THE EFFECTS OF POLYOLS AND SELECTED STARCH SOURCES ON THE METABOLISM AND MILK PRODUCTION OF DAIRY COWS

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by

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SUMMARY

1. Aspects of digestion, energy metabolism and endocrinology were discussed in relation to nutrient supply and substrate utilisation for milk synthesis.

2. In Experiments 1 and 2 xylitol digestion and metabolism was investigated using animals fitted with rumen and intestinal cannulas. In Experiment 1 the rate of xylitol disappearance from the liquid phase of rumen digesta and the post-ruminal absorption of xylitol was measured in 3 sheep. The disappearance of a single dose of 60g xylitol given into the rumen in 1 l CrEDTA solution indicated that more than 95% of the xylitol escaped ruminal fermentation under these conditions. Less than 1% of xylitol given at levels of up to 60g/d by continuous intraruminal infusion was recovered at the duodenum. When xylitol was infused intraduodenally in amounts ranging from 15 to 60g xylitol/day approximately 35% of the dose was absorbed prior to the ileum.

In 3 non-lactating cows (Experiment 2) intraduodenal administration of 200g xylitol increased blood plasma glucose concentration. The response was smaller and more transient than that observed to 236g glucose given intraduodenally and indicated that 40-60% of the xylitol had been converted to glucose. Still smaller increases in plasma glucose concentration observed after 200g xylitol was given intraruminally were consistent with there having been a limitation to xylitol absorption imposed by dilution of the dose within the rumen and by the rate at which xylitol passed from the rumen.

3. Experiments 3 and 4 were of change-over design and were commenced in cows during weeks 2-3 of lactation. In each experiment isoenergetic diets which were designed to differ in the amount and type of glucogenic nutrients which they supplied were given. In Experiment 3, 8 cows were used to compare 4 diets in a duplicated Latin Square. A basal hay/dairy concentrate diet (45:55 on a dry matter (DM) basis) was given alone or with one of 3 supplements which supplied additional 20MJ ME/d. Supplementation with barley or ground maize resulted in similar increases (2.2 and 2.5kg/d respectively) in milk production, no changes in milk composition, and increases in blood plasma glucose concentrations and reductions in ketone body concentrations as compared with those for the unsupplemented diet. When the basal diet was supplemented with a xylitol/barley mixture supplying 500g xylitol/d the responses showed trends which were similar to those to the other supplements but which were comparatively small. The mean increase in milk yield when this supplement was added was 1.4kg/d.

Three diets were compared in Experiment 4. These were applied to 8 animals in a sequence dictated by a Youden Square. The diets were silage-based (51% of total DM) and contained soyabean meal and either barley, barley treated with an acidified-formalin preservative reagent to reduce the rate of ruminal breakdown of starch, or a combination of a xylitol-enriched polyol mixture absorbed onto palm kernel meal and barley. The mixture supplied 578g polyol/d including 329g xylitol/d. Milk yields, milk composition and blood plasma metabolite and hormone concentrations did not differ significantly between treatments.

The results of Experiments 3 and 4 indicated that during early lactation differences in the amounts of propionic acid and glucose absorbed from the gut of the order which could be achieved by commercially practicable modifications to dietary carbohydrate supply were in general unlikely to markedly alter milk production. The responses for the diets containing xylitol with or without other polyols were not those which would have been predicted from the results of Experiments 1 and 2 and indicated that during a relatively short period of exposure to polyols the rumen microbial population may have developed a greater ability to ferment xylitol.

4. The effects of dietary inclusion of polyols were investigated further in Experiment 5 in cows which, being freshly calved, were likely to be especially prone to energy and glucose insufficiencies. A hay/dairy concentrate (42:58 on a DM basis) basal diet was given. This was supplemented with either a polyol mixture absorbed onto palm kernel meal (supplying 410g polyol/d including 98.4g xylitol/d) or an isoenergetic amount of barley. Each diet was offered to a group of 10 cows from 2 weeks prior to calving to the 8th week of lactation. Six of the 10 animals receiving polyol developed clinical ketosis. None receiving barley supplement became clinically ketotic.

Dry matter, organic matter and nitrogen digestibilities did not differ between the 2 diets when measured in sheep (Experiment 6). In Experiment 7, 4 rumen-cannulated sheep received a hay/dairy concentrate diet for a 2-week control period. Polyol mixture was then substituted for concentrate in the diet so that, as in Experiment 5, polyol supplied 2.7% of total DM. This diet was given for a 3-week period after which the control diet was given for a

further 2 weeks. Total VFA concentration and especially acetate and propionate concentration was reduced and the ratio of the combined concentrations of acetate and butyrate to propionate was increased from an initial control value of 3.85 to 4.84 during the period of polyol inclusion. Effects were generally largest 14 days after the introduction of polyol and were partially reversed by day 21.

The marked hyperketonaemia and poor performance associated with the diet containing polyol in Experiment 5 were similar to the responses to xylitol observed in Experiment 3, but more extreme. Together with the effects of dietary inclusion of polyols on fermentation pattern (Experiment 7) these results demonstrated that not only are polyols unlikely to contribute to the success with which commercial products in which they are included in small amounts are used in the prophylactic treatment of clinical ketosis, but that in animals in negative energy balance polyols can induce effects which are detrimental to animal health and performance.

5. The results were discussed in relation to the effects which the precarious glucose status of the dairy cow in early lactation and the peculiarities of nutrient metabolism associated with this physiological state might have been expected to have on the relationships between dietary energy supply and milk yield and composition.

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SECTION I

INTRODUCTION

waste-products volatile fatty acids (VFA), which are absorbed and used by the host.

The microbial species present in the rumen differ in the main substrates which they ferment, and can be divided accordingly into cellulolytic, amylolytic, dextrinolytic and saccharolytic species, hydrogen utilisers and protozoa (Baldwin & Allison, 1983). By virtue of their ability to digest cellulose and hemicellulose the microbes release otherwise unobtainable nutrients to the host. The microbes degrade some dietary protein to ammonia and incorporate this, together with ammonia from endogenous sources of urea, into bacterial protein. They also extensively modify dietary lipid and themselves synthesise lipids de novo. The microorganisms also contribute to the host animal's vitamin supply.

Dietary components, bacteria and bacterial waste-products not absorbed from the reticulo-rumen flow through the omasum, where electrolytes and water are removed, to the abomasum. This is the true stomach, so called because it corresponds in function to the fundic and pyloric regions of the non-ruminant stomach. It is in the abomasum that the digesta are first subjected to the digestive processes of the host, mediated by the secretion of pepsin and hydrochloric acid.

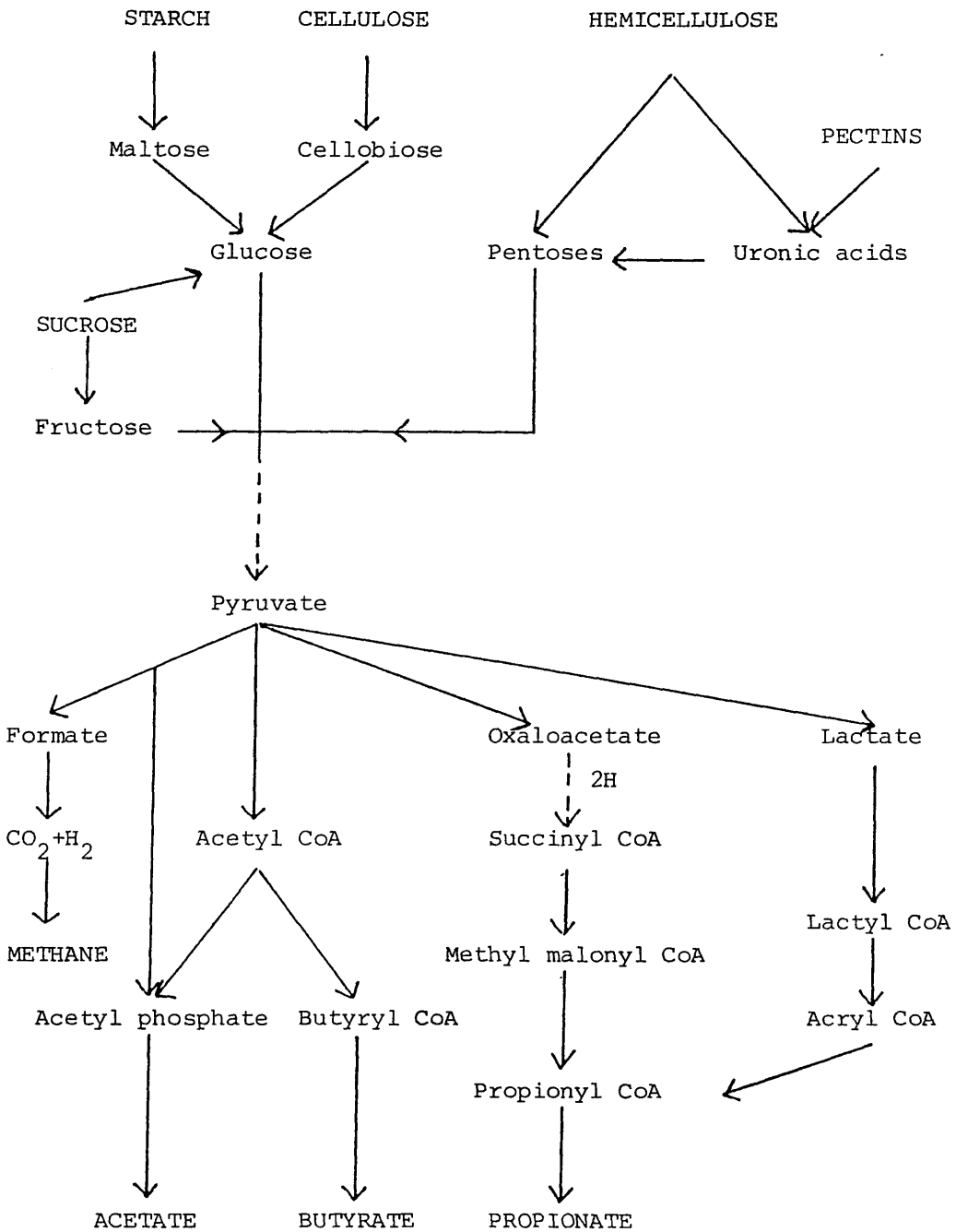
From the abomasum digesta flow into the duodenum, where bile and pancreatic enzymes are primarily responsible for the breakdown of bacteria and undegraded food residues to sugars, long-chain fatty acids and amino acids, prior to absorption. The contribution made by microbial fermentation to digestion in the small intestine is assumed

to be small because of the relatively high rate of passage of digesta through this region and the relatively low number of bacterial cells present, 10^5-10^6 cells/ml compared with 10^{10}-10^{11} cells/ml in the rumen (Harfoot, 1981).

Undigested food residues pass from the small intestine to the caecum and colon, where further microbial fermentation and some absorption of VFA occurs.

