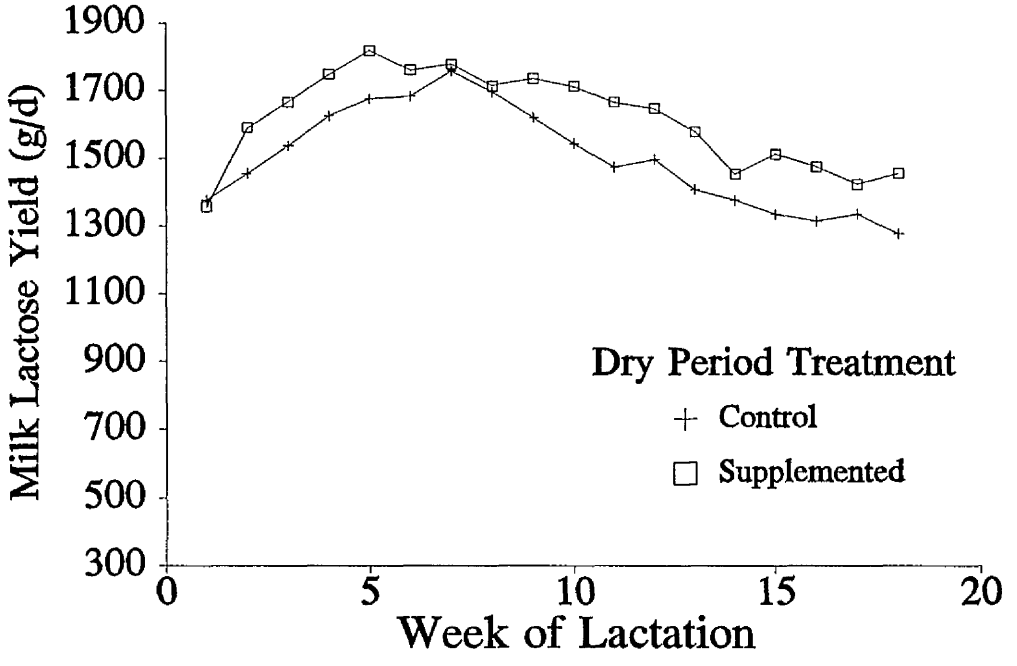


Figure 6.8 Model mean daily milk lactose yields for each dry period treatment.

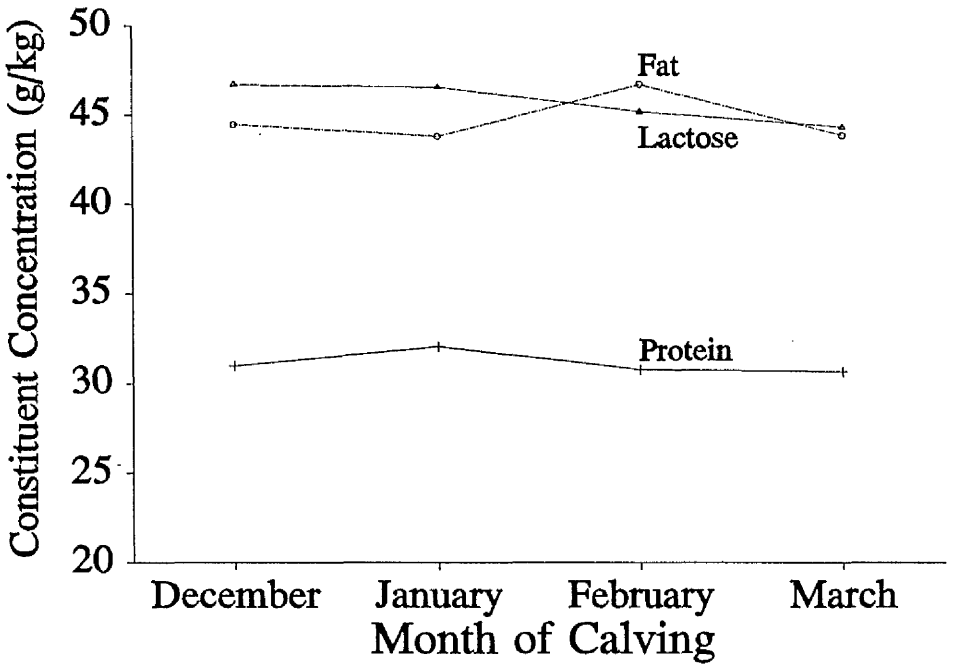


Figure 6.9 Model mean effects of month of calving on milk constituent concentrations.

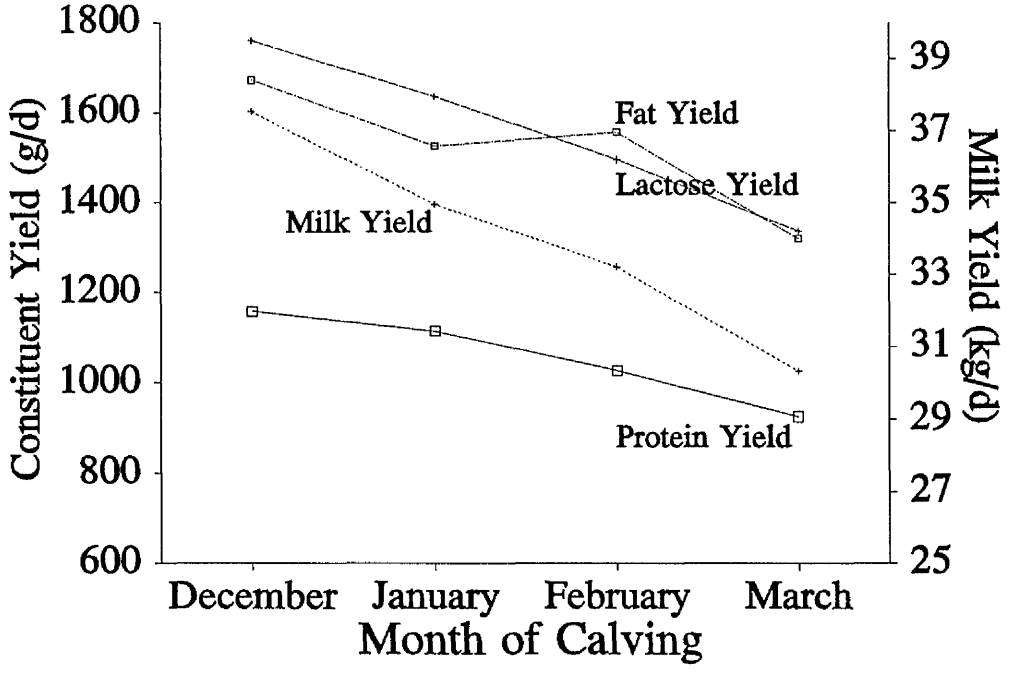


Figure 6.10 Model mean effects of month of calving on daily milk yields and daily yields of milk constituents.

Table 6.11 Mean daily intakes of silage and total dry matter (DMI), crude protein (CPI), and metabolisable energy (MEI) and urinary allantoin plus uric acid to creatinine ratios (AU/C) of 12 animals in early lactation.

	Dry Period Treatment			
	Control	Supplemented	SED	F Probability
Silage DMI, kg/d	13.8	14.8	0.66	0.202
Total DMI, kg/d	19.2	20.1	0.66	0.202
CPI, kg/d	3.4	3.6	†	
MEI, MJ/d	200	209	†	
AU/C	3.04	3.16	0.167	0.501

† Not determined.

6.5.4 Early Lactation

6.5.4.1 Feed Intakes

Feed intakes were not measured for animals in the main dairy unit. However, *ad libitum* intakes of silage mixes were measured for animals housed in the metabolism unit in early to peak lactation when being fed the formulated quantities of parlour concentrate (3.2 kg/d) and beet blend concentrate (3.0 kg). Mean daily intakes of silage mix and total dry matter intakes, plus calculated intakes of crude protein and metabolisable energy of these animals are presented in Table 6.11. Total daily DMI may have differed slightly for animals in the main dairy cubicle unit since the amounts of beet blend concentrate that was actually fed by the dairy staff was not weighed accurately for individual animals.

There was no significant difference in the total intake of DM of cows housed during early lactation in the metabolism unit. Both groups of cows ate significantly more of the silage mix that was offered in the morning (and contained the barley/fish meal mix) than of the silage mix that was offered in the afternoon. Mean silage mix DM intakes were 8.9 and 5.4 kg DM/d (SED 0.42; $P<0.001$) for morning and afternoon mixes respectively.

6.5.4.2 Milk Production

Table 6.12 gives a summary of the milk yields and composition obtained from the early lactation animals. There were no effects of dry period treatment on milk production in this group of animals. Therefore, there were no significant differences due to treatment seen in the ratios of protein/fat, protein/lactose or fat/lactose.

Table 6.12 Treatment mean milk yields (mean of 7 days) and milk protein, fat and lactose concentrations (weighted mean of 2 days) and yields of 12 animals, weeks 5-12 of lactation.

	Dry Period Treatment			
	Control	Supplemented	SED	F Probability
Milk Yield, kg/d	37.4	37.6	1.94	0.941
Protein, g/kg	31.1	30.4	1.43	0.629
Fat, g/kg	38.5	40.3	2.27	0.463
Lactose, g/kg	50.1	49.5	0.67	0.374
Protein Yield, g/d	1157	1141	54.0	0.786
Fat Yield, g/d	1427	1511	119.0	0.514
Lactose Yield, g/d	1867	1858	75.9	0.912

Table 6.13 Treatment mean values of major milk nitrogenous constituents from 12 cows in weeks 5-12 of lactation.

	Dry Period Treatment			
	Control	Supplemented	SED	F Probability
Milk Yield, kg/d	37.4	37.6	1.94	0.941
Crude Protein, g/kg	30.5	30.1	1.23	0.758
True Protein, g/kg	28.6	28.1	1.23	0.663
Casein, g/kg	24.2	23.2	0.98	0.375
Whey[†], g/kg	4.45	4.83	0.285	0.241
Non-Urea NPN, g/kg	0.089	0.097	0.0057	0.234
Urea, g/kg	0.430	0.471	0.0207	0.105
Crude Protein Yield, g/d	1135	1131	49.4	0.940
True Protein, g/d	1066	1055	47.2	0.831
Casein Yield, g/d	901	874	41.6	0.536
Whey Yield, g/d	165	182	7.58	0.075
Non-Urea NPN Yield, g/d	3.2	3.6	0.15	0.083
Urea Yield, g/d	16.2	17.7	1.35	0.307

[†] Whey calculated as True Protein - Casein.