Digestion of carbohydrates. Carbohydrates comprise 68-80% of the dry matter in the diets of dairy cattle (Rook, 1976). The carbohydrate is present as simple sugars and as polymers - starch, pectin, cellulose and hemicellulose. Dietary sugars are almost completely fermented in the rumen (Beever, Thomson, Pfeffer and Armstrong, 1971) and little of the starch present in the food normally escapes microbial fermentation (MacRae & Armstrong, 1969). Although the digestibility of cellulose and hemicellulose varies with level of feeding and extent of lignification, about half of that in the diet is generally degraded by the rumen organisms (Mitchell, Little, Karr & Hayes, 1967; Watson, Savage, Brown & Armstrong, 1972). The pathways of microbial degradation of carbohydrate have been discussed recently (Baldwin & Allison, 1983) and are outlined in Fig. 1.01. It has been estimated that 60% of the digestible energy of most diets is fermented in the rumen (Sutton, 1976). The initial product of starch and cellulose breakdown is glucose, which undergoes glycolysis. Pentoses resulting from hemicellulose degradation enter the same pathway via fructose-6-phosphate and glyceraldehyde-3-phosphate. The pyruvate so formed is present in the rumen in very low concentrations as it is rapidly metabolised to VFA, CO_2 and

Fig. 1.01 Metabolic pathways of carbohydrate metabolism in the rumen (After MacDonald, Edwards & Greenhalgh, 1973).



methane. VFA have been calculated to contribute 48% of the dietary energy digested by frequently-fed lactating cows (Sutton, 1976). The major VFA are acetate, propionate and n-butyrate with small amounts of n-valerate, isovalerate and isobutyrate. These are absorbed across the rumen wall.

Dietary carbohydrate escaping degradation, together with microbial starch, passes into the small intestine. There the starch is subjected to the digestive enzymes of the host. Pancreatic juices from mature cattle possess strong amylase and weak maltase activities (Siddons, 1968). Both enzymes are present in the mucosa of the duodenum, jejunum and ileum, in each case the highest levels being in the jejunum (Hembry, Bell & Hall, 1967), where the pH of digesta has been shown to be near optimal for the enzymes (Lennox & Garton, 1968). Armstrong and Beever (1969) reasoned that maximal starch hydrolysis is likely to occur in the proximal half of the jejunum. Any starch escaping digestion there is likely to be fermented by the increasing number of microorganisms at the distal end of the small intestine, or subsequently in the caecum.

The post-ruminal digestion of cellulose and hemicellulose, being the result of microbial fermentation, is confined to the caecum and colon (Mitchell et al., 1967; Watson et al., 1972). Absorption of VFA from this region has been shown in sheep (Myers, Jackson & Packett, 1967). The remaining fermentation products are lost with the food residues in the faeces.

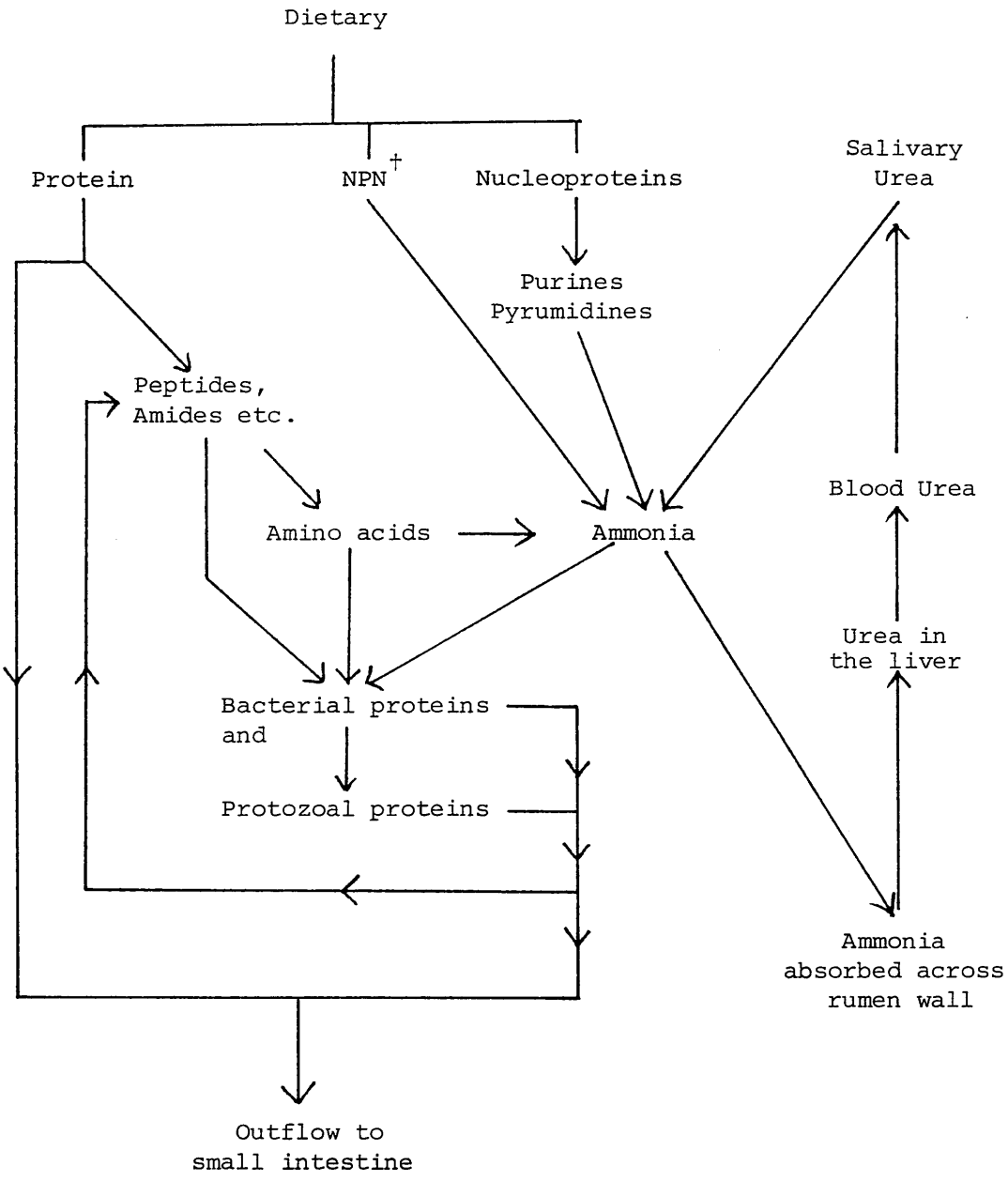
Digestion of nitrogenous compounds. Ruminal degradation of dietary nitrogenous compounds contributes to the supply of nitrogen for the synthesis of microbial protein, as described in the summary of

ruminal nitrogen metabolism given in Fig. 1.02. Proteolytic bacteria were found to comprise 38% of the total population isolated from the bovine rumen, and to include species of Butyrivibrio, Succinivibrio, Selenomonas and Bacteroides (Fulghum & Moore, 1963). The concentration of free amino acids in the rumen is very low (Annison, 1956). Under normal conditions the contribution made to the nitrogen uptake of the host by direct absorption of amino acids from the rumen is insignificant, as illustrated in sheep by the absence of an increase in α-amino nitrogen concentration in portal or arterial blood after the introduction of casein hydrolysate into the rumen (Annison, 1956). Instead, the amino acids are rapidly deaminated to ammonia, which constitutes the major source of nitrogen for the synthesis of bacterial protein (Bryant & Robinson, 1962). Dietary sources of non-protein nitrogen, for example urea, can be utilised for protein synthesis by bacteria possessing urease activity; 35% of bacterial species in sheep have been shown to be ureolytic (Jones, Macleod & Blackwood, 1964). Amino acids are also fermented to yield VFA (El-Shazly, 1952).

As much as 70% of the protein present in the rumen may be of microbial origin (Hungate, 1966). Protozoa, which have been estimated to engulf 40% of ruminal bacteria (Abe & Kandatsu, 1969), possess powerful intracellular proteases (Kudo, 1966), which enable them to meet most of their amino acid requirement from bacterial protein.

The efficiency with which dietary nitrogen is utilised by rumen microorganisms depends upon the concurrent availability of the energy required for synthetic processes (Hagemeister, Kaufmann & Pfeffer, 1976). A nitrogen to sulphur ratio of less than 10 is

Fig. 1.02 An outline of nitrogen metabolism in the rumen (After Church, 1976)



† Non-protein nitrogen

normally necessary to ensure that the level of sulphur is not limiting for microbial synthesis of methionine and cysteine (Hume & Bird, 1970). Much of the ammonia not used for synthetic purposes by microorganisms is absorbed from the rumen, and passes to the liver to be converted to urea. Some of this is recycled into the rumen in saliva (Somers, 1961) and through the rumen wall (Houpt & Houpt, 1968). Urea recycling has been suggested to be equivalent to 12% of the dietary nitrogen intake (Satter & Roffler, 1977). The remaining urea is excreted in the urine.

Modification of dietary nitrogen sources by rumen microorganisms results in the proteins entering the small intestine being less variable in amount and quality than those ingested (Bergen, Purser & Cline, 1968). The digestion of these proteins is initiated in the highly acidic conditions of the abomasum by the peptic enzymes of the abomasal secretions. Of the total nitrogen of the nitrogenous digesta at the duodenum 64-68% is amino acid-nitrogen and 5-10% is ammonia (Clarke, Ellinger & Phillipson, 1966). The apparent digestibility of amino acids in the small intestine ranges from 50 to 80% (Coelho da Silva, Seeley, Thomson, Beever & Armstrong, 1972; Salter & Smith, 1977). The acidic conditions which extend to the lower jejunum in sheep (Lennox & Garton, 1968; Kay, 1969) are thought likely to inhibit the activity of the pancreatic enzymes to such an extent that some α-amino nitrogen may pass undigested at the large intestine, where further degradation may make a small contribution to protein supply, through recycling of urea to the rumen from the caecum and colon (Kay, 1969).

Digestion of lipids. The lipids present in ruminant diets undergo rapid and complete hydrolysis in the rumen (Garton, Lough & Vioque, 1961) by non-selective bacterial lipases to release unesterified fatty acids. The glycerol produced is rapidly fermented to VFA, principally propionate (Hobson & Mann, 1961). Hydrolysis of the ester linkages is a prerequisite for hydrogenation (Keeney, 1970). Unsaturated C_{18} acids, which constitute a high proportion of the residues of the lipids present in ruminant feedstuffs, are extensively hydrogenated by the rumen bacteria (Bickerstaffe, Noakes & Annison, 1972). Protozoa may be dependent on some form of symbiotic relationship with bacteria if they are to hydrogenate fatty acids (Girard & Hawke, 1978). The predominant end-products of hydrogenation are stearic (80%) and trans isomers of octadecanoic acids (12%) (Bickerstaffe et al., 1972). These fatty acids are readily incorporated into microbial structural lipids together with those, including odd-numbered and branched-chain acids, synthesised de novo by both bacteria and protozoa (Emmanuel, 1974, 1978). There is little, if any, ruminal degradation of long-chain fatty acids and acids of C_{16} chain length or longer do not appear to be absorbed in significant amounts from the rumen (Garton, 1969).

As a consequence of the contribution from de novo synthesis the amount of fatty acids flowing to the duodenum exceeds dietary intake (Sutton, Storry & Nicholson, 1970; Knight, Sutton, Storry & Brumby, 1978). Knight et al. (1978), by measuring the incorporation of [1-^{14}C] acetate infused intraruminally in sheep receiving hay and concentrates, estimated microbial lipid to contribute 75% of the flow of fatty acids into the duodenum. The fatty acids of the intestinal contents are predominantly unesterified, but are closely associated

with the particulate matter of the digesta, as they are in the rumen (Ward, Scott & Dawson, 1964). Lennox and Garton (1968) showed the uptake of these fatty acids and the hydrolysis and uptake of esterified fatty acids to be virtually complete before the ileum. This absorption is dependent upon the formation of micelles in the presence of bile and is greatly reduced in the absence of pancreatic juice (Heath & Morris, 1963).

THE EFFECT OF DIET ON PRODUCTS OF DIGESTION

Carbohydrate. Dietary carbohydrate forms the main energy source for rumen microorganisms. Thus, the relative amounts of soluble carbohydrate, starch, hemicellulose and cellulose available in the rumen influence the balance of microbial species present, and hence the pattern of VFA production. Leng and Brett (1966) have suggested that, at least for forage diets, the molar proportions of the VFA in the rumen liquor are a good indication of the relative rates of their production. When forage diets are given these proportions are typically 64-74% acetate, 15-20% propionate and 8-16% butyrate (Thomas & Rook, 1977).

The influence of dietary composition on rumen metabolism has been summarised recently (Baldwin & Allison, 1983). Amylolytic microbes, such as are likely to be present in the rumen in increased numbers when the diet consumed contains high levels of starch, produce considerably more propionate than the cellulolytic species, which tend to predominate when high-forage diets are offered. In the latter situation acetate production is further increased by the

proliferation of saccharolytic organisms, which compete favourably for soluble carbohydrates and the products of starch hydrolysis. Thus, the relative production of acetate is generally decreased by a reduction in the proportion or availability for ruminal degradation of structural carbohydrate in the diet. Obversely, as the proportion of concentrate given is increased so is that of propionate and/or butyrate in the VFA mixture present in the rumen (Donefer, Lloyd & Crampton, 1962; Storry & Rook, 1966). Similarly, the addition of cereal concentrates to silage diets was found by Thomas, Kelly, Chamberlain and Chalmers (1980) to reduce the proportion of acetate, increase that of butyrate and produce little increase or occasionally a decrease in the proportion of propionate formed. The extent to which fermentation pattern is changed by altering the forage to concentrate ratio in the diet is modified by the feeding level and the physical and chemical characteristics of the forage and concentrates.

Level of feeding has little effect on the molar proportions of VFA produced when forages alone are given (Williams & Christian, 1956; Bath & Rook, 1963), but where diets consist of mixtures of hay and concentrates increased feeding level tends to reduce digestibility (Moe, Reid & Tyrrell, 1965) and to modify rumen fermentation, increasing the proportion of propionate or butyrate at the expense of acetate (Bath & Rook, 1963). A sudden introduction, at high levels, of diets containing a large proportion of readily-degradable carbohydrate favours the development of 'high propionate' types of fermentation (Thomas & Rook, 1977). This may be accompanied by an accumulation of lactic acid and a fall in pH, which disrupts the balance of the microbial population, reducing the numbers of

protozoa and methanogenic bacteria (see Schwartz & Gilchrist, 1975); in lactating cows there is an associated depression in milk fat synthesis.

With diets consisting solely of forage, alteration of the physical form of the diet has little effect. However, grinding or pelleting or heat-treatment of forages in mixed diets tends to increase the flow of cellulose and hemicellulose to the small intestine and reduce the molar proportion of acetate in the rumen (Balch, Broster, Rook & Tuck, 1965). The starch of cereal grain is highly digestible (Karr, Little & Mitchell, 1966; Topps, Kay & Goodall, 1968) but some processing of the cereal is normally necessary as cattle are inefficient at digesting whole grains (Wilson, Adeeb & Campling, 1973). Ruminal starch digestion may vary from 40% to in excess of 90%, depending on the degree of processing, the proportion of grain in the diet and the type of cereal offered (Waldo, 1973).

Milling, rolling, heating, expanding and micronizing of cereals increases rumen degradability. For example, steam-flaking can increase rumen digestibility of ground sorghum from 42% to 83% (McNeil, Potter & Riggs, 1971). The changes in VFA production associated with such an increase in rumen digestibility are similar to those which occur when the proportion of concentrates in the ration is increased; that is a reduction in the ratio of the molar proportion of acetate to that of propionate. The latter relationship is modified by cereal type; the reduction in acetate:propionate ratio associated with an increase in the level of cereal inclusion is smaller when a relatively poorly-degraded cereal such as maize is given (Sutton, Oldham & Hart, 1980). In this case there is instead

an increase in the duodenal flow and possibly in the faecal excretion of starch.

As a consequence of these factors the amount of starch, including that of microbial origin, passing to the duodenum varies from less than 5% to over 50% of that consumed (Sutton, 1976). As much as 83% of the starch entering the small intestine in cattle receiving diets containing ground maize is apparently digested before reaching the caecum (Armstrong & Beever, 1969). Although the negative correlation between starch content and pH of digesta at the terminal ileum is suggestive of some microbial fermentation of starch (Mayes & Orskov, 1974) and in exceptional circumstances a little starch may be lost in the faeces (Karr et al., 1966), a major part of the starch appears to be absorbed as glucose. Thivend (1973) showed replacement of hay with maize in the diet to be associated with increased glucose in the portal blood in lambs and dietary additions of ground maize and molassine meal increased the concentration of reducing sugars in the mesenteric vein draining the central third of the ileum in cows (Symonds & Baird, 1975). Furthermore Janes, Weekes and Armstrong (1985a) have recently shown in sheep fitted with re-entrant cannulas that starch disappearing from the small intestine when the animals received maize-based diets did so as glucose, and that as little of this was metabolised in the intestinal epithelium a large proportion entered the mesenteric venous blood. Assuming starch disappearing from the small intestine to be absorbed as glucose, Armstrong and Smithard (1979) calculated the contribution to the overall glucose requirement of the lactating cow made via this route for diets in which the major grain source was either barley or maize. These contributions were estimated to be

15-19% and 61-77% respectively, and in the former case the starch was suggested to originate entirely from microbial sources.

Nitrogenous compounds. The amino acid supply to the ruminant is derived solely from the absorption from the small intestine of amino acids originating from microbial protein synthesised in the rumen, undegraded or protected dietary proteins which pass through the rumen and endogenous secretions. The amino acid composition of the microbial protein varies little with diet (Weller, 1957), but the diet influences the amount of microbial protein synthesised in the rumen.

When the availability of energy in the rumen is insufficient the incorporation of ammonia released from dietary proteins into microbial protein is inefficient and ammonia is absorbed from the rumen and the nitrogen 'lost' as urea excreted in the urine. Conversely, where insufficient nitrogen is available the ATP generated from carbohydrate fermentation is not used efficiently because microbial growth is restricted. Maximal microbial protein synthesis has been shown in vitro (Satter & Slyter, 1974) and subsequently in vivo (Okorie, Buttery & Lewis, 1977) to be supported by a rumen ammonia concentration of about 5mg/100ml. In a study involving frequent sampling of large numbers of cattle receiving different diets, Satter and Roffler (1975) found mean ruminal ammonia concentration to exceed 5mg/100ml with diets containing 11-14% crude protein. This concentration of ammonia was reached at higher levels of dietary crude protein for rations of higher energy content.

Increases in the crude protein content of the diet up to at least 16% and in the case of silage-based diets as high as 24%

(Gordon, 1977) may be associated with an increase in the digestibility of the ration (see Oldham, 1980), with the possibility of a concurrent increase in feed intake. The extent to which digestibility is enhanced appears to be influenced by protein source. Digestibility was higher when isonitrogenous rations containing soya bean or peanut meal rather than cotton seed meal were given to cows (Van Horn, Zometa, Wilcox, Marshall & Harris, 1979).

The maximum incorporation of available ammonia with respect to the energy supplied has been calculated to be 33g and 22g of protein nitrogen synthesised/kg of organic matter apparently digested in the rumen for forage and cereal diets respectively (McMeniman, Ben-Ghedalia & Armstrong, 1976). The reduced efficiency of microbial synthesis with cereal diets may be the result of reduced rates of dilution of the liquid phase in the rumen (Kennedy, Christopherson & Milligan, 1976), low pH, or the presence of relatively large numbers of protozoa (Dennis, Arambel, Bartley & Dayton, 1983). Protozoa engulf bacteria and increase the recycling of nitrogen within the rumen (Abe & Kandatsu, 1969) and protozoa themselves are sequestered in the rumen so that only a limited amount of protozoal protein passes to the small intestine (Weller & Pilgrim, 1974).

The nitrogen required by the microorganisms can be supplied by the degradation of dietary protein or non-protein sources of nitrogen. The contribution made by dietary proteins is dependent on their ruminal degradability, which tends to be higher for proteins which are readily soluble (McDonald, 1952; Hendrickx & Martin, 1963). Estimates by Miller (1973) show feedstuffs to differ widely in the ruminal degradability of their protein, from 90% for barley to 30% for Peruvian fish meal. Satter and Roffler (1977) assumed that for

most diets 60% of dietary protein is degraded. Degradability may be reduced as the level of feeding and hence the rate of passage of digesta is increased, the effect being particularly pronounced for more resistant proteins. Feeding level does not appear important for highly soluble proteins. For example, Miller (1973) reported 75% of a sunflower seed meal supplement to be degraded in the rumen whether fed to lambs at 1.25 or 2.5 times maintenance.

The ruminal supply of ammonia may be supplemented by dietary addition of non-protein nitrogen (NPN), which is particularly beneficial when added to diets which are low in protein, or more precisely rumen-degradable protein, and high in energy. Once rumen ammonia concentration exceeds 5mg/100ml further supplementation of the diet with NPN is not advantageous (Satter & Slyter, 1974). Some NPN sources, for example urea, are rapidly hydrolysed (Pearson & Smith, 1943) and produce high peaks in the ruminal concentration of ammonia, which is consequently wastefully utilised. Attempts to avoid this have included the use of alternative NPN forms, for example biuret, which is more slowly hydrolysed (Schaadt, Johnson & McClure, 1966) or the use of inhibitors to urease (Brent & Adepoju, 1967).

The most effective way of altering the composition of the amino acid supply to the host is by the use of dietary protein sources which make a substantial contribution to the protein passing to the small intestine, and which differ in amino acid composition from microbial protein. The rumen degradability of dietary protein can be reduced by a variety of physical or chemical treatments. Heat treatment of forage, thereby denaturing and decreasing the solubility of the protein, results in an increased nitrogen flow at the duodenum

and uptake from the small intestine (Beever, Thomson & Cammell, 1976). Likewise, grinding and pelleting forages increases the amount of protein escaping ruminal degradation (Osbourn, Beever & Thomson, 1976). However, care must be taken to avoid a reduction in digestibility of the protein in the small intestine, as has been shown to occur when clover is heat-treated and processed (Beever, Thomson & Harrison, 1971). Chemical agents including aldehydes and tannins, by forming reversible cross-linkages with amino and amide groups, decrease the solubility of proteins at the pH of the rumen. The acidic conditions of the abomasum then release the protein to the host. The results of formaldehyde treatment of plant proteins are variable, however, and overprotection of dietary protein with high rates of application of formaldehyde reduces protein digestion in the small intestine. Faecal nitrogen excretion is generally elevated when formaldehyde-treated proteins are given (Chalupa, 1975).