Table 6.13 summarizes the effects of dry period treatment on the nitrogenous fractions of milk measured in early lactation. The only effects seen were slight increases in the yields of whey proteins and non-urea NPN from the supplemented animals. There were no

significant differences between animals from the two groups, although the proportion of true protein that was casein was significantly lower ($P<0.05$) for the supplemented group, with treatment means of 0.85 and 0.83 (SED 0.005) for control and supplemented groups respectively.

Relative protein efficiencies were determined by dividing protein output by protein intake of animals during early lactation. No difference due to dry period treatment was seen in the efficiency of production of any of the milk nitrogenous compounds when this was done.

Milk whey protein fractions were investigated by HPLC, although actual concentrations could not be determined. However, by analysing peak heights no significant differences in the concentrations of milk immunoglobulins, milk serum albumin, α-lactalbumin or ß-lactoglobulin were seen.

Mean milk concentrations and yields of pyruvic, lactic, uric and hippuric acids are presented in Table 6.14. There was a slight trend towards higher pyruvic acid concentrations in milk from supplemented animals. The concentrations of other acids determined were not significantly different between the two dry period groups.

Table 6.14 Treatment mean concentrations and yields of various organic acids from 12 animals in early lactation.

	Dry Period Treatment			
	Control	Supplemented	SED	F Probability
Lactic, mg/kg	371	348	99.6	0.821
PD*, mg/kg	29.8	32.1	3.25	0.504
Hippuric, mg/kg	7.4	7.0	1.14	0.775
Pyruvic, mg/kg	44.7	50.0	2.54	0.091
Lactic Yield, g/d	13.6	13.0	3.53	0.883
PD Yield, g/d	1.11	1.20	0.146	0.575
Hippuric Yield, g/d	0.28	0.26	0.046	0.709
Pyruvic Yield, g/d	1.68	1.88	0.111	0.137

* PD, purine derivatives, measured by the technique of Marsili *et al.* (1981) as uric acid, but also assumed to contain a contribution of allantoin. See Section 2.3.7.

Table 6.15 Treatment mean milk mineral concentrations and yields from 12 animals in early lactation.

	Dry Period Treatment			
	Control	Supplemented	SED	F Probability
Sodium, g/kg	0.36	0.35	0.014	0.421
Potassium, g/kg	1.57	1.56	0.081	0.950
Chlorine, g/kg	0.83	0.80	0.076	0.792
Calcium, g/kg	1.16	1.09	0.035	0.090
Phosphorus, g/kg	0.99	0.92	0.049	0.219
Magnesium, g/kg	0.10	0.10	0.003	0.335
Na Yield, g/d	13.5	13.1	1.14	0.747
K Yield, g/d	59.3	58.5	5.79	0.896
Cl Yield, g/d	31.2	30.3	2.76	0.402
Ca Yield, g/d	43.6	43.1	2.76	0.402
P Yield, g/d	36.8	34.4	2.52	0.385
Mg Yield, g/d	3.76	3.63	0.287	0.663

Mean milk concentrations and yields of potassium, sodium, chloride, calcium, magnesium and phosphorus are given in Table 6.15. There were no significant differences due to the effects of either of the two dry period treatments in any of these variables, although there was a slight trend for the calcium concentration to be increased in the milk of control cows.

6.5.4.3 Purine Derivative Excretion

Urinary purine derivative (PD) excretion measured in the early lactation animals. Mean figures for allantoin plus uric acid to creatinine ratios (AU/C) for the 12 animals in early lactation are presented in Table 6.11. There were no significant differences between dry period groups in either these or in allantoin/creatinine or uric acid/creatinine ratios, although in all three, morning excretion rates were significantly lower than afternoon excretion rates ($P<0.001$) with mean values of 2.63 and 3.56 for am and pm respectively. The proportion of the AU/C that was allantoin was the same for both dry period groups, with a grand mean value of 0.92.

Table 6.16 Summary of blood metabolic profile analyses from 12 animals in early lactation (BOHB=ß-hydroxybutyrate; NEFA=non-esterified fatty acids).

	Dry Period Treatment			
	Control	Supplemented	SED	F Probability
Protein, g/l	83.8	87.7	2.41	0.165
Albumin, g/l	39.3	40.1	0.56	0.195
Globulin*, g/l	44.5	47.6	2.00	0.184
Urea, mM	2.84	3.13	0.142	0.096
Glucose, mM	3.85	3.87	0.118	0.846
BOHB, mM	0.79	0.76	0.135	0.806
NEFA, mM	0.25	0.23	0.017	0.385
Mg, mM	1.09	1.13	0.054	0.565
P, mM	1.81	1.77	0.060	0.523

* Globulin calculated as Protein - Albumin.

6.5.4.4 Metabolic Profiles

Metabolic profile analyses of the 12 animals in early lactation are presented in Table 6.16. There were no significant differences for any of the metabolites between dry period treatment groups, although blood urea was slightly higher (P=0.096) from supplemented animals.

6.5.5 Mid-Lactation Experimental Concentrate Challenge

The results presented below were obtained from the mid-lactation changeover design experiment using 6 pairs of experimental animals removed from the main experimental group and fed different diets of grass silage and 2 lactation concentrates.

6.5.5.1 Feed Intakes

Table 6.17 shows the mean composition of the silage and the two concentrates used during the experiment. The analysis of concentrate samples collected during the collection weeks were the same, i.e. there was no change in concentrate analysis during storage. Table 6.18 shows the mean daily intakes of silage dry matter, total dry matter, crude protein, metabolisable energy and estimated fermentable metabolisable energy for each of the two diets. There was an increase in silage intake on the HPLF concentrate, leading to increases in the intakes of total DM and ME. The intakes of CP and FME were also

greater on the HPLF concentrate, which was expected due to the composition of the concentrate. FME was estimated from the ME and acid hydrolysis ether extract (AHEE) contents of the concentrate (with concentrate FME = ME - 0.33 x AHEE) and silage ME (with silage FME = 0.71 x ME), assuming additivity (TCORN, 1992).

Table 6.17 Composition of the silage and concentrates fed to 12 animals during the changeover experiment (for concentrates: L=Low, H=High, P=Protein, F=Fat).

		Concentrates	
	Silage	HPLF	LPHF
Dry Matter, g/kg	259	857	865
Organic Matter, g/kg DM	908	917	878
Crude Protein, g/kg DM	191	236	194
Metabolisable Energy, MJ/kg DM	11.3	13.2	13.2
Neutral Detergent Fibre, g/kg DM	448	151	257
Acid Detergent Fibre, g/kg DM	269	83	168
Starch, g/kg DM	29	272	69
Water Soluble Carbohydrates, g/kg DM	36	105	138
Acid Hydrolysis Ether Extract, g/kg DM	70	42	102
In vitro Organic Matter Digestibility, g/kg OM	787	-	-
Potassium, g/kg DM	22.3	16.5	19.0
Calcium, g/kg DM	7.2	9.3	14.8
Phosphorus, g/kg DM	3.0	5.9	6.3
Magnesium, g/kg DM	2.9	2.7	4.2
Sodium, g/kg DM	5.3	3.0	5.2
pH*	3.9	-	-
D-Value*, g/kg DM	703	-	-

* Silage alone.

Table 6.18 Mean daily intakes of silage and total dry matter, crude protein and metabolisable energy and estimated intakes of ERDP, DUDP (TCORN, 1992) and fermentable ME, from 12 animals on the experimental lactation diets (Diet=Effect of Diet; DP=Effect of dry period treatment).

	Control		Supplemented			F Probability		
	HPLF	LPHF	HPLF	LPHF	SED	Diet	DP	D.DP
Silage DMI, kg/d	13.4	12.6	12.8	12.6	0.50	0.019	0.631	0.132
Total DMI, kg/d	20.2	19.6	19.7	19.6	0.50	0.036	0.631	0.132
CPI, kg/d	4.2	3.8	4.1	3.8	†			
ERDPI, kg/d	2.29	2.24	2.23	0.24	†			
DUDP, kg/d	0.58	0.50	0.57	0.50	†			
MEI, MJ/d	241	234	235	234	†			
FMEI, MJ/d	188	169	184	169	†			

† Not determined.

6.5.5.2 Milk Production

Tables 6.19 and 6.20 give the mean treatment effects on milk yield and composition and major milk nitrogenous fractions respectively. Feeding the HPLF ('protein enhancing') concentrate significantly increased milk protein concentrations by almost 2 g/kg compared to the LPHF concentrate ('protein depressing'). There were no other significant effects of diet on basic milk composition although milk fat was lower on the HPLF diet than on the LPHF diet (P=0.066). Milk yields were almost exactly the same on both diets. No significant effects of dry period treatment were seen on milk yields or protein, fat and lactose concentrations or yields, despite differences between treatment of similar sizes as those due to immediate dietary effects. This is partly due to the experimental design, in which some effects can be assessed more accurately than others; a lot of variation can be attributed to individual animals, and this is taken into account in the Latin Square part of design (i.e. the lactation diets) but not the split plot part (i.e. dry period pairs). Ignoring the animal pairing in the statistical analysis leads to even larger SEDs and hence lower levels of significance.

The ratios of protein/fat, protein/lactose and fat/lactose were all significantly affected by lactation diet. For HPLF and LPHF diets respectively, mean protein/fat ratios were 0.76 and 0.70 (SED 0.013; $P<0.01$), protein/lactose ratios were 0.71 and 0.67 (SED 0.010; $P<0.01$), and fat/lactose ratios were 0.93 and 0.96 (SED 0.013; $P<0.05$).

Table 6.19 Effect of concentrate, dry period treatment, and the interaction of diet with dry period treatment on milk yield and composition of animals during the changeover experiment (DP=Effect of dry period treatment).

	Control		Supplemented			F Probability		
	HPLF	LPHF	HPLF	LPHF	SED	Diet	DP	D.DP
Yield, kg/d	33.4	32.7	34.6	34.7	1.91	0.551	0.425	0.370
Protein, g/kg	33.1	31.2	31.4	29.5	1.50	<.001	0.306	0.991
Fat, g/kg	41.9	42.9	43.0	44.3	2.20	0.066	0.577	0.737
Lactose, g/kg	45.4	45.4	46.0	45.2	0.55	0.458	0.948	0.427
Protein, g/d	1097	1010	1085	1025	45.5	<.001	0.969	0.353
Fat, g/d	1383	1382	1485	1538	72.1	0.356	0.114	0.338
Lactose, g/d	1516	1482	1581	1569	95.6	0.409	0.445	0.691

Table 6.20 Effect of concentrate, dry period treatment, and the interaction of diet with dry period treatment on concentration and yields of milk nitrogenous fractions from animals during the changeover experiment (DP=Effect of dry period treatment).