In lactating cows 50-60% of the total protein entering the duodenum is likely to be of bacterial origin, depending on the type of diet and the source of dietary protein (Hagemeister et al., 1976). The major nitrogenous components of rumen bacteria are 75-85% protein and 13-19% nucleic acids (Smith, 1975). The latter are almost totally absorbed in the small intestine but are of little nutritional value to the host (Coelho da Silva, Seeley, Beever, Prescott & Armstrong, 1972). The digestibility of protozoal protein is higher than that of bacterial protein but protozoa constitute a relatively small proportion of the microbial protein entering the small intestine (Weller & Pilgrim, 1974). Smith (1975) concluded that 60-70% of microbial protein entering the duodenum is digested in the small intestine, with a further 10-20% fermented in the large

intestine where uptake of nitrogen is mostly in the form of ammonia (Ulyatt, Dellow, Reid & Bauchop, 1975). The digestibility of undegraded dietary protein varies widely with source and is influenced by treatments applied to protect the protein from ruminal degradation. Again, residual protein passing to the caecum and colon will be fermented and part absorbed as ammonia.

There is evidence of preferential uptake of essential amino acids in the small intestine (Ben-Ghedalia, Tagari, Bondi & Tadmor, 1974) and this is consistent with the demonstration in vitro that when rumen bacteria are incubated with proteolytic enzymes essential amino acids tend to be released more rapidly than non-essential amino acids (Burris, Bradley & Boling, 1974).

Lipids. Lipid usually comprises 5% or less of ruminant diets (Palmquist & Jenkins, 1980), though lipids of dietary origin entering the duodenum are augmented by those contributed from de novo synthesis by rumen microorganisms. The increase in amount of fatty acids entering the small intestine compared with that consumed is greater with high-concentrate diets and more so as the level of feeding is increased (Sutton et al., 1970).

Dietary additions of fat may adversely affect the rumen microbial population, thereby reducing the digestibility of cellulose and protein (Brooks, Garner, Gehrke, Muhrer & Pfander, 1954), the production of acetate and voluntary food intake (Bines, Brumby, Storry, Fulford & Braithwaite, 1978). The mechanism(s) by which this is brought about are incompletely understood. Fatty acids inhibit the growth of cellulolytic bacteria in vitro (Henderson, 1973), but

as discussed by Devendra and Lewis (1974) other mechanisms may also operate. These include the coating of dietary fibre by lipid, thereby preventing microbial attack, and impairment of the rumen microbes through effects on their cell wall permeability and metabolism. The effects of fatty acids are reduced where there are high dietary concentrations of cations which form insoluble complexes with the fatty acids, preventing their binding to dietary fibre and microorganisms (El Hag & Miller, 1972).

Effects of added lipid supplements on digestibility are variable but Palmquist and Jenkins (1980) concluded that for dairy cows in early lactation fat may safely be added at a level corresponding to 5% of the total ration. It is suggested that under these circumstances effects on the rumen population are minimised, particularly if calcium intake is sufficient for the formation of insoluble soaps, as a result of the high level of food intake and the rapid rumen turnover rate. The level at which fats can be included in the diet without detrimental effects on digestibility and intake can be increased if they are given as natural, unextracted seeds or in some 'protected' form which passes through the rumen intact, for example, as minute fat droplets coated with a layer of formaldehyde-treated casein (Scott, Bready, Royal & Cook, 1972).

THE EFFECTS OF THE SUPPLY OF ENERGY SUBSTRATES ON METABOLISM

Glucose metabolism. Under normal dietary conditions, and particularly in animals receiving high-forage diets, only small

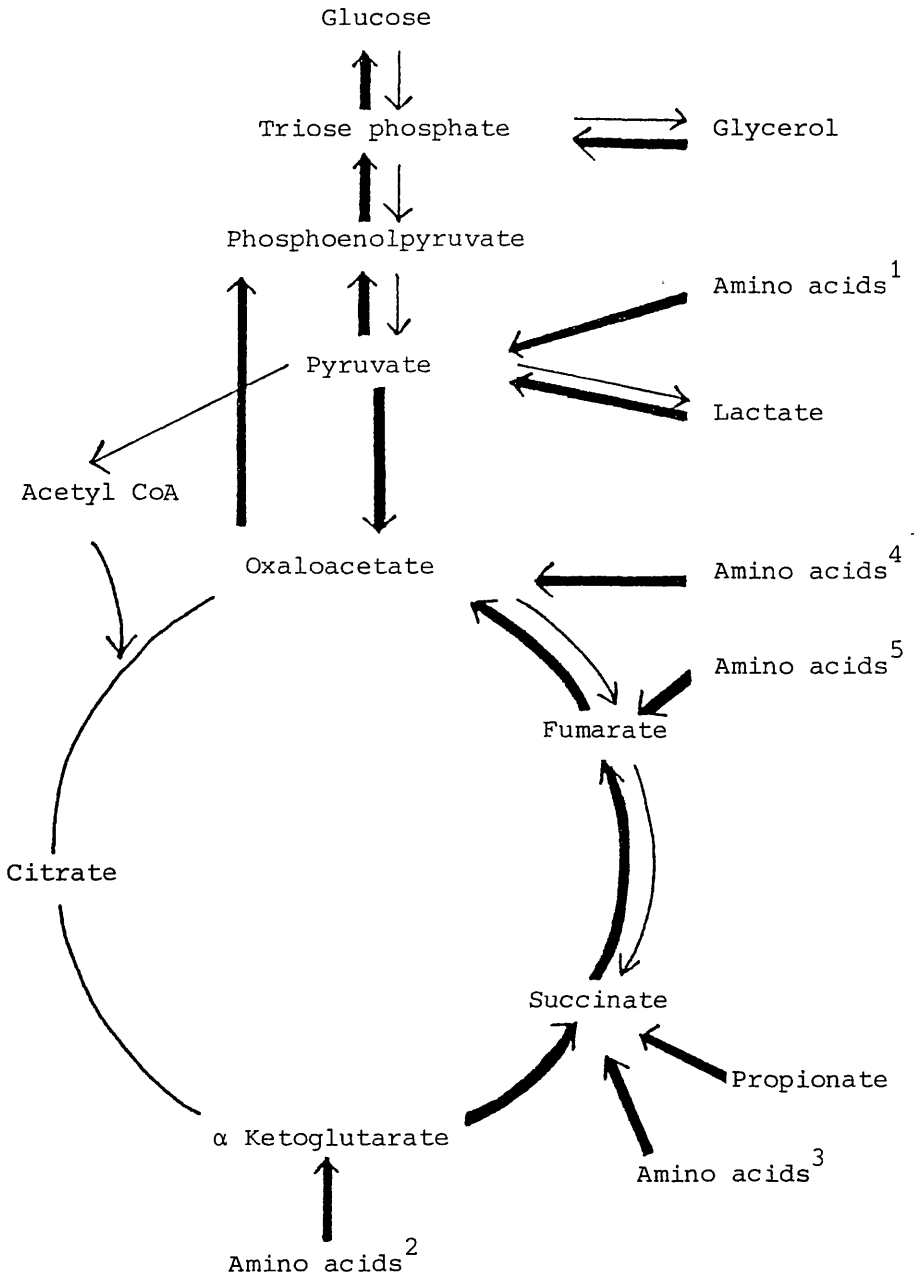
amounts of glucose are absorbed directly from the ruminant digestive tract (Heald, 1951; Bensadoun, Paladines & Reid, 1962). However, on a metabolic liveweight basis, glucose utilisation rates show glucose to be quantitatively almost as important in ruminants as in non-ruminants (Annison & White, 1961; Ballard, Hanson & Kronfeld, 1969). Glucose is essential for ruminant brain and erythrocyte metabolism (Lindsay, 1980) and as a precursor for muscle glycogen, and it is also utilised for the generation of NADPH required for lipogenesis. Moreover, glucose requirements are greatly increased during late pregnancy and lactation by the additional demands for foetal growth and lactose synthesis respectively (McDowell, 1983).

Normally, 90-100% of the glucose supply to ruminant tissues is derived by gluconeogenesis (Lindsay, 1978) from propionate and amino acids absorbed from the digestive tract, glycerol from triglyceride breakdown, lactate from brain, erythrocyte and muscle glycolysis and amino acids from protein turnover (Fig. 1.03). Acetate is not gluconeogenic but is an alternative substrate to glucose for oxidation in skeletal and cardiac muscle, adipose tissue, liver, kidney and the lactating mammary gland and for lipogenesis (Annison & Linzell, 1964; Holdsworth, Neville, Nader, Jarret & Filsell, 1964; Bird, Chandler & Bell, 1981).

Approximately 85% of gluconeogenesis occurs in the liver (Bergman, Katz & Kaufman, 1970) and the remainder in the kidney (Kaufman & Bergman, 1971).

Propionate, the only major ruminal VFA capable of contributing to glucose synthesis (see Bergman, 1973), may contribute as much as 40% of the total glucose produced in the fed animal. The other glucogenic VFAs, isobutyric and valeric acid contribute about 5%

Fig. 1.03 Pathways of gluconeogenesis (shown by heavy arrows) in the liver (After Leng, 1970)



[1] Alanine, Threonine, Glycine, Serine, Cysteine

[2] Arginine, Histidine, Glutamine, Proline

[3] Isoleucine, Valine, Methionine

[4] Aspartate, Asparagine

[5] Phenylalanine, Tyrosine

(Lindsay, 1978). The utilisation of propionate for renal gluconeogenesis is low in cattle (Shreeve, 1974) and most of the glucose synthesised from propionate is produced in the liver, which extracts 80-85% of the propionate available in the portal vein (Baird, Lomax, Symonds & Shaw, 1980). About 50% of this is converted to glucose through oxaloacetate and pyruvate and the remainder used to form other metabolites or oxidised to CO_2 (Bergman, Roe & Kon, 1966).

Propionate produced in the rumen also contributes to the supply of glucogenic precursors as lactate, which is formed by propionate metabolism in the rumen wall during absorption. However, observations suggest that the conversion of propionate to lactate is unlikely to be substantial (Weigand, Young & McGilliard, 1972; Weekes & Webster, 1974); Ash and Baird (1973) suggested that propionate is metabolised primarily in the liver as a result of autoregulation of the activation of VFA in rumen epithelium and liver. Lactate produced in the rumen wall and small amounts absorbed directly from the rumen normally comprise about half of the hepatic uptake of lactate in dairy cows, the remainder being derived endogenously, together with pyruvate, via the Cori cycle (Baird, Symonds & Ash, 1975). The contribution of lactate to hepatic gluconeogenesis in sheep is about 15% of the total glucogenic precursors (Annison, Lindsay & White, 1963). In contrast to this, lactate is the predominant metabolite removed by the kidney and may account for as much as 60% of net renal glucose output (Kaufman & Bergman, 1974). Thus, the total contribution of lactate to glucose synthesis in the body as a whole is normally approximately 28% (Lindsay, 1978).

The second important exogenous source of glucogenic substrate is amino acids absorbed from the small intestine. Many of the major amino acids, lysine, leucine and tryptophan being notable exceptions, can contribute to glucose synthesis via pyruvate or TCA cycle intermediates (see Rook & Thomas, 1983). Most of the amino acids in the portal blood are removed by the liver, the uptake of some exceeding the amount absorbed from the small intestine (see Table 1.01), the difference representing a net movement from peripheral tissues to the splanchnic viscera (Wolff, Bergman & Williams, 1972). Estimation of the glucogenicity of amino acids is complicated by hepatic protein synthesis and degradation and, in isotopic tracer studies, by the randomisation of some ^{14}C within the TCA cycle. However, Bergman and Heitmann (1978), in summarising the work done in this field, concluded that fed ruminants derive a minimum of 15% of their glucose from amino acids, and that alanine and glutamine contribute 40-60% of this.

As the rate of triglyceride turnover in adipose tissue is normally slow little glycerol is released to become available for gluconeogenesis. Approximately 50% of that removed by the liver and kidneys is utilised for glucose synthesis, contributing about 5% of the total glucose supply in the fed animal (Bergman, Starr & Reulein, 1968).

Lipid metabolism. While the liver is the most important site for gluconeogenesis, it is of minor importance in lipogenesis. Ballard et al. (1969) suggested that this may be a consequence of the relative activities in hepatic tissue of the enzymes involved in oxaloacetate utilisation. In the ruminant liver the high activity of

Table 1.01 Net uptake and release of some plasma amino acids and ammonia by portal-drained viscera and liver of fed sheep.

Amino acid	Portal viscera		Liver	
	Removed	Added	Removed	Added
Glycine		1.5	3.9	
Alanine		2.3	3.2	
Glutamine	1.5			
Glutamate	0.2			1.1
Serine		1.1	1.2	
Arginine		0.3	0.9	
Citrulline		0.5	0.1	
Ornithine		0.3		0.6
Leucine		1.1	0.4	
Isoleucine		0.7	0.3	
Valine		0.9	0.5	
Threonine		0.7	0.8	
Tyrosine		0.6	0.9	
Phenylalanine		0.8	1.1	
Ammonia		29.0	30.3	

Values in mmol/hr. All rates, except citrulline and valine removal by liver, were significantly different from zero. Mean weight of sheep was 50kg and all were fed 800g alfalfa pellets per day at 1 hour intervals. Mean portal and hepatic plasma flow was 1.3 and 1.6 l/min.
(From Bergman and Heitmann, 1978)

phosphoenolpyruvate carboxylase and low activities of NAD-malate dehydrogenase and NADP-malate dehydrogenase, as well as the low level of NADH favour gluconeogenesis whereas conditions in adipose tissue encourage lipogenesis, and in the ruminant this tissue is the major site for the uptake, synthesis and storage of lipids. As Bauman and Davis (1975) pointed out, the separate location of gluconeogenesis and lipogenesis avoids competition for precursor substrates, reducing equivalents and energy.

The main source of exogenous fatty acids taken up by adipose tissue appears to be the triglycerides of plasma lipoproteins (Vernon, 1980). Fatty acids are made available by the action of lipoprotein lipase, and are readily incorporated into adipose tissue triglycerides. The fatty acids are predominantly saturated, regardless of the nature of dietary fat, as a result of extensive hydrogenation within the rumen (Dawson & Kemp, 1970).

Ruminant adipose tissue metabolism also involves the storage of excess energy as endogenously synthesised fatty acids and this tissue is the site of 90% of such synthesis in the non-lactating ruminant (Ingle, Bauman & Garrigus, 1972). These fatty acids are derived almost totally from acetate (Baldwin, Reichl, Lewis, Smith, Yang & Osborne, 1973), which is incorporated into fatty acids much more rapidly than glucose. The low utilisation of glucose for lipogenesis is associated with relatively low activities of two key enzymes, ATP citrate-lyase and NADP-malate dehydrogenase (Bauman, 1976). Vernon (1980) points out, however, that it is unclear whether this causes the low glucose utilisation or whether the low enzyme activities are simply a consequence of the generally low plasma glucose availability in ruminants, especially as diets which increase

glucose availability also increase ATP citrate-lyase activity and the rate of glucose utilisation for lipogenesis (Ballard, Filsell & Jarret, 1972). Lactate (Prior, 1978) and pyruvate (Whitehurst, Beitz, Pothoven, Ellis & Crump, 1978) can contribute to lipogenesis, but at a lower rate than acetate. Butyrate and β-hydroxybutyrate, which is formed during absorption of butyrate from the rumen, can as CoA esters replace acetyl CoA as primers for fatty acid synthesis in the ruminant mammary gland (Moore & Christie, 1979) and probably in adipose tissue also (Bauman, 1976).

NADPH necessary to support fatty acid synthesis is generated by the metabolism of glucose in the pentose phosphate pathway and glucose and acetate in the isocitrate dehydrogenase cycle (Bauman, 1976). Glucose is also the major precursor of glycerol-3-phosphate required for fatty acid esterification (Vernon, 1980).

Pothoven, Beitz and Zimmerli (1974) found palmitic, stearic and oleic acids to be the major fatty acids synthesised de novo in bovine adipose tissue slices.

The energy and hormonal status of the animal influence the relative rates of lipogenesis and lipolysis and hence the balance between fatty acid storage and mobilisation in adipose tissue. Lipolysis involves the hydrolytic cleavage of triglycerides to free fatty acids (FFA) and glycerol. Initiation of this in the non-ruminant involves the activation of a cyclic AMP-dependent protein kinase which then activates a hormone-sensitive lipase. Although the levels of these enzymes have not been measured in ruminants, indirect evidence suggests that the control of lipid mobilisation in ruminants and non-ruminants is similar (Bauman, 1976). Mobilised lipid is predominantly in the form of long-chain

FFA (Frederickson & Gordon, 1958). These are mainly removed by the liver (Stein & Shapiro, 1959), which extracts about 10% of the FFA supplied in the plasma (Katz & Bergman, 1969). The rate of β-oxidation of these fatty acids is thought to be dependent upon their availability (Fritz, 1961) but the capacity of the ruminant liver for complete oxidation of long-chain fatty acids appears to be limited. The greater proportion of fatty acids is incompletely oxidised, yielding ketone bodies and acetate (Connelly, Head & Williams, 1964), which are used as energy sources in skeletal muscle, kidney, mammary gland and gut tissues (Baird, 1977). This proportion appears to increase as the availability of FFA for hepatic oxidation increases (Mayes & Felts, 1967).

Endocrine status. Insulin and glucagon, the major hormones involved in the short-term regulation of metabolism and nutrient utilisation, together with only growth hormone of those having longer-term actions and which are involved to a lesser extent, show patterns of secretion clearly related to nutrient status (Bassett, 1980). Thyroxine may also modify metabolism in the longer term, as indicated by the negative correlation between changes in the concentration of this hormone and changes in milk yield during lactation (Hart, Bines & Morant, 1979), but patterns of secretion of thyroxine do not appear to be related to nutrient absorption (Bines, Hart & Morant, 1983).

The regulation of pancreatic endocrine activity appears to be the result of complex and incompletely understood interactions between direct neural stimulation and inhibition of the islet cells, local paracrine effects, hormones released from the gastrointestinal tract and circulating metabolite levels (Bassett, 1980). By effects

operating in some or all of these ways, diet and the end-products of digestion are of major significance in the stimulation of hormone release.

Both insulin and glucagon secretion are stimulated by feeding (Bassett, 1972): Bassett (1980) suggested that the control mechanisms involved are likely to be the same for the two hormones. The initial increase in secretion associated with ingestion is thought to be mediated by reflex neural stimulation of the pancreatic islets (Bassett, 1980). However, Bassett, Weston and Hogan (1971) found the magnitude of the insulin response in sheep receiving a variety of diets to be positively correlated with the amount of organic matter digested and this suggests that there is some direct relationship between secretion and nutrient availability.

The importance of VFA in eliciting the release of pancreatic hormones is equivocal. Despite the insulinogenic actions of propionate and butyrate when administered intravenously (Manns & Boda, 1967) and their stimulation of glucagon release from the autotransplanted ovine pancreas (Arcus, Ellis, Kirk, Beavan, Donald, Hart & Holland, 1976), Bassett (1975) argues that at physiological levels VFA are unlikely to be important regulators of insulin and glucagon secretion, as the amounts of propionate and butyrate normally absorbed from the rumen are rapidly and almost completely removed by the liver, while acetate, the only VFA reaching the peripheral plasma in substantial amounts is not insulinogenic (Manns & Boda, 1967). However, Bines and Davey (1978) found, particularly for high-concentrate diets, that the levels of organic acids in jugular plasma were increased after feeding while Ross and Kitts (1973) found increases in insulin concentration to coincide with

post-prandial increases in plasma propionate and butyrate concentrations. Recently too, Brockman (1982) has shown, by infusing propionate and butyrate at physiological levels into the hepatic portal veins in sheep, that both propionate and butyrate increase plasma insulin, while butyrate, but not propionate, increases plasma glucagon concentration. The lack of a clear dose-response relationship within physiological limits led the author to suggest a modifying, rather than a regulatory, role for these VFA with respect to insulin and glucagon secretion. Further evidence for the importance of VFA in the stimulation of insulin secretion is provided by the results of intraruminal infusion of a mixture of acetic, propionic, butyric and iso-valeric acids in cows (Bines, Hart & Napper, 1980; Bines & Hart, 1982). The mixture was infused 30 minutes after feeding hay, such that VFA concentrations in peripheral blood corresponded to those in animals receiving hay plus 7kg of concentrate. While infusion of the complete VFA mixture elicited an insulin response similar to that found with the concentrate diet, omission of propionate, which had provided 27% of the energy of the VFA mix, reduced the insulin response by 70%, suggesting an important role for propionate in the regulation of insulin secretion. Under normal circumstances changes in plasma glucose concentration do not appear to regulate insulin secretory response to ingested nutrients (see Bassett, 1978), which is not unexpected in view of the small amount of glucose absorbed from the ruminant digestive tract.

The end-products of carbohydrate digestion may also influence the release of gut hormones, including enteroglucagon. Benzins and Manns (1979) found that concentrations of this hormone, a partial antagonist to glucagon, increased in the plasma of cattle given

increasing proportions of barley in their diet. Furthermore, these authors reported that, of a range of metabolites infused intraduodenally, only glucose stimulated enteroglucagon release and it was postulated that this hormone had a role in the inhibition of gluconeogenesis in animals receiving diets leading to a high uptake of glucose from the small intestine.

This work also showed that intraduodenal infusion of amino acids increased plasma insulin and glucagon levels; but again, as the levels of amino acids reaching the peripheral system in the post-prandial period are not normally greatly increased (Wolff et al., 1972), Bassett (1980) argued that plasma amino acid level is unlikely to be an important physiological regulator of pancreatic endocrine secretion at this time. However, as insulin concentration does appear to be closely related to protein digestion within the intestine (Bassett et al., 1971), an indirect effect, mediated by gastrointestinal hormones may be involved.

The release of growth hormone from the pituitary is episodic (Hove & Blom, 1973; Tindal, Knaggs, Hart & Blake, 1978), and because of the large variability in growth hormone levels in adult ruminants the relationships between growth hormone and metabolite levels are unclear, especially as they appear to result from a complex interaction of effects. In general, however, growth hormone secretion appears to be inversely related to energy status and therefore to be influenced by food intake.