	Control		Supplem'ted			F Probability		
	HPLF	LPHF	HPLF	LPHF	SED	Diet	DP	D.DP
Crude Protein, g/kg	31.8	30.3	30.6	28.8	1.43	<.001	0.368	0.687
True Protein, g/kg	30.0	28.5	28.6	27.0	1.36	0.002	0.325	0.768
Casein, g/kg	25.3	23.7	23.3	22.1	1.09	<.001	0.156	0.525
Whey[†], g/kg	4.69	4.79	5.29	4.85	0.441	0.620	0.306	0.431
Non-Urea NPN, g/kg	0.067	0.067	0.063	0.052	0.0019	0.907	0.427	0.877
Urea, g/kg	0.49	0.46	0.53	0.51	0.037	0.259	0.186	0.897
Crude Protein Yield, g/d	1055	982	1057	1000	47.0	0.004	0.832	0.617
True Protein Yield, g/d	992	924	989	936	43.3	0.003	0.914	0.595
Casein Yield, g/d	838	771	807	768	40.4	0.013	0.664	0.413
Whey Yield, g/d	154	153	182	168	10.2	0.405	0.012	0.458
Non-Urea NPN Yield, g/d	2.15	2.09	1.99	1.82	0.592	0.833	0.280	0.928
Urea Yield, g/d	16.5	15.1	18.4	17.5	1.64	0.248	0.165	0.771

[†]Whey calculated as True protein - Casein.

From Table 6.20 it can be seen that the significant increase in crude protein concentration achieved on the HPLF diet was due to an increase in casein. The proportion of true protein that was casein was not significantly affected by lactation diet, but was significantly greater in milk from the control dry period treatment animals (control vs supplemented, 0.84 vs 0.82; SED 0.004; $P<0.01$). This was mostly due to significantly lower yields of whey proteins from the control dry period animals.

A summary of treatment mean effects on milk organic acids is given in Table 6.21. No significant effects of any of the treatments were seen. Table 6.22 summarizes the treatment effects on milk mineral concentrations and yields. There was a slight increase in the milk potassium concentration on the HPLF concentrate, and significant increases in the calcium and phosphorus concentrations on this concentrate.

Table 6.21 Treatment mean effects on milk organic acid concentrations and yields for 12 animals during the changeover experiment (DP=Effect of dry period treatment).

	Control		Supplemented			F Probability		
	HPLF	LPHF	HPLF	LPHF	SED	Diet	DP	D.DP
Acetic, mg/kg	83.3	97.6	115.0	103.5	25.19	0.909	0.436	0.315
Lactic, mg/kg	1099	1015	1532	1181	360.7	0.445	0.265	0.635
PD*, mg/kg	56.8	59.9	64.6	54.3	7.64	0.388	0.874	0.131
Hippuric, mg/kg	19.7	18.4	18.4	19.0	4.31	0.913	0.902	0.781
Pyruvic, mg/kg	91.9	84.9	127.7	126.4	36.87	0.818	0.288	0.875
Acetic Yield, g/d	2.73	3.21	4.07	3.63	0.969	0.962	0.359	0.324
Lactic Yield, g/d	35.5	34.5	54.5	41.7	13.4	0.488	0.228	0.552
PD Yield, g/d	1.90	1.99	2.25	1.89	0.388	0.409	0.695	0.176
Hippuric Yield, g/d	0.62	0.62	0.65	0.66	0.149	0.965	0.782	0.986
Pyruvic Yield, g/d	3.01	2.82	4.53	4.46	1.418	0.796	0.277	0.877

* PD, purine derivatives, measured by the technique of Marsili *et al.* (1981) as uric acid, but also assumed to contain a contribution of allantoin. See Section 2.3.7.

Table 6.22 Treatment mean effects on milk mineral concentrations and yields for 12 animals during the changeover experiment (DP=Effect of dry period treatment).

	Control		Supplemented			F Probability		
	HPLF	LPHF	HPLF	LPHF	SED	Diet	DP	D.DP
Sodium, g/kg	0.42	0.40	0.39	0.38	0.025	0.378	0.420	0.767
Potassium, g/kg	1.64	1.54	1.66	1.63	0.051	0.094	0.227	0.420
Chlorine, g/kg	0.91	0.88	0.93	0.91	0.060	0.315	0.724	0.877
Calcium, g/kg	1.27	1.19	1.22	1.15	0.062	0.039	0.436	0.875
Phosphorus, g/kg	1.01	0.92	0.96	0.87	0.046	0.006	0.300	0.905
Magnesium, g/kg	0.11	0.11	0.10	0.11	0.006	0.314	0.538	0.980
Na Yield, g/d	13.8	13.0	13.4	13.4	1.12	0.139	0.977	0.206
K Yield, g/d	55.1	50.8	57.5	56.4	4.16	0.101	0.349	0.291
Cl Yield, g/d	30.8	29.0	31.9	31.6	2.75	0.210	0.522	0.345
Ca Yield, g/d	42.2	38.8	42.4	39.8	2.88	0.027	0.831	0.720
P Yield, g/d	33.3	29.9	33.2	30.3	2.15	0.004	0.957	0.718
Mg Yield, g/d	3.53	3.55	3.54	3.64	0.291	0.476	0.875	0.622

Table 6.23 Relative protein efficiencies of milk nitrogenous constituents (milk protein output/crude protein intake, g/kg).

	Control		Supplemented			F Probability		
	HPLF	LPHF	HPLF	LPHF	SED	Diet	DP	D.DP
Crude Protein	253	262	260	267	10.1	0.136	0.564	0.854
True Protein	238	247	243	250	9.2	0.119	0.647	0.844
Casein	201	206	198	205	8.7	0.288	0.795	0.828
Whey	37.0	40.9	44.9	44.9	2.58	0.400	0.009	0.381
Non-Urea NPN	0.52	0.56	0.49	0.48	0.155	0.949	0.384	0.876
Urea	3.95	4.03	4.53	4.68	0.410	0.656	0.113	0.884

Table 6.23 gives a summary of the relative protein efficiencies (milk protein output/crude protein intake) for each diet. It is worth noting that no differences were seen between the lactation diets for any of the variables, but that the animals that underwent the supplemented dry period treatment produced significantly more whey protein per kilogram CPI ($P<0.01$).

6.5.5.3 Metabolic Profiles

Table 6.24 gives a summary of the metabolic profile data of blood collected from the 12 animals in the changeover experiment. Blood urea, glucose, NEFA, and phosphorus were all significantly affected by diet: glucose, urea and P were greater on the HPLF diet, whereas NEFA was greater on the LPHF diet. There were no significant effects due to dry period treatment, although there was a slight trend for control animals to have higher blood glucose concentrations (P=0.096). The interaction effects between dry period treatment and lactation diet were significant for albumin, magnesium and glucose.

In order to test the partitioning of urea into milk, the ratio of milk urea concentration to blood urea concentration was calculated. The mean ratios for control HPLF, LPHF, and supplemented HPLF and LPHF respectively were 2.98, 3.40, 2.85 and 3.20 (SED 0.238); the effect of dry period treatment was not significant, but the LPHF concentrate caused a significantly greater concentration of milk urea per unit blood urea than the HPLF concentrate ($P<0.05$).

Table 6.24 Summary of effects of diet and dry period treatment on blood plasma metabolites from 12 animals in the changeover experiment (DP=Effect of dry period treatment; BOHB=ß-hydroxybutyrate; NEFA=Nonesterified fatty acids).

	Control		Supplemented			F Probability		
	HPLF	LPHF	HPLF	LPHF	SED	Diet	DP	D.DP
Protein, g/l	79.7	78.7	79.4	78.3	2.74	0.208	0.898	0.930
Albumin, g/l	38.4	38.5	38.8	37.8	0.82	0.106	0.842	0.041
Globulin*, g/l	41.3	40.2	40.6	40.5	2.12	0.436	0.928	0.508
Urea, mM	2.76	2.24	3.12	2.65	0.227	<.001	0.129	0.782
Glucose, mM	4.14	4.12	4.08	3.76	0.118	0.021	0.095	0.036
BOHB, mM	0.92	0.87	0.92	0.98	0.134	0.960	0.690	0.258
NEFA, mM	0.21	0.26	0.21	0.26	0.010	<.001	0.839	0.920
Mg, mM	1.16	1.09	1.09	1.14	0.029	0.834	0.534	0.041
P, mM	1.90	1.76	1.83	1.70	0.102	0.002	0.530	0.938

* Globulin=Protein - Albumin.

6.5.5.4 Live Weights and Condition Scores

There were no significant differences in animal live weights between treatments (grand mean 600 kg). Similarly, there were no significant differences in CS between the 12 animals from the two dry period groups, with a grand mean of 2.25 at the start of the first experimental period. Changes in condition scores over each period were similar for all animals, with an increase of half a point for most animals over the first experimental period and a decrease of the same amount over the second period.

6.5.6 Metabolism Unit Animals

Inspection and comparison of the lactation data presented in Table 6.10 for all animals, and Tables 6.12 and 6.19 for the animals housed in the metabolism unit at two points during lactation reveals certain inconsistencies. In other words, the data of the smaller group does not match that of the whole group. To investigate this further, the whole lactation data were reanalyzed to week 15 (from which point the metabolism unit animals were no longer milk sampled in the main dairy unit) separating metabolism unit animals from the remainder of the group. Table 6.25 summarizes the results of this analysis.

Table 6.25 Comparison of results for the lactation (weeks 1-15) of animals occasionally housed in the metabolism unit with the rest of the experimental animals following the control or supplemented dry period treatment.

	Met. Unit				Rest			
	Control	Suppl'd	SED	χ^2 *P*	Control	Suppl'd	SED	χ^2 *P*
Milk Yield, kg/d	37.0	36.3	0.81	0.343	32.0	35.9	0.73	<.001
Protein, g/kg	33.1	32.3	0.43	0.048	30.7	31.6	0.31	0.001
Fat, g/kg	47.5	48.0	1.62	1.000	44.2	41.8	1.18	0.121
Lactose, g/kg	47.7	47.6	0.37	1.000	44.8	45.1	0.31	0.125
Protein Yield, g/d	1218	1170	27.3	0.054	981	1124	23.5	<.001
Fat Yield, g/d	1736	1750	71.4	0.584	1413	1496	57.8	0.051
Lactose Yield, g/d	1762	1729	42.1	0.439	1444	1623	35.9	<.001

From Table 6.25, it can be seen that the data collected from the metabolism unit group of animals whilst in the main dairy unit were quite different from the rest of the experimental group. There was no significant difference in milk yields of the metabolism unit group due to dry period treatment as there was for the whole group (Figure 6.11), as seen in Tables 6.12 and 6.19, and the control cows had a significantly higher mean milk protein concentration (Figure 6.12) and protein yield - the opposite of the effects seen for rest of the experimental group. With the effects of the metabolism unit group removed from the lactation data, similar trends in the effects as seen for the whole group are found, but to a greater degree. Of particular note are the extremely high fat concentrations (Figure 6.13) of the metabolism unit animals, which increased the mean concentrations in Table 6.10; the mean fat concentrations of the rest of the animals are reduced to more normal levels when the effect of this is removed.

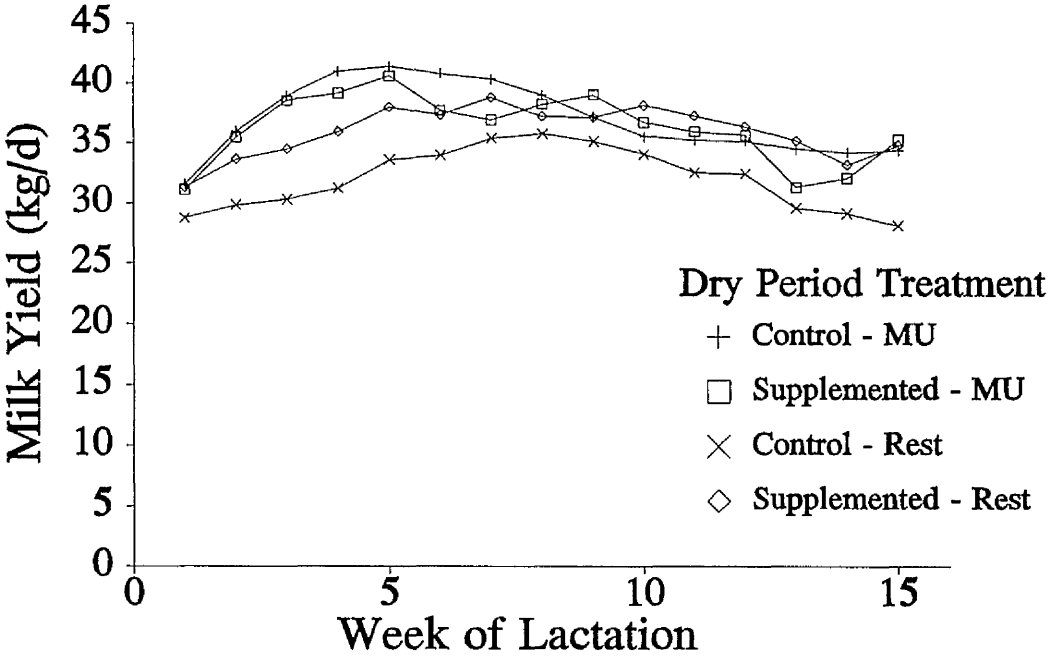


Figure 6.11 Comparison of model mean effects of dry period treatment on milk yields of animals used for metabolism unit studies (MU) and the rest of the group.

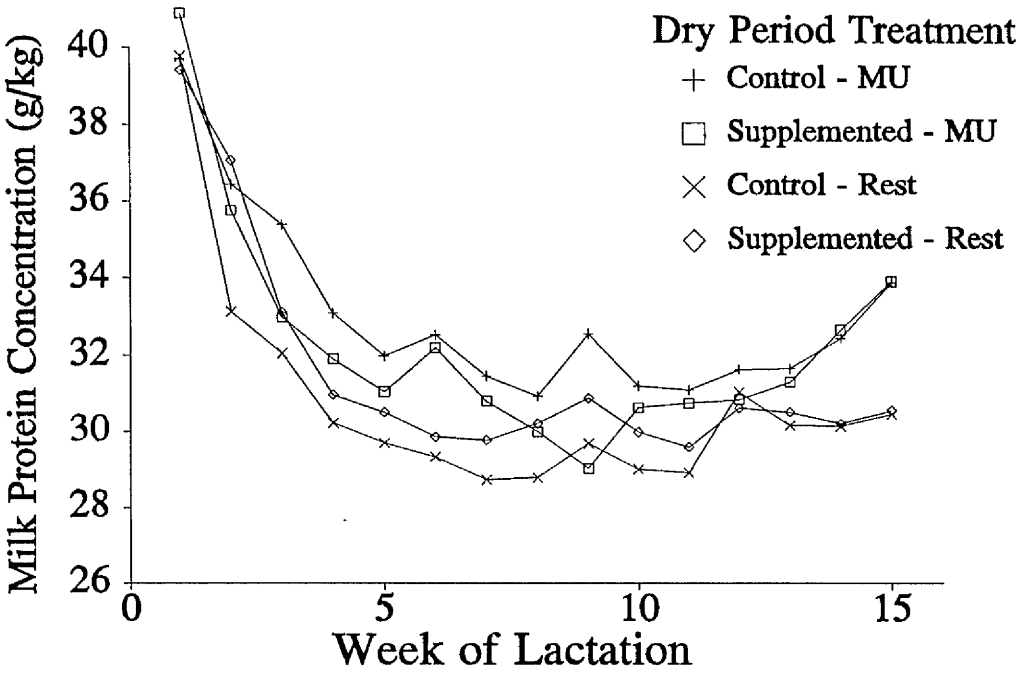


Figure 6.12 Model mean effects of dry period treatment on milk protein concentrations of animals used in metabolism unit studies (MU) and the rest of the group.

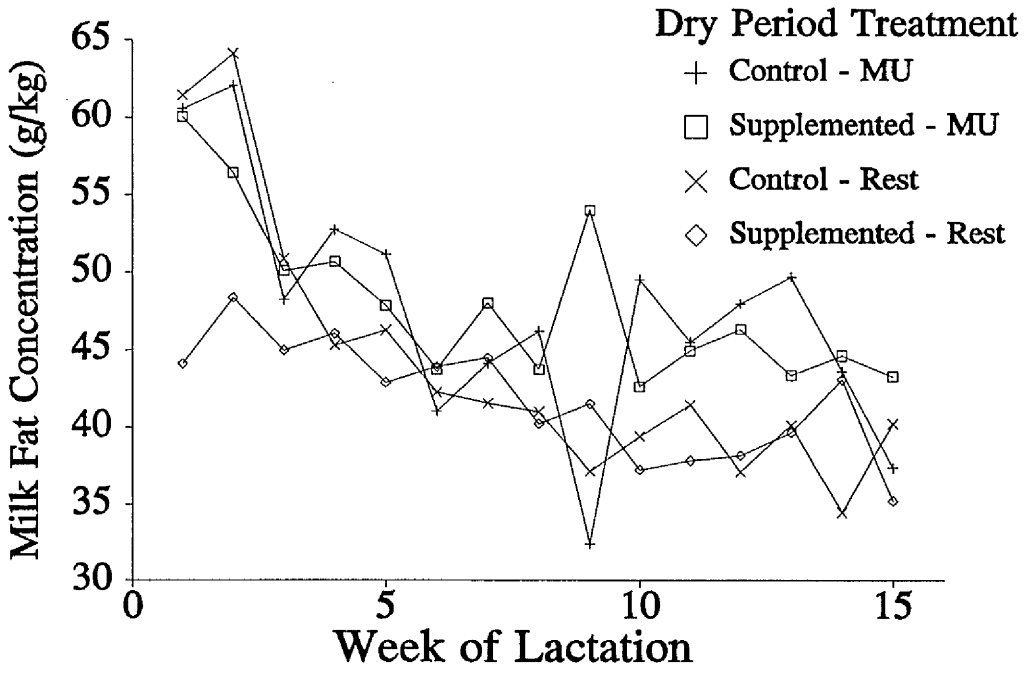


Figure 6.13 Model mean effects of dry period treatment on milk fat concentrations of animals used in metabolism unit studies (MU) and the rest of the group.

6.6 DISCUSSION

6.6.1 Effect of Dry Period Treatment

This experiment was designed to test the hypothesis that feeding a protein supplement during the dry period could increase the concentration of milk protein during the subsequent lactation and to examine the effects of dry period treatment in more detail than was possible in the experiment described in Chapter 5. Milk composition was affected by dry period treatment, but not in the same way as previously; the greatest effect was that of an increase in milk yield from those animals fed the protein supplemented diet during the dry period, which lasted to at least week 18 of lactation.

Since the lactation diet was very similar during this experiment to that fed during the previous experiment, the difference between the two years' experiments was caused by either differences in the dry period diet or the state of the animals' reserves at the start of the experiment. The supplemented dry period diet was formulated to supply the predicted requirement of ERDP whereas the control diet was predicted to supply an excess of ERDP. Both diets were predicted to supply metabolisable energy requirements. During this experiment both diets, and the control diet in particular, were of better quality than the two diets available to the animals during the previous experiment. It would have been interesting to have compared experimental animals with non-experimental animals fed a control diet like that of the previous experiment, but this was not possible since the remaining Auchincruive dry cows were not housed in the Auchincruive dry cow unit but were run with lactating animals and received the same diet as the (control) experimental cows.

The feed intakes recorded on a group fed basis during the dry period compare well with the feed intakes recorded using individual animals, particularly those of the control animals. Silage intake of the supplemented animals was slightly higher than formulated, but was a consequence of the lack of control with which the amounts of silage could be weighed out using the feeder wagon and differences in dry matter content between the pre-trial analysis and the analysis of the silage actually fed. Straw intakes of the supplemented animals confined in the metabolism unit were higher than those of the group as a whole, and may be partly due to the slightly lower amounts of silage offered, and differences in the way in which straw was presented. As in the previous year, animals consumed less straw than predicted by the rationing software.