Thus growth hormone levels have been shown to fall rapidly in the post-prandial period, remaining low for several hours before increasing prior to the next meal (Hove & Blom, 1973; Bassett, 1974; Driver & Forbes, 1978). Recent studies in goats suggest that the

initial suppression of growth hormone secretion during feeding may be neurally-effected following stimulation of stretch receptors in the cranial rumen (Tindal, Blake, Simmonds, Hart & Mizuno, 1982). The pattern of growth hormone secretion suggests that there is also some relationship with levels of circulating metabolites (Weekes & Godden, 1980), though the influence of VFA absorbed from the rumen during the post-prandial period is unclear. McAtee and Trenkle (1971) failed to show an effect of intravenously infused VFA, whereas others have found an apparent inhibition of growth hormone secretion in response to infusion of VFA intraruminally (unpublished results cited by Bines & Hart, 1982) or intravenously (Hertelendy & Kipnis, 1973). Bassett (1974) found minimum growth hormone levels to coincide with maximum plasma VFA concentrations after feeding. Bassett's work showed too an inverse relationship between plasma growth hormone and FFA concentrations, which is consistent with the proposal that decreasing FFA levels may provide a stimulus to growth hormone secretion (Hertelendy & Kipnis, 1973; Reynaert, de Paepe, Marcus & Peeters, 1975). It is uncertain whether growth hormone secretion is influenced by plasma glucose levels, although there are indications that an increase in growth hormone secretion may be associated with a rapid decrease in plasma glucose levels (Trenkle, 1971). Bassett (1975) has suggested that these effects may be mediated by effects on the secretion of hypothalamic stimulatory and inhibitory hormones, including somatostatin.

In contrast to the metabolites already discussed, there is some evidence of a positive association between the products of protein digestion and growth hormone secretion. Although intravenous infusion of pharmacological levels of amino acids has been shown to

increase plasma growth hormone levels (McAtee & Trenkle, 1971), the concentration of this hormone was not significantly correlated with total plasma amino acid concentration in sheep receiving a variety of diets (Bassett et al., 1971). However, the presence in the small intestine of products of protein digestion appears to influence growth hormone secretion. Abomasal infusion of casein increased plasma growth hormone levels in goats (Oldham, Hart & Bines, 1978). Moreover a growth hormone response, in the absence of changes to milk yield and composition, in cows given protected casein indicated the elevated growth hormone concentration to be a direct result of an increase in α-amino nitrogen in the small intestine, rather than an indirect effect of an apparent energy deficit resulting from increased milk synthesis (Oldham, Hart & Bines, 1982).

The regulation of energy metabolism. The processes involved in energy metabolism are regulated by substrate availability and thus to a large extent by dietary inputs, as well as by hormonal status, which is itself interrelated with nutrient availability.

The rate of gluconeogenesis and the relative contribution of each precursor to glucose supply is primarily determined by the availability of glucogenic substrates for hepatic uptake (Annison et al., 1963; Bergman et al., 1968; Judson & Leng, 1973), and thus to a large extent by the mixture of end-products of digestion. Consequently, gluconeogenesis is maximal in the post-absorptive ruminant (Thompson, Bassett & Bell, 1978), whereas during food deprivation the rate of gluconeogenesis is reduced (Kronfeld & Simesen, 1961; Baird, Reid, Lomax, Symonds, Roberts & Mather, 1977), as is the relative contribution from propionate, which is available

only by absorption from the digestive tract, whilst there is a compensatory increase in the relative contribution of endogenous lactate, pyruvate and glycerol (Baird et al., 1977). Likewise, dietary conditions which encourage high levels of ruminal propionate production tend to increase the relative contribution made by this substrate to glucose supply (Wiltrout & Satter, 1972). However, this relationship may be modified if the ratio of glucogenic VFA in the rumen to glucose released in the small intestine is concurrently reduced. Judson, Anderson, Luick and Leng (1968) found in sheep that a smaller proportion of glucose synthesised in the liver was derived from propionate when the amount of glucose absorbed directly from the small intestine was increased.

Substrate supply also directly influences adipose tissue metabolism, although Vernon (1980) has suggested hormonal changes to be more important in the regulation of lipid metabolism. Like gluconeogenesis, lipogenesis is maximal during the post-absorptive period (Brockman, 1978a) and is reduced during food deprivation, when the availability of ruminal acetate is reduced (Pothoven & Beitz, 1975). Although the pattern of ruminal VFA production in animals receiving high-concentrate diets is likely to reduce acetate availability for lipogenesis, the associated hormonal changes are likely to be the major influence on adipose tissue metabolism. Dietary supplementation with lipid, which increases the availability of preformed fatty acids to adipose tissue, generally suppresses de novo synthesis (Hood, Cook, Mills & Scott, 1980).

Experiments in vitro suggest that the rate of lipolysis is insensitive to variations in the availability of glucose, acetate, propionate or acetoacetate, although β-hydroxybutyrate appears to inhibit lipolysis (Metz & van den Bergh, 1972).

The importance in the control of energy metabolism of endocrine responses to nutritional and physiological status is less well understood, as the interpretation of plasma hormone levels is complicated by a number of factors. These include variation in the numbers and binding affinities of hormone receptors and in some cases the episodic or circadian patterns of hormone release and the influences of day length, temperature and adaptation to environment, as well as the interrelationships between hormones (see Trenkle, 1978; Weekes & Godden, 1980). Bassett (1975), however, suggested that in animals in the fed state, hormonal modification of energy metabolism might be expected to maximise gluconeogenesis from propionate and the oxidation and entry into lipogenesis of acetate, while directing amino acids towards tissue synthesis. The actions of glucagon and insulin, which dominate the regulation of metabolite utilisation in the liver and peripheral tissues respectively (Brockman, Bergman, Joo & Manns, 1975; Bauman, 1976; Lomax, Baird, Mallinson & Symonds, 1979), are important in the achievement of this.

Insulin acts on the liver to inhibit gluconeogenesis (Prior & Christenson, 1978) and glucose release but has little effect on glucose uptake, which is negligible in the ruminant, as is consistent with the virtual absence of hepatic glucokinase activity (Ballard et al., 1969). These effects, which seemingly offer no benefit, particularly during the post-prandial period, are however counteracted by concurrent increases in substrate supply and glucagon concentration. Glucagon promotes hepatic uptake of amino acids, lactate (Brockman et al., 1975) and propionate (Brockman, 1978b) and, when insulin levels are low, stimulates lipolysis in adipose tissue, thereby releasing glycerol and increasing the availability of

this substrate for gluconeogenesis (Brockman et al., 1975). It appears that, in addition to stimulating hepatic uptake of glucogenic substrates, glucagon increases their conversion to glucose, at least in the sheep, by increasing the activity of pyruvate carboxylase (Brockman & Manns, 1974; Brockman, 1978b).

It is through actions exerted in non-hepatic tissues that insulin has important effects on energy metabolism, although in adipose tissue these are thought to be achieved in the main indirectly (Prior & Smith, 1982). Glucose uptake and utilisation by peripheral tissues is increased in vitro (Yang & Baldwin, 1973) and in vivo (West & Passey, 1967) by insulin. In adipose tissue this appears to increase the availability of NADPH and glycerol-3-phosphate, thereby encouraging fatty acid synthesis and esterification (Prior & Smith, 1982). Insulin also stimulates adipose tissue lipoprotein lipase, thereby increasing uptake of preformed fatty acids and acetate (Emery, 1979). In addition, Vernon (1980) has suggested that the above changes, together with insulin-induced reduction in lipolysis demonstrated to occur in ruminants following insulin administration (Yang & Baldwin, 1973), might be expected to lead to a decrease in the concentration of long-chain fatty acids. This would then activate acetyl-CoA carboxylase and hence increase lipogenesis. The importance of this has yet to be clearly demonstrated in the ruminant, although Pothoven and Beitz (1975) found that levels of acetyl-CoA carboxylase were reduced during starvation in steers, and that this coincided with reduced lipogenesis. This led the authors to suggest that acetyl-CoA carboxylase was limiting fatty acid synthesis under these conditions - the activities of the NADPH generating enzymes were reduced by

starvation but the responses were delayed and small. The sensitivity of acetyl-CoA carboxylase to dietary energy supply has since been confirmed by Scott and Prior (1977), and increased activities of various NADP-linked dehydrogenases has been shown to be associated with consumption of high-concentrate diets (Martin, Wilson, Cowan & Sink, 1973).

Although glucagon is lipolytic in ruminants (Bolton, Weekes, Godden & Armstrong, 1983), under normal physiological conditions this effect is weak and is easily offset by the antilipolytic action of insulin (Brockman, 1976, 1978a).

Thus it is the ratio of insulin to glucagon rather than their absolute concentrations, which appears to be important in ensuring a balance between the anabolic actions of insulin on adipose tissue and muscle metabolism and the stimulation by glucagon of catabolism and gluconeogenesis (Unger, 1971). Bassett (1975) suggested that a reduced insulin:glucagon ratio when energy supply is insufficient would maximise gluconeogenesis and minimise utilisation of glucose in the peripherial tissues, whereas when surplus energy is available the ratio would be increased and the anabolic actions of insulin predominate, thus maintaining glucose homeostasis.

Growth hormone has a range of effects on protein, carbohydrate, fat and mineral metabolism which appear to be mediated by different moieties of the growth hormone molecule (see Hart, 1980). Though the mechanisms involved are poorly understood, the major effects of this hormone appear to be to enhance protein deposition and inhibit proteolysis in skeletal muscle while opposing the anabolic effects of insulin with respect to glucose and fatty acid metabolism. Whether growth hormone directly stimulates

gluconeogenesis is unclear (McDowell, 1983). However, the diabetogenic actions of the hormone inhibit glucose uptake by peripheral tissues (Wallace & Bassett, 1966; Hart, 1983) and have been shown to antagonise insulin-stimulated lipogenesis from acetate and glucose in ovine adipose tissue in vitro (Vernon, 1978). Subcutaneous injections of growth hormone increase plasma FFA concentrations in dairy cows (Williams, Lee, Head & Lynch, 1964; Peel, Bauman, Gorewit & Sniffen, 1981). The proposed lipolytic action of growth hormone is supported by the positive correlation between plasma levels of growth hormone and FFA that exists throughout lactation (Smith, Hansell & Coppock, 1976; Hart, Bines, Morant & Ridley, 1978; Cowie, Forsyth & Hart, 1980). Thus the ratio of insulin to growth hormone concentrations might be expected to be an important influence on the utilisation of energy metabolites (McDowell, 1983).

Other hormones which may influence energy metabolism include thyroid hormones, which may do so by stimulating gluconeogenesis (Heitzman, Hibbitt & Mather, 1971) or indirectly by altering metabolic rate. Although various workers have suggested a role for prolactin in promoting lipogenesis (Swan, 1976; Bauman, Eisemann & Currie, 1982) others have failed to find evidence to support this (Hart et al., 1978).

ENERGY METABOLISM DURING LACTATION

Requirements of the mammary gland. The mammary gland extracts precursors from the blood for the synthesis of milk lactose, for the milk fatty acids, about half of which are synthesised in the gland

and half of which are absorbed preformed, and for more than 90% of the milk crude protein (Thomas, 1975).

Glucose is required for lactose synthesis (Barry, 1964) and, as lactose is the main osmotic constituent in milk, glucose availability is an important determinant of milk yield (Linzell & Peaker, 1971). It has been calculated that a cow producing 40kg of milk requires 3045g of glucose each day (Elliot, 1976). Some glucose is converted to glycerol for milk triglyceride synthesis, but little glucose is incorporated into fatty acids (Bauman, Brown & Davis, 1970). Glucose oxidation accounts for 29-49% of CO_2 production by the mammary gland in goats (Annison & Linzell, 1964). It has been suggested however, that this is to a large extent the result of metabolism via the pentose phosphate pathway, thereby providing NADPH for synthesis of fatty acids de novo, rather than the production of ATP (Smith, Crabtree & Smith, 1983).