The daily MEI of the dry supplemented animals was only two thirds of the MEI of the control animals. Despite random allocation of animals to dry period treatments, at the start of the dry period the control group had a slightly higher mean condition score than the supplemented group. It can be concluded from the loss of condition and blood NEFA concentrations of the supplemented cows that their lower intakes of ME did not fully meet their ME requirements. This again indicates that the control dry period diet was of a better quality during this experiment than it was during the initial study, when both groups of animals calved at similar condition scores.

In Section 5.6, it was conjectured that the dry cows were eating to meet protein requirements rather than energy requirements and, more specifically, that the supplemented dry cows did not need to eat as much DM because their protein requirements were being met more efficiently by the provision of the prairie meal as a protein supplement. This is possible considering the high demands on the pregnant cow during late gestation. It would explain the tendency for dry cows to typically fatten on relatively poor quality grass silage. In the initial study, undegradable protein intake may have been particularly important due to the very poor quality silage that was being fed as a control diet. In this study, the control diet was of a much better quality, but despite this the protein supplemented animals consumed an estimated 100 g/d more UDP than the control animals. Purine derivative excretion data suggests that there was no difference in microbial protein supply to animals on either of the dietary treatments. However, additional UDP would not have been of any benefit to the animals if the basal diet was adequate to support foetal growth or if the protein reserves were not depleted at the start of the dry period. The control animals had significantly higher blood urea concentrations during the dry period than the supplemented animals, which is indicative of inefficient capture of RDP (Lewis, 1957); the supplemented animals had a lower blood urea concentration because of the low quantities of silage that they consumed. However, more importantly, the blood albumin levels of the two groups did not differ significantly either during the dry period or during lactation. Sykes (1976) found blood albumin levels to be a good indicator of protein reserves in sheep and during the initial study the supplemented animals had higher blood albumin concentrations than the control animals. In this study, blood albumin levels did not differ between groups, and both groups had levels similar to those of the supplemented animals in the previous study. From the above arguments, it therefore seems likely that despite the increased UDP supply to the supplemented animals during the dry period, they

gained no advantage over the control animals in terms of the repletion of labile protein reserves because either the diet of the control animals was sufficient to meet their protein requirements or their protein reserves were not depleted.

During the following lactation, the response to the supplemented diet was, like that of the initial study, increased milk solids yields. However, unlike the initial study, the mean milk yields also increased, which meant that the concentrations of milk solids were similar for both dry period groups and only that of protein concentration differed significantly - although in real terms the difference in protein concentration was very small between groups. The increase in milk yields was apparently brought about by increased lactose yields - no difference was seen in the concentrations of soluble milk minerals during early lactation. Similarly, increased protein yields were responsible for the slightly increased protein concentration, but increased fat yields meant that the fat concentration was not significantly different between groups. In order to achieve these increases in milk solids yields, it was necessary to either have increased the supply of precursors to the mammary secretory tissue - from increased blood concentrations, increased mammary blood flow, or increased uptake by the mammary gland - or to have increased the efficiency of utilisation of these precursors by the secretory tissue. An increased supply to the mammary gland requires a source, and this may have been the diet or body stores. During early lactation, no differences were seen in the relative protein efficiencies of milk production (i.e milk output/milk input). If body labile protein reserves were playing a part in the production of milk protein, it might be expected that an increase in the relative efficiency of milk protein production would be seen, i.e. more protein would be seen to be produced by animals whose body protein was being channelled into milk than by animals where milk protein was being produced from precursors derived solely from the diet. This was not seen, although there is some concern that the animals on which this measurement was made were not representative of the whole group in this respect.

A major question is the effect of differences in condition score at calving, where the control group were almost half a point fatter than the supplemented group. The control group received an estimated 37 MJ ME/d more than the supplemented group; with this sort of difference in energy intake, an even greater difference in CS might have been expected. Why this did not happen is not clear. Garnsworthy (1988) found differences in lactation performance in animals that differed in condition score at calving, but only when the CS

was greater than 1-2 points. It is unlikely that the difference in CS between the two groups in the present study was great enough to have caused the effects seen. Both dry period groups had high blood BOHB concentrations during lactation, reflecting the use of fat stores for gluconeogenesis; the control group had particularly high BOHB concentrations at 6 weeks after calving, probably due to a greater utilisation of body fat. By week 10 of lactation, the blood concentrations of NEFA and BOHB were similar, suggesting that by that time the role of fat reserves in the control animals was no longer important. Nevertheless, the differences in milk yield and yields of protein and lactose continued to at least week 18, indicating that some factor other than the animals' fat reserves was influencing milk production, at least later in lactation.

A potential cause of the differences in milk yield and composition seen during the lactation is differences in DM intake between groups. During early- and mid-lactation, no difference in dry matter intake of the metabolism unit animals was seen which could be attributed to dry period treatment. However, during early lactation the patterns of milk production from the whole group of lactating experimental animals were not observed in the smaller group of animals used for the intake study work, and upon further investigation it was revealed that the 'whole lactation' data of the smaller group of animals was quite different to that of the rest of the experimental group. It is therefore difficult to extrapolate the conclusions with any confidence to all experimental animals on the basis of the data collected from the animals studied in the metabolism unit. The reason for the discrepancy is unclear. During the dry period, only 9 of the 12 animals used during lactation were housed in the metabolism unit. Of those animals, individual intake data match the data collected on a group basis in the main dairy unit. Furthermore, dry animals and animals housed in early lactation in the metabolism unit received the same diet as those in the main unit. Only in later lactation did the 12 metabolism unit animals show similar trends in milk yields as the remainder of the group. One factor that could possibly have been the cause of the difference in results between the main and metabolism unit groups was the timing of their starting on the experiment. The metabolism unit group was composed of animals that were amongst the first to start the experiment, and their body reserves may therefore may have been in a different state to those that started later, depending on the diets received before the experiment. However, the reason for the discrepancies still remains unclear.

Small differences in the metabolic profile data of the two groups other than those discussed above were seen, although there were no significant effects of dry period treatment. Blood urea concentrations were slightly higher in the supplemented dry period group as a whole, particularly during early lactation; this was also observed in the group used for the intake study at this time, when no significant difference in the intakes of DM or CP were seen. The trend for increased mean blood urea concentrations was reflected in the increase in mean milk urea concentration, supporting the work of other authors (Oltner and Wiktorsson, 1983; Roseler, Ferguson, Sniffen and Herrema, 1993). Urea is rapidly cleared from the blood, with a half-life of about 90 minutes (Lindsay, 1976) and effects on blood and milk urea concentrations may therefore differ slightly if milk acts as a buffer. This was seen in the changeover experiment where the ratios of milk and blood urea concentrations differed significantly with treatment. Changes in blood globulin concentrations over the periparturient period are consistent with other reports (Boisclair, Grieve, Allen and Curtis, 1987), and the reduced concentrations before calving were probably due to their use in colostrum formation. The effect of dry period treatment was not significant, although the three animals that were prone to mastitis infections may have been responsible for slight differences in the blood globulin concentrations. There were no apparent effects of dry period treatment on the health of the cows during the subsequent lactation, although the low numbers of animals used in the study and the number of illnesses encountered make it impossible to assess with any accuracy.

The most interesting effect of dry period treatments was the increased milk yields achieved by the cows subjected to the supplemented dry period treatment. Lactose concentrations were the same for the two groups of animals, and with the absence of significant differences in milk concentrations of potassium, sodium or chlorine during the either early or mid-lactation, lactose production was likely to be the major factor controlling milk yields. In Section 4.6, a hypothesis was put forward to explain the decrease in milk protein concentration which is often seen when fat is fed to dairy cows. Glucose, as an energy source, may be spared from utilisation for *de novo* fatty acid synthesis, since dietary fatty acids can be incorporated directly into milk fats (Banks, Clapperton and Kelly, 1980). This allows more lactose to be produced leading to an increase in milk volume. In this study, differences in milk yield were brought about by effects occurring before lactation commenced, and since neither the intakes of DM (at least of the metabolism unit animals) nor fat were affected by dry period treatment, the effect must be

due to some other factor. Blood glucose levels were similar between dry period groups during lactation, and therefore the difference in lactose output by the mammary gland may be due to a partitioning effect within the mammary secretory tissue. Using the mean production figures in Table 6.16, it can be seen that the ratio of fat to lactose is higher from control animals than from supplemented animals; that is, some 3% more lactose was produced per unit fat by the supplemented cows, despite increased fat yields by the same animals. Blood NEFA concentrations were significantly lower during lactation for the supplemented cows compared to control cows; blood NEFA concentrations have been correlated to their use for fat production (Miller, Reis, Calvert, DePeters and Baldwin, 1991a). When the effect of extremely high milk fat concentrations of the 12 metabolism unit cows was removed (Table 6.25), milk fat concentrations were seen to be higher from the control cows, as would be expected with an increased supply of NEFA to the mammary gland. Blood acetate is also used for milk fatty acid production and increased dietary forage tends to increase milk fat (Sutton and Morant, 1989), and although it was not measured, it is unlikely that blood acetate levels differed between the two groups owing to the similarity of their feed intakes. The supplemented cows could not provide extra fatty acids from body reserves since they had less body fat at the start of lactation; what caused the increased milk yields from the supplemented group of cows, therefore, remains unclear. Furthermore, it is difficult to explain the very high milk fat concentrations seen in the 12 metabolism unit animals and why these were different to the other animals.

Although the results of the present study do not provide clear evidence of the way in which the action of the dry period treatment was mediated, the results of two other studies provide interesting comparisons. The work of Stelwagen *et al.* (1992) investigated the effect of prepartum bST injections on the performance of heifers during their first lactation. Injections of a moderate dose (20 mg/d) of bST increased mean milk yields by about 4 kg/d compared to saline treated (control) animals and significantly increased the yields of protein and lactose. Fat concentration was slightly lower since fat yields were unaffected by bST treatment. Stelwagen *et al.* (1992) hypothesised that the effect of the bST was to enhance the proliferation of mammary secretory cells leading up to lactation, a theory for which they provide no evidence to support, but which has been shown to influence milk yield to a great extent (see Knight and Wilde, 1987). However, where bST has been shown to increase milk yields through stimulation of mammary growth, yields

were reduced once treatment was withdrawn (Knight and Wilde, 1987). Whether prepartum bST treatment could produce residual effects such as those reported by Stelwagen *et al.* (1992) through mammary proliferation is less certain; it is more likely that Stelwagen *et al.* (1992) managed to increase the animals' protein reserves since the body weight gain of the bST treatment heifers was some 300 g/d more than that of the control animals. Secondly, Chew *et al.* (1984a) found significant increases in milk yield during at least the first 22 weeks of lactation following the intravenous infusion of arginine to dairy cows in late gestation. They too attributed this to increased mammogenesis following the dramatic but transient increases in growth hormone, prolactin and insulin with arginine infusion, and while this cannot be excluded, neither can increases in the protein reserves of the animals which would be expected with increased circulating levels of growth hormone. Changes in mammogenesis in the present study cannot be excluded as the cause of differences in milk production in the present study since they were not measured. One of the original ideas which prompted the start of the investigation and the conduction of the initial study described in Chapter 5 was that by increasing the amount of protein available to the animal, the mammary gland may become 'accustomed' to the increased availability of milk protein precursors in the blood and proliferate to take advantage of this. Without a measure of the number of secretory cells or enzyme activity, it is not possible to be certain that this did not occur in this study. Although mammogenesis occurs leading up to parturition, foetal growth probably accounts for a greater proportion of the demand on the cow for nutrients. When Hook, Odde, Aguilar and Olson (1989) fed cows high or low protein levels leading up to parturition they found no difference in calf birth weight between treatments. However, as in the present study, significantly higher milk yields from the animals fed the high protein prepartum diet were observed during the following lactation, for which no explanation was offered, although the influence of protein reserves is suspected. The work of Chew *et al.* (1984a) and Stelwagen *et al.* (1992) suggest the possible involvement of endocrine regulation of the maintenance of labile protein reserves, perhaps by influencing the relative rates of protein synthesis and degradation.

The effect of month of calving was significant only for lactose yield, although there were marked downward trends in the yields of milk and all of the milk solids from December to March. Seasonal variation in milk yield is well known and Wood (1969) calculated that dairy cows give less milk during the winter months and more during the summer months

because of the quality of feeding. The present figures do not follow this trend, with December calving animals yielding the most milk. Part of the reason for this could be the way in which the present figures were calculated, i.e. over the course of lactation up to 18 weeks only. A comparison of the milk yields from 12 animals used for the metabolism unit studies and the remainder of the group (Figure 6.11) shows that those 12 (amongst the first to calve) had a faster increase to peak lactation than the rest of the group. Despite this, animals that calved earliest reached peak lactation in mid-January and those that calved latest peaked after turnout, after which an increase in milk yields is expected. It is unclear why these trends were observed, although changes in feed quality may have had a large effect.

To investigate the effect of diet on rumen efficiency, urinary purine derivative excretion was used as an index of microbial protein yield. Neither during the dry period nor during early lactation were there significant differences in the mean daily PD excretion rates when expressed as a ratio to creatinine, suggesting the delivery of similar quantities of microbial protein to the duodenum in both groups. This implies that the extra UDP supplied to the supplemented animals in the form of prairie meal was the only source of extra protein for those animals, and represented an increase in the supply of metabolisable protein compared to the control animals. In other words, the increased intake of silage by the control animals did not increase their protein intake beyond that of the supplemented animals, which could have explained the lack of differences in milk production. Interestingly, there were significant differences between times of sampling for the supplemented group during the dry period and for both groups during early lactation, with higher ratios in the afternoon in both cases. This is explained by the feeding regimes: during the dry period, the supplemented animals were fed their silage and prairie meal in the morning, and during lactation, all animals consumed more concentrates in the morning. In both cases, increases in the morning feed intake resulted in increases in afternoon PD excretion due to elevated microbial protein yields. Such diurnal variation was seen in the experiments described in Chapters 3 and 4 and demonstrates the sensitivity that the purine derivative technique offers, even when PD excretion is measured against creatinine excretion.

6.6.2 Effect of Lactation Diet

This part of the experiment was designed to investigate the interaction effect of lactation diet on dry period treatment. The effects of the two concentrates, formulated to increase

and decrease milk protein concentration, were as expected: milk protein concentration was increased by the HPLF concentrate with no difference in milk yields compared to the LPHF concentrate. The increase in milk crude protein concentration was brought about by an increase in the casein concentration - whey protein and non-protein nitrogen milk concentrations were not affected by lactation diet. The effect of dry period treatment was, however, not as expected in the animals used for this part of the experiment, and few interaction effects were seen, possibly because of this.

The increase in silage intake on the HPLF concentrate meant that total dry matter intakes were significantly higher on that diet. This increased the intakes of crude protein and ME of the animals on the HPLF concentrate even further than would have been achieved if the intake of DM of the diets was the same, due to the higher CP and FME concentrations with the HPLF concentrate. This increase in protein supply to the animal led to an increase in the milk protein concentration, much of which was due to an increase in casein production. The relative protein efficiencies were not affected by dietary crude protein intake (which might be expected under the law of diminishing returns if milk protein production was near its limit), and there did not appear to be any difference in the efficiency of utilisation of dietary protein in the production of milk casein (which might be expected if protein reserves were supplying milk protein precursors), but see later.

Milk fat concentrations were higher (P=0.066) on the LPHF diet. This could be a reflection of the higher blood NEFA concentrations of animals fed this diet, which may be available for incorporation directly into milk fat (Banks *et al.*, 1980; Miller *et al.*, 1991a). However, the total daily production of fat was not affected by either of the lactation diets, despite differences in dietary fat content (and thus a potential reduction in fibre fermentation to acetate), and therefore differences in concentration appears to be due to differences in milk yields. Neither lactose production (yield) or concentration were significantly affected by any of the treatments (despite a significant increase in blood glucose concentration on the HPLF diet), and so the increase in the availability of free fatty acids was probably the main cause of the increase in milk fat rather than changes in cellular partitioning of glucose. This led to the significant differences in the fat/lactose ratios of milk from the two lactation diets.

There was a tendency for milk potassium concentrations to be higher from the animals fed

the HPLF concentrate. The daily yield of potassium was slightly higher from these animals also (P=0.101). Milk production, however, was unaffected by diet and some factor must therefore have balanced the osmotic potential of potassium to draw water into milk. This was not apparently any of the other major osmotic components of milk - sodium, chloride or lactose - since neither concentrations nor yields of these components were significantly affected by dietary treatment, although changes in the concentrations of these occurred in the right direction for a combined effect to have taken place. Calcium and phosphorus concentrations were significantly higher in the milk of animals fed the HPLF concentrate, but this was probably due to the increased casein concentrations since these minerals are mostly bound to casein in milk and thus have little contribution to the osmotic potential of milk.

Whey protein production (i.e. yield) was significantly higher from supplemented animals and was the only significant effect of dry period treatment. A similar increase in whey protein yield from supplemented animals was seen during the early-lactation study. Moreover, the relative protein efficiency of whey protein was significantly increased in the supplemented dry cows, indicating the possible involvement of proteins from body reserves. In Section 3.6 the possibility of the increase in whey protein yield being due to a greater expression of α-lactalbumin to help with increased lactose production was proposed. However, there were no significant differences in the concentrations of α-lactalbumin, or of ß-lactoglobulin, serum albumin and immunoglobulins in the milk of early lactation animals. Differences in the daily whey protein yields during early lactation must therefore have been a combination of slightly higher concentrations and slightly higher milk yields from the supplemented animals. Later in lactation, the same supplemented animals again yielded more whey proteins, indicating a real effect, although again it was apparently due to slight increases in both concentration and yield. Determination of the different whey proteins was not carried out in this part of the experiment and so it is not possible to tell which of the whey proteins was increased in the milk of the supplemented animals. A significant interaction effect between dry period treatment and lactation diet was seen for blood albumin concentrations, with supplemented animals on the LPHF diet having the lowest. This result cannot be explained, unless, for some reason, there was an increase in the secretion of serum albumin into the milk of these animals.

Milk urea concentrations were not affected by diet, despite a significant increase in the silage intake of those animals fed the HPLF concentrate. Following the arguments discussed above, the RDP was apparently utilised by the rumen population to a similar extent in animals on all treatments. The RDP:FME ratios of the two concentrates were deliberately formulated to be similar, but since silage intake was increased with concentrate HPLF, estimates of the actual ERDP:FME ratios achieved were slightly different at 12.2 and 13.2 for diets HPLF and LPHF respectively. In Chapter 4, milk purine derivative excretion was seen to be significantly correlated with urinary purine excretion, and it was shown that it could be used for a similar, although less sensitive purpose. If the ERDP:FME ratios had been the same for both lactation diets, one would have expected an increase in milk PD excretion on the HPLF diet due to the increased intakes of dietary CP. However, this was not seen, and is possibly explained by the similar ERDP intakes achieved on all diets. The milk PD excretion on this study was some 2-4 times the excretion seen in the experiment in Chapter 4 whereas the urea concentrations were very similar. The difference between experiments is unlikely to be caused by differences in endogenous PD production since, although the contribution that the mammary gland makes to milk PD excretion is unknown, it is likely to be related to protein production and in both experiments milk protein production (and milk yields) were similar. Microbial protein production by the rumen was therefore probably more efficient on the present diets than on the Chapter 4 diets, and relative protein efficiencies (i.e. g milk protein output/kg protein intake) were approximately 40% higher on the present study. This does not explain the lack of difference in milk PD excretion where one is expected, but suggests that further investigation into the use of milk PD excretion as an indicator is needed to validate it as an index of microbial protein production.

None of the organic acids measured in milk were affected by either lactation diet or dry period treatment. Taking milk hippuric acid as an index of rumen lignin degradation, there was apparently no difference between treatments. Similarly, using other milk organic acid concentrations as indices of mammary metabolism, there was no apparent difference between treatments.

6.7 CONCLUSIONS

The effect of dry period diet in this experiment was different to that of the previous study. An increase in milk yield of 2-3 kg was achieved by feeding a small amount of protein supplement to dry cows. Milk protein concentration was also increased slightly, and hence protein yield was increased significantly. No difference in feed intakes were seen between animals from the two dry period treatments, although those animals on which intakes were studied did not appear to be a satisfactory representative subsample. Other factors, however, indicated that there were no significant differences in dry matter intakes.

In mid-lactation, when two differing lactation diets were fed to examine the interaction between dry period treatment and lactation diet, the two diets performed as expected, with dietary intakes of protein and energy-yielding compounds causing the anticipated effects on milk composition. The effects of lactation diet on milk production confirm the possible mechanisms by which milk protein concentration may be altered through dietary manipulation, and in particular through the supplementation of grass silage using typical levels of concentrate feeding. There was, however, no evidence for an interaction between dry period treatment and lactation diet. The absence of this may have been due to an absence of dry period treatment effects in the animals used.