Acetate is taken up in substantial amounts by the bovine mammary gland (Bickerstaffe, Annison & Linzell, 1974) and is, in goats, the source of 23-27% of mammary gland CO_2 (Annison & Linzell, 1964; Annison, Linzell, Fazakerly & Nichols, 1967). It is probably the major supplier of energy to the gland, as it is oxidised to CO_2 only via the TCA cycle (Davis & Bauman, 1974). Acetate together with β-hydroxybutyrate, which makes a relatively small contribution, and mainly of C_4-units (Palmquist, Davis, Brown & Sacham, 1969), is utilised for the synthesis of short and medium-chain fatty acids de novo (Annison & McDowell, 1980). Thus Bickerstaffe et al. (1974) found that in lactating cows 25-50% of milk triglycerides were derived from acetate while 35-80% were derived from preformed long-chain fatty acids transferred from plasma triglycerides. FFA

are taken up by the gland, but in the fed animal the amounts are matched by FFA simultaneously released from triglycerides hydrolysed by lipoprotein lipase during the uptake of lipids by mammary tissue (Bickerstaffe et al., 1974). Long-chain fatty acids do not appear to contribute to mammary gland energy supply in the fed animal (Annison et al., 1967), but during early lactation (Kronfeld, 1965) and fasting (Linzell, 1967), when arterial FFA concentration is elevated, there is a net uptake of FFA across the gland and long-chain fatty acids are oxidised to supply energy for milk synthesis.

Mammary uptake of essential amino acids is sufficient to provide for their secretion in milk protein (Bickerstaffe et al., 1974). Some non-essential amino acids are extracted in amounts in excess of requirements for milk protein, while the uptake of others is inadequate. Thomas (1975) suggested that, in view of evidence for the interconversion of amino acids and their de novo synthesis from glucose and acetate within the udder (Mepham, 1971) requirements for milk protein synthesis are met by these processes.

Metabolic adaptation to lactation. The mechanisms by which metabolism is modified through endocrinological changes to meet the demands for milk synthesis during lactation appear to involve the combined effects of homeostasis and what has been termed homeorhesis (Bauman & Currie, 1980). The latter is defined as the integration of the metabolism of body tissues to support a particular physiological state.

Although the dietary intake of glucogenic substrates is normally high during early lactation, Smith et al. (1976) and

Schwalm and Schultz (1976) found plasma glucose concentrations to be reduced in cows at this time, reflecting the high demand for glucose for lactose synthesis. These authors suggested that the low glucose concentration in part explained the concurrent reduction in plasma insulin concentration. The reduced rate of insulin secretion also appears to be a consequence of a decrease in the sensitivity of the pancreas to insulinotrophic agents in lactating cows (Lomax et al., 1979). Insulin uptake by the mammary gland (Beck & Tucker, 1978) may also contribute to the reduction in the plasma concentration of this hormone. The effects of these changes may be augmented by a decrease in adipocyte insulin receptor numbers as found during fat mobilisation during pregnancy in sheep (Vernon, Clegg & Flint, 1981) and possibly an increase in receptor numbers on mammary cells, as found in rats at parturition (O'Keefe & Cuatracasas, 1974). Homeorhetic roles for progesterone and prolactin in initiating and maintaining these changes were discussed by Bauman and Currie (1980), but the importance of these hormones in this respect has yet to be demonstrated clearly in ruminants.

The effects of reduced insulin concentrations are interrelated with those of glucagon and growth hormone status. There is some evidence that glucagon levels may increase at the onset of lactation (Manns, 1972) and increased growth hormone concentration has been noted in high-yielding cows (Hart et al., 1978). Thus the combined changes in insulin, glucagon and growth hormone secretion during early lactation, which tend to reduce the ratios of insulin to both glucagon and growth hormone, appear to encourage the utilisation of available nutrients for milk synthesis. At the onset of lactation adipose tissue lipoprotein lipase activity and hence triglyceride

fatty acid uptake is suppressed, as is triglyceride synthesis (Shirley, Emery, Convey & Oxender, 1973). The active form of hormone-sensitive lipase is increased (Sidhu & Emery, 1979) and this together with the decreased activities of the enzymes of glyceride synthesis and possibly of glycerol-3-phosphate dehydrogenase (Baldwin & Smith, 1971) promotes the release of FFA , both by increasing lipolysis and almost entirely suppressing re-esterification (Metz & van den Bergh, 1977). Moreover, at least in vitro, glucose fails to stimulate re-esterification of fatty acids in early lactation (Metz & van den Bergh, 1977). As tissue uptake of FFA is largely proportional to plasma FFA concentration (Lindsay, 1975) stimulation of adipose tissue mobilisation enhances hepatic and peripheral utilisation of FFA, indirectly increasing the availability of triglycerides and acetate and β-hydroxybutyrate at the mammary gland. Concurrent increases in mammary gland lipoprotein lipase and NADP-dependent dehydrogenases (Shirley et al., 1973) ensure rapid uptake of triglyceride fatty acids (Annison et al., 1967) and their esterification within the udder.

Increased fat mobilisation also releases additional glycerol for gluconeogenesis (Metz & van den Bergh, 1977), the rate of which is raised during lactation (Bergman & Hogue, 1967; Baird, Lomax, Symonds & Shaw, 1980). The high rate of gluconeogenesis is also in part a result of enhanced glucose synthesis from lactate (Baird et al., 1978) which was suggested by Baird et al. (1978) to be a consequence of a reduced insulin:glucagon ratio. However, Hart (1983) concluded that clear definition of a role for this ratio must await further information on the pattern of glucagon secretion during early lactation. Although tissue protein is mobilised at this time

(Trigg, Jury, Bryant & Parr, 1980) the contribution made to gluconeogenesis by amino acids during early lactation has been estimated by Oldham (1978) and Bruckental, Oldham and Sutton (1980) to be no more than 2-3%. These workers reason that this arises because demands for protein, as well as for glucose, are increased during lactation. As Lindsay (1971) pointed out, the increased rate of gluconeogenesis during lactation is likely to be to some extent a consequence of the concurrently high dietary intake of glucogenic substrates.

Increased gluconeogenesis, combined with a reduction in the proportion of total glucose turnover accounted for by oxidation in peripheral tissues (Bennink, Mellenberger, Frobish & Bauman, 1972) and the insensitivity of glucose uptake by the mammary gland to insulin concentration (Laarveld, Christensen & Brockman, 1981) combine to enhance glucose supply for milk synthesis.

Substrate availability for glucose homeostasis and milk synthesis is also likely to be augmented by the actions of adrenalin and noradrenalin. Catecholamines suppress insulin secretion, increase glycogenolysis and lipolysis, stimulate glucagon secretion and increase gluconeogenesis (Bassett, 1970, 1971; Bloom, Edwards & Vaughan, 1973). Metz and van den Bergh (1977) showed adipose tissue lipolysis to increase in sensitivity to catecholamine stimulation during early lactation in dairy cows, when the numbers of β-adrenergic receptors on adipocytes are high (Jaster & Wegner, 1981).

The metabolic effects of end-products of digestion on milk yield and composition. The secretory capacity of the mammary gland is

dictated by the amount and activity of the mammary tissues and by the blood supply to the gland. These factors are largely hormonally-regulated and the patterns of hormonal secretion during lactation are associated with characteristic changes in milk yield and composition. As milk is isoosmotic with blood plasma (Wheelock, Rook & Dodd, 1965) milk yield is closely related to the amount of lactose synthesised, this being the major milk constituent to contribute to osmotic activity. Milk composition is therefore largely determined by the rates of fat and protein secretion relative to that of lactose. The post-peak lactational decline in milk yield is typically accompanied by increases in fat and protein contents (see Rook, 1961). These lactation trends are modified by diet, which affects precursor supply for milk synthesis both directly and through effects on the endocrine control of nutrient partitioning between the mammary gland and body tissues. Insufficient information is available at the present time to permit the relationships between specific dietary substrates, digestion products, effects on metabolism and effects on milk production to be quantified but several clear trends are apparent.

As suggested by Rook and Balch (1961) and supported by studies involving intraruminal infusion of propionate (Rook, Balch & Johnson, 1965) or intraduodenal infusion of glucose (Farhan & Thomas, 1977; Clark, Spires, Derrig & Bennink, 1977) end-products of digestion are unlikely to greatly influence lactose synthesis through direct effects on glucose availability. Plasma glucose concentration is normally homeostatically controlled within a narrow physiological range such that it rarely falls below about 2.2mmol/l, the point at which glucose availability may become limiting for lactose synthesis

(Rook, Storry & Wheelock, 1965; Rook & Hopwood, 1970). Similarly, although increased yields of milk and all milk constituents following intraruminal infusion of acetate suggested a general effect related to energy supply to the mammary gland (Rook & Balch, 1961), Rook and Thomas (1983) thought it unlikely that acetate availability and milk secretion are directly related. Plasma acetate concentration is known to fluctuate widely under conditions where the rate of milk secretion is constant.

Although infusion studies have given broad indications of the relationships between specific digestion products and milk synthesis (Thomas & Chamberlain, 1984), the effects of dietary factors such as the composition of the ration and the level of feeding rarely produce a specific change in the availability of a single digestion product. A comparison made between barley- and maize-based concentrates offered to cows as either 60% or 90% of the ration (Sutton et al., 1980) failed to clarify the relationships between digestion products, lactose synthesis and milk yield, but rather emphasised the complexity of the situation. As Sutton (1981) concluded, the response to an increase in the proportion of maize in the diet was in agreement with the infusion studies of Rook and Balch (1961), in that a diet-induced reduction in acetate production in the absence of a change in propionate production was associated with a reduction in milk and lactose yield. However, when the level of inclusion of barley in the diet was increased the associated increase in rumen propionate production was accompanied by increased yields of milk and of lactose. This response led Sutton (1981) to propose that milk yield might depend on the absolute rate of propionate production in the rumen.

Dietary inputs, through effects on the pattern of end-products of digestion, also have important effects on the synthesis of milk fat. The specific increase in fat secretion observed in response to intraruminal infusions of acetate or butyrate (Rook & Balch, 1961) suggested the availability of acetate and β-hydroxbutyrate at the mammary gland to be a limitation on intra-mammary de novo synthesis of short- and medium-chain fatty acids. Indeed, depressed milk fat secretion frequently has been demonstrated to be associated with high intakes of readily-fermentable carbohydrate (Flatt, Moe, Hooven, Lehmann, Orskov & Hemken, 1969; Broster, Sutton & Bines, 1979; Sutton et al., 1980). As discussed earlier these diets generally lead to a fermentation pattern characterised by a reduced acetate plus butyrate:propionate ratio. In animals receiving such diets mammary uptake of acetate and β-hydroxybutyrate is reduced in line with the reduction in their plasma concentrations (Annison, Bickerstaffe & Linzell, 1974).