Chapter 7

General Discussion

Short and Long Term Effects of Diet on the Concentration of Protein in the Milk of Dairy Cows

7.1 INTRODUCTION

The experiments described in this work indicate two major ways in which milk protein may be influenced by dairy cow nutrition: short term feeding and long term management strategies. Both may be used by the dairy farmer to increase milk protein concentration, which is important for increasing both profits and the efficiency of nitrogen utilisation.

7.2 SHORT TERM FEEDING

One major factor which has emerged as being central to the objective of increasing milk protein concentration in the dairy cow is protein supply to the animal. Without a supply of precursors, the mammary gland is unable to produce milk protein. Two dietary components that have been demonstrated to be important to this end are crude protein and fermentable metabolisable energy. The balance of the two has to be maximised to increase the protein supply to the animal and reduce ruminal inefficiencies which result in an expensive waste of nitrogen. The FME supply should be sufficient to ensure maximum capture of rumen degradable crude protein and this is one reason why fat is not a good energy yielding compound in a lactation diet, if an increase in milk protein concentration is the aim.

In Chapter 3, starch (barley) and fibre (sugar beet pulp) as sources of concentrate energy were compared. When the effects of microbial protein yield were taken into account (due to differences in the RDP content of the diets) there was no difference between the two as energy yielding compounds for milk protein production because of the availability of the two to the rumen microbe population. In Chapter 4, fat was compared to carbohydrates in lactation diets, and two effects were seen. The first was a reduction in the microbial protein yield from the rumen on a high fat/low digestible carbohydrate diet compared to a low fat/high digestible carbohydrate diet of the same ME density. This would have led to a decreased protein supply to the animal if it had not been for compensation due to increased dry matter intakes. The second effect was a change in the partitioning of glucose in the mammary gland between fatty acid synthesis and lactose (and possibly ATP) synthesis: dietary fatty acids were incorporated directly into milk fat resulting in a greater glucose availability for lactose and ATP production. The increase in lactose production and milk soluble ion transport increased milk yield, and, with no increase in milk protein production, resulted in a reduction in the milk protein concentration. In Chapter 3 it was seen that when the energy intake was similar, but the form in which the energy was

provided differed, significant differences in the milk fat/lactose ratios were seen. A presumed increase in the rumen acetate:propionate ratio in animals fed the fibrous diets led to an increased fat/lactose ratio - acetate being used to a greater extent in milk fat production than glucose.

One of the general problems associated with forage for milk protein production is the tendency towards a high acetate production. It is possible that this was not so evident in the feeding experiments presented here because of the high level of concentrate feeding. A large proportion of the volatile fatty acids formed in the rumen are used directly by stomach tissues (approximately 30%, 50% and 90% of the acetate, propionate and butyrate respectively (Parker, 1990; Britton and Krehbiel, 1993)). Starchy concentrates that tend towards causing a higher propionate fermentation therefore may not supply as much energy to the animal's peripheral tissues since a greater proportion is used by the stomach tissues. This must be balanced with the need for precursors of hepatic gluconeogenesis (e.g. propionate and amino acids) and if an enhanced propionate fermentation reduces the use of amino acids for this purpose then an increase in milk protein production may be achieved. Finally, if dietary nitrogen is degraded and not captured within the rumen, the animal absorbs a lot of ammonia which must be detoxified by the liver. Ureagenesis needs certain amino acids, glutamate and aspartate in particular (Reynolds, 1992), and thus an inefficient capture of RDP represents not only a loss of microbial protein but also a potential loss of α-amino nitrogen that could otherwise be used for milk protein.

To the milk producer, this means that an appropriate supplement must be fed in order to achieve an efficient utilization of the crude protein provided by grass-silage since much of this is in the form of ammonia. This should include a readily fermentable source of ME and possibly a balanced supply of additional protein. To achieve a high yield of milk with a high concentration of milk protein, a balance in the supply from the gut of energy- and protein-yielding compounds must be sought in order to supply the mammary gland with the most efficient balance of nutrients. Extensive clearance of propionate from the portal blood for gluconeogenesis, and little hepatic utilisation of acetate (Armentano, 1992) results in mammary availability of glucose and acetate. Dietary fatty acids, which bypass the liver in the lymphatic system, are also available for incorporation into milk. Amino acids that escape hepatic metabolism are available for incorporation into milk protein. Unfortunately, the relative proportions of these and other nutrients that are needed for the

production of milk for a given quality are largely unknown. However, with the use of certain markers such as milk urea, milk citric acid and purine derivative excretion, the efficiency of milk production may be monitored very easily.

In all the short term feeding experiments reported here, milk urea concentrations (used as an estimate of rumen inefficiency) were low compared to many figures reported in the literature. This suggests that the high milk protein concentrations seen were partly due the sparing of amino acids from processes such as ureagenesis. Microbial protein capture, as estimated by the urinary purine derivative to creatinine ratios, was relatively low on all four diets described in Chapter 3, but was higher on the diets described in Chapter 4. By using a combination of both milk urea concentration and urinary PD excretion, it was possible to explain differences in microbial protein production in terms of differences in RDP supply in the first experiment and differences in FME supply in the second. The rumen has such an important influence on the performance of the dairy cow that it is extremely useful to be able to characterise it in such an easy way.

Similarly, energy partitioning within the mammary gland, and its role in controlling milk volume, can be partially characterised by measuring the citric acid concentration and investigating the fat/lactose ratio. In Chapter 4, *de novo* synthesis of fatty acids was apparently increased when animals were fed a low fat diet. There were no differences in the fat/lactose ratios between diets despite this, suggesting that the production of fat and lactose was not limited by energy supply, but that preformed fatty acids were used for fat production in preference to *de novo* synthesis. In Chapter 3, fat/lactose ratios were affected by diet, with more fat produced per unit lactose on the fibrous concentrates. This indicates a more fundamental level of energy partitioning within the cell, since the fatty acid supply to the mammary gland is likely to have been similar for all diets. A greater supply of acetate to the mammary gland on the fibrous diets allowed an increase in fat production, whereas a greater supply of glucose increased lactose production. Similarly, the fat/lactose ratios obtained in the milk of animals that were fed the two lactation rations described in Chapter 6 were significantly higher on the low protein/high fat concentrate, where the carbohydrate source was predominantly fibrous. In this case, an increase in NEFA supply allowed a further increase in the efficiency of fat production without altering lactose production.

The two contrasting lactation diets fed in part of the experiment described in Chapter 6 were formulated to test some of effects seen in the previous experiments. By feeding a silage based diet supplemented with a high protein/low fat concentrate, higher silage intakes were achieved and, most importantly, milk protein concentrations were increased by almost 2 g/kg without affecting milk yield.

7.3 LONG TERM EFFECTS

In Chapters 5 and 6, large changes in milk protein production were shown to be brought about by simple changes in dairy cow management during the dry period. A small amount of high quality protein fed to the animals with a restricted quantity of forage increased the yield of milk protein during the subsequent lactation. The effects of the treatment are not clearly understood, but it is hypothesised that they are likely to be caused by maintaining and/or replenishing the animals' labile protein reserves during the last stages of pregnancy when they might otherwise be depleted in order to support foetal growth.

In the experiment described in Chapter 5, animals fed a protein supplement during the dry period produced similar quantities of milk as animals fed a control diet of silage only; the consequence of increased milk protein yields was therefore increased milk protein concentrations. In the experiment described in Chapter 6, similarly supplemented dry cows produced significantly more milk than control animals, and therefore exhibited only slightly higher milk protein concentrations. The effects on milk production lasted to about week 25 of lactation of the first experiment, and at least until week 18 of the second. In the first of the two experiments, feed intakes were not recorded but evidence from blood metabolic profiles and milk composition suggested that there were no effects of dry period treatment on feed intake. In the second experiment, detailed feed intake measurements were made at two points during the lactation on a proportion of animals, and no effect of dry period treatment was seen.

A comparison of the milk protein concentrations from the animals between the two years (Figure 7.1) reveals that the milk protein concentrations of the animals in the second year were midway between those of the first year. The protein yields (Figure 7.2) of the two groups (control and supplemented) differed significantly each year by about 70-100 g/d, with those of both second year groups being higher than those of the first year. In particular, the first year control group had the lowest protein yields of all. It is interesting

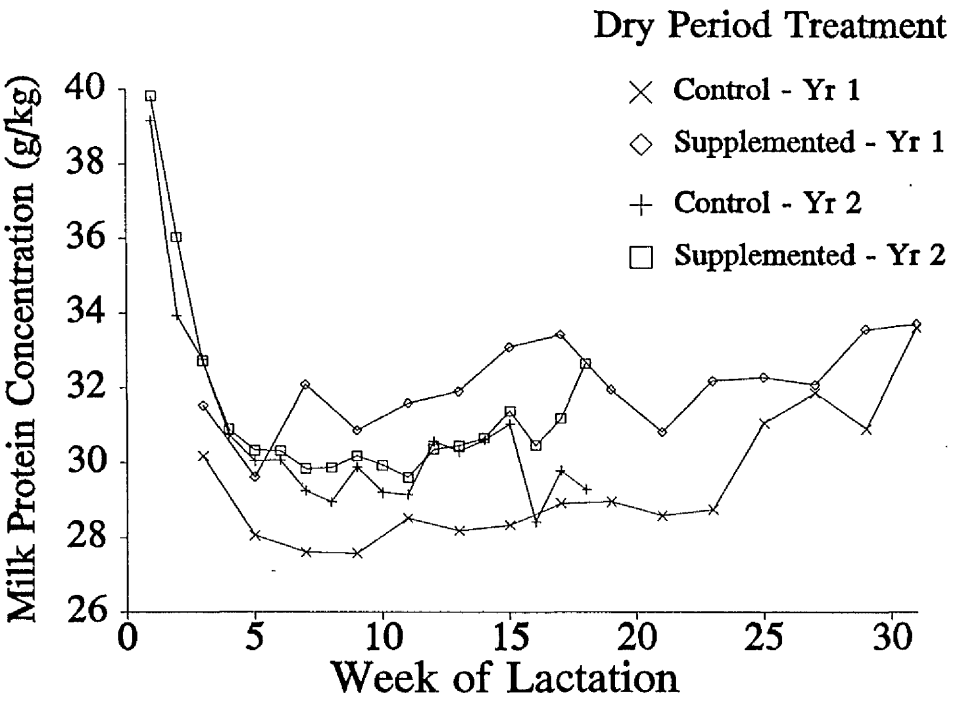


Figure 7.1 Comparison of the two years' protein concentration data from animals that were fed the control or supplemented dry period diets.

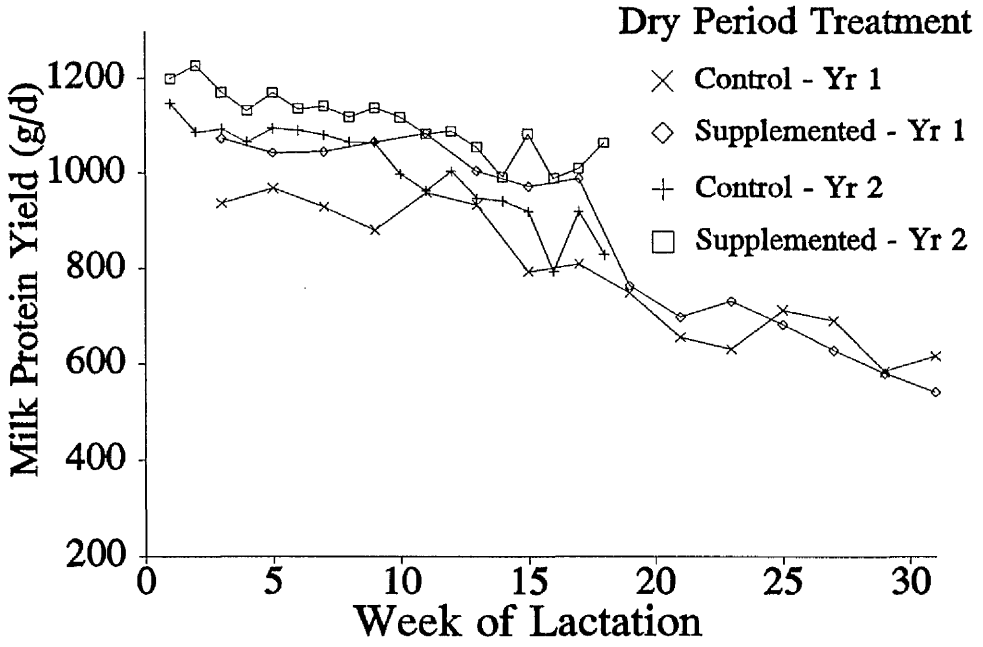


Figure 7.2 Comparison of the two years' protein yield data from animals that were fed the control or supplemented dry period diets.

to compare the blood albumin concentrations (Figure 7.3) with the milk protein concentrations, since the differences between groups for both variables are similar. If blood albumin concentrations are assumed to be an indication of the potential milk protein

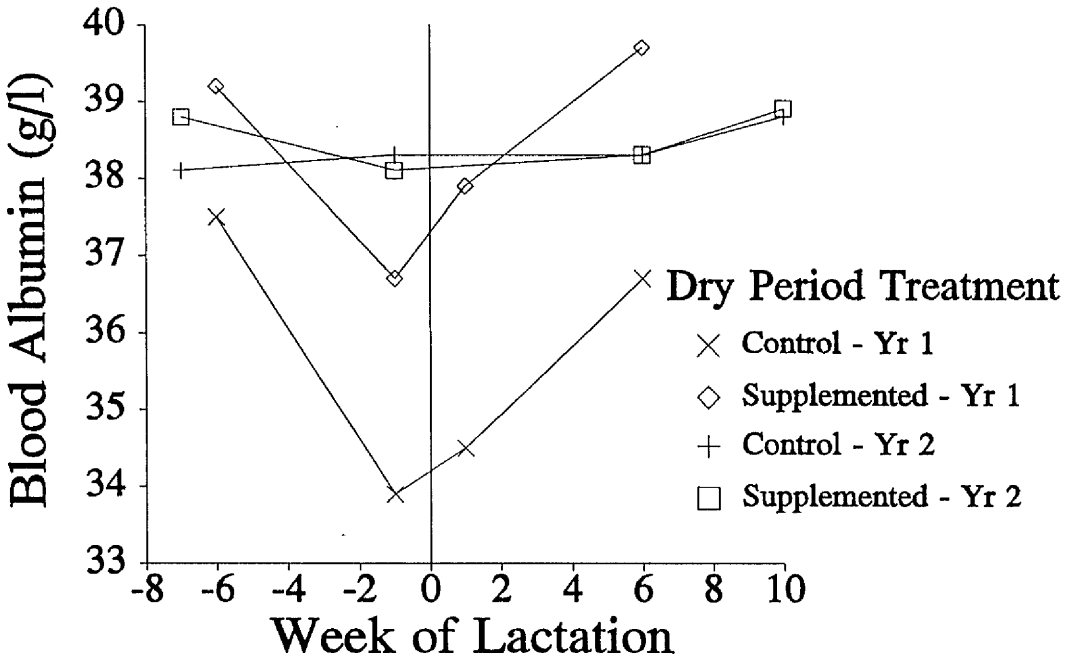


Figure 7.3 Blood albumin concentrations at different points during lactation from animals that were fed the control or supplemented dry period diet.

precursors in the blood, then there is a good relationship between the two. It also indicates that the protein reserves of the control group of animals may not have been as deplete as expected.

7.4 CONCLUSIONS

It has been shown that milk protein concentrations can be increased through dietary manipulation of the dairy cow, by both short and long term effects. It is apparent that milk protein production is not tied to that of lactose or fat, although the concentration of protein in milk depends on the partitioning of cellular energy into these two components. In order to increase milk protein concentration, one of the most important things is to achieve an efficient utilisation of rumen degradable protein. This will maximise microbial protein production, and reduce the amount of ammonia absorbed by the animal. This is turn will reduce ureagenesis and therefore spare amino acids from this process. Carbohydrates as a source of energy are better than fat in this respect, particularly those leading to a high propionate fermentation since, particularly at low concentrate levels, the effects of different fermentation patterns on milk protein concentration cannot be ignored. With a high propionate fermentation the amount of amino acids used for gluconeogenesis may be reduced. In the longer term, the nutrition and health of the dairy cow in late

pregnancy was shown to be particularly important for maximal milk production and milk protein concentration. The nutrition of the non-milking dairy cow is frequently forgotten; animals are fed a high ME, low metabolisable protein diet, and are unable to perform at their best during the subsequent lactation. The term 'dry cow' is not a good one.

7.5 FUTURE RESEARCH PROPOSALS

In any work of this kind, more questions are raised than are answered. It is therefore useful at this point to indicate the direction in which it would be valuable to proceed with research from the areas covered in this thesis, assuming the bounds of money and time are not too restrictive.

The most interesting finding of this thesis is the potential of residual effects of dry period feeding. The influence of protein reserves are strongly suspected, although the demonstration of this was not possible. Therefore, as a continuation of this work it would be valuable to perform nitrogen balance studies on animals which had been fed to replete or deplete the supposed labile protein reserves. To investigate the source of protein used to support foetal growth, to demonstrate the incorporation of body nitrogen into milk protein, and perhaps to indicate the length of time that protein reserves may influence lactation, pulse-chase experiments with ^{15}N-labelled compounds could be carried out. Diet selection may be a way in which dairy cows can regulate the nutrients needed to replete to appropriate levels their body reserves of fat and protein, and it would be interesting to investigate this area of research. Furthermore, the quality of the blood amino acid profile has been shown to be important for milk protein production; a potential research area would be the study of amino acid profiles of animals with depleted or replete protein reserves and the effect of this on milk protein production.

Similarly, mammary tissue protein turnover and its relationship to milk protein production is little understood. The mammary gland, being a very highly active organ, would be expected to have a very high tissue turnover rate, and recent evidence has suggested that milk protein production may utilise the products of mammary tissue degradation. This may lead to a reduced efficiency of protein production, and again stable isotopic work could be useful in quantifying the involvement of mammary protein turnover.

It has been demonstrated in the literature that there has been relatively little success in

increasing milk protein concentration by the infusion of certain, supposedly limiting, amino acids. This implies that the amino acid profiles chosen for infusion are not, in fact, limiting. There is therefore a potentially large amount of work needing to be done to identify the factors that were provided by the diets described in the thesis. Recent evidence has suggested that small peptides may be more important than originally envisaged. Future detailed work should therefore include peptides as possible sources of amino acids for milk protein production.

Milk protein concentration is a function of milk protein production and milk yield. Energy partitioning has been shown to be important in terms of fat production, and has been implicated in the control of milk volume. A more detailed study of energy partitioning, including the use of amino acids as a glucose source, would be beneficial in the understanding of the control of milk protein concentration. Those milk minerals which also help to control milk volume have been shown to vary to a greater extent than frequently considered in the literature. Again, an understanding of factors that control the flow of potassium, sodium and chloride into milk - and which may be influenced by intramammary energy partitioning - would help to understand the control of milk protein concentration.

Non-invasive techniques for studying dairy cow metabolism have the obvious advantages of welfare, cost and scale. As indicators of rumen function purine derivative excretion in urine and milk, and urea excretion in the milk have both been shown to be extremely valuable. In order to develop the purine derivative technique further, several questions need answering. The first major area to investigate would be the effect of diet - composition and presentation - on the extent of diurnal variation in PD excretion in the milk and urine of dairy cows. This could easily be achieved with the simple use of different feeding, sampling and milking regimes. The second question, in relation to PD excretion in milk, is the extent to which it is modified by the endogenous contribution of mammary metabolism. This may be investigated by the use of blood vessel catheterisation and the calculation of arterio-venous differences across the mammary gland. The rate of endogenous purine salvage may be investigated again using stable isotopes and pulse-chase procedures. It is likely that the production of endogenously derived purine derivatives is correlated with milk protein production, and this may offer the possibility of a correction factor if it is needed. Milk orotic acid concentration may be a useful alternative correction

factor since it is easily measured and may be present in milk in direct proportion to its utilisation in RNA production.

Finally, an area of research that has not been considered in this thesis is that of endocrine partitioning of nutrients. The mammary gland needs certain hormones for lactation, although there is little evidence to suggest that circulating hormones are directly responsible in the control of lactation, but this does not preclude the indirect partitioning effects of the endocrine system. Very little work to date has investigated the effects that nutrient intake has on the partitioning of those nutrients between the various parts of the animal, i.e. are certain nutrients, or certain nutrient profiles, partitioned towards lactation more effectively than others? It is suspected that hepatic metabolism may have an important influence on this, and therefore detailed *in vivo* investigations with lactating cows may be a good starting point for such research.

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