The direct effect on milk fat secretion of reducing the availability of precursors for intramammary synthesis of fatty acids is augmented by indirect, hormonally-mediated effects on adipose tissue metabolism and hence the availability of long-chain fatty acids for transfer to milk fat. Evidence for endocrine involvement includes the observed reduction in triglyceride fatty acid uptake by the mammary gland in cows receiving low-roughage diets (Annison et al., 1974) and the failure of intraruminal infusions of acetate or butyrate to redress completely the reduction in milk fat secretion in cows receiving such diets (Rook & Balch, 1961). Moreover, normal milk fat secretion can be almost completely restored by intravenous infusion of triglycerides or dietary supplementation with protected

fats (Storry, Brumby, Hall & Johnson, 1974) and the re-establishment of a normal fermentation pattern in animals changed from a low-roughage to a normal diet preceeds the complete recovery of milk fat secretion by 1-2 weeks (Storry & Sutton, 1969). It is the elevated ruminal propionate production associated with low-roughage diets which is thought to be important in eliciting the hormonal changes. The decreased synthesis of milk fat is unlikely to be mediated by an antiketogenic action of propionate, as β-hydroxybutyrate entry rate is not reduced when high-concentrate diets are given, and only 8% of the carbon in milk fatty acids is estimated to be derived from that source (Palmquist, Davis, Brown & Sacham, 1969). Similarly, any influence of increased availability of propionate is unlikely to be important as peripheral concentrations are not significantly increased when high-concentrate diets are given (Storry & Rook, 1965a). The depression of milk fat secretion has been argued to be an indirect effect of increased glucose availability and consequent stimulation of insulin secretion (McClymont & Vallance, 1962). This theory is supported by: the demonstration of increased glucose entry rate and insulin secretion in cows fed high-concentrate rations (e.g. Evans, Buchanan-Smith, Macleod & Stone, 1975); the increased activities of lipoprotein lipase (Benson, Askew, Emery & Thomas, 1972) and of enzymes associated with lipogenesis (Opstvedt, Baldwin & Ronning, 1967) in adipose tissue of cows fed high-concentrate diets; the observation that consumption of such diets increases the incorporation of plasma fatty acids into adipose tissue rather than mammary triglycerides (Palmquist & Conrad, 1971); and the tendency for milk fat synthesis to be reduced as plasma glucose concentration is increased (Storry &

Rook, 1965b; Fisher & Elliot, 1966). Moreover, the observations of Sutton et al. (1980) were consistent with this theory: milk fat secretion was reduced when the level of inclusion of barley in the diet was increased. The demonstration of a similar response to increased dietary inclusion of maize led Sutton (1981) to postulate that the insulin response is associated with an increase in the supply of glucogenic precursors, be it as ruminal propionate or starch in the small intestine, and a decreased supply of lipogenic precursors, namely acetate and butyrate.

It is, however, difficult to reconcile the glucogenic theory of McClymont and Vallance (1962) with the demonstrated failure of exogenously administered glucose or propionate to suppress milk fat yield to the extent seen in spontaneously developed extreme depression of milk fat secretion (Storry & Rook, 1965c; Fisher & Elliot, 1966; Frobish & Davis, 1977a). This led Frobish and Davis (1977b) to propose that a reduction in vitamin B_{12} production by the rumen microorganisms coupled with the increased propionate availability gave rise to the milk fat depression. As ruminal B_{12} is the host's sole source of this vitamin, which is required as a cofactor for propionate metabolism, these authors postulated that in animals receiving high-concentrate diets insufficiency of B_{12} could lead to impaired hepatic metabolism of propionate and an accumulation of methylmalonic acid, which would in turn inhibit milk fat synthesis. Similar changes have been shown to occur in ruminant hepatic tissues (Smith, Osborne-White & Russell, 1969; Somers, 1969; Smith & Marston, 1971) but as Vernon (1980) explained, any inhibitory actions of methylmalonyl CoA would be expected to decrease the rate of fatty acid synthesis in both adipose tissue and the mammary gland,

yet this does not occur (Palmquist & Conrad, 1971). The involvement of B_{12} deficiency in the development of severe milk fat depression in the ruminant has yet to be demonstrated (Elliot, Barton & Williams, 1979).

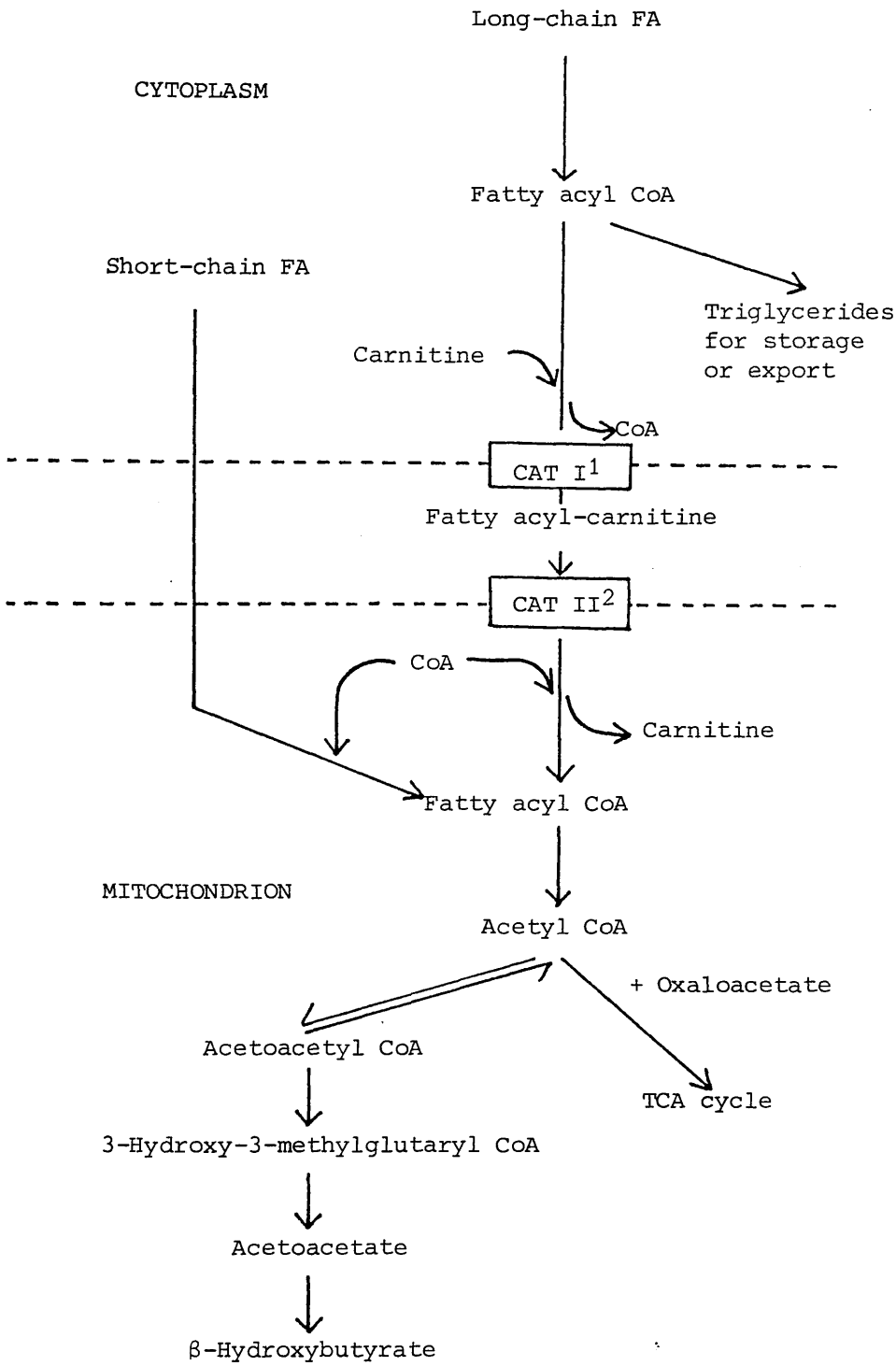
Ketosis. Maximal food intake is rarely achieved by the dairy cow until several weeks after peak lactation (Flatt, 1966; Bauman & Currie, 1980). Thus during the first 6 to 8 weeks of lactation the high-yielding animal is predisposed to the development of a degree of carbohydrate insufficiency, which is exacerbated by the hormonally-mediated priority assigned to milk secretion and the high demands, especially for glucose, of the mammary gland. Although gluconeogenesis is not impaired (Kronfeld, 1971), plasma glucose concentration is reduced and there is an associated decline in plasma insulin concentration post-partum (Schwalm & Schultz, 1976). Extreme energy imbalance precipitates the development of clinical ketosis, the onset and severity of which are closely associated with the degree of hypoglycaemia (Cote, Curtis, McSherry, Robertson & Kronfeld, 1969); the disorder is also characterised by elevated plasma FFA concentrations and hyperketonaemia (Alder, Wertheimer, Bartana & Flesh, 1963).

In a recent review of bovine ketosis Baird (1981) explained that β-hydroxybutyrate output across the splanchnic bed normally arises from approximately equal contributions from the metabolism of butyrate in the rumen epithelium and from the metabolism of butyrate and FFA in the liver. There is no significant splanchnic output of acetoacetate, as hepatic uptake balances gut production. Blood plasma ketone body levels in ruminants are normally high and the

β-hydroxybutyrate to acetoacetate ratios are greater than 10:1 (Baird, Hibbit, Hunter, Lund, Stubbs & Krebs, 1968; Koundakjian & Snoswell, 1970). Both substrates are utilised by skeletal muscle (Jarret, Filsell & Ballard, 1976), kidney (Kaufman & Bergman, 1971) and lactating mammary gland (Kronfeld, Raggi & Ramberg, 1968). However, under conditions of increased FFA mobilisation, such as energy undernutrition during early lactation, hepatic output of β-hydroxybutyrate is increased and there is a net production of acetoacetate by the liver. During ketosis hepatic ketogenesis appears to exceed the capacity of peripheral tissues for ketone body uptake (Bergman & Kon, 1964), which may be impaired by the low insulin concentrations in the blood (Brockman, 1979).

The increase in hepatic ketogenesis is partly a consequence of the increased availability of FFA for hepatic uptake, but there are evidently changes within the liver which increase the proportion of FFA converted to ketone bodies. This proportion has been shown to increase from 30% to 80% during the development of ketosis in sheep (Katz & Bergman, 1969). Although the changes in FFA metabolism in the liver associated with ketosis are incompletely understood, this syndrome is generally thought to be a consequence of increased hepatic FFA oxidation in the absence of sufficient oxaloacetate to facilitate oxidation via the TCA cycle of the acetyl CoA so formed (Hibbit, 1979; Baird, 1982), thereby favouring ketogenesis, as outlined in Fig. 1.04. Indeed Baird, Heitzman & Hibbit (1972) found the concentrations of oxaloacetate together with those of other intermediates in the TCA cycle to be reduced when ketosis was induced by starvation in lactating cows. However, as Baird (1982) pointed out, it is the concentration of oxaloacetate within the mitochondria

Fig. 1.04 Pathways of FFA metabolism in the ruminant liver (After Baird, 1982)



[1] Carnitine acyltransferase I.

[2] Carnitine acyltransferase II

(the site of TCA cycle activity) which is likely to influence the rate of acetyl CoA entry into the TCA cycle, and this has yet to be measured in the bovine liver. The successful treatment of bovine ketosis by administration of glucocorticoids or glucose, both of which suppress ketonaemia and increase hepatic concentrations of glycogen and TCA cycle intermediates (Baird, 1977) supports the theory that ketogenesis is regulated by the supply of glucogenic intermediates.

There has been little investigation in ruminants of other regulatory mechanisms of hepatic ketogenesis. Zammit (1981) discussed evidence in rats for an inverse relationship between fatty acid synthesis within the cytosol and the entry of long-chain fatty acids, as acyl carnitine esters, into mitochondria prior to oxidation. Enzyme activities in starved rats suggested this regulation to be mediated by changes in the concentration of malonyl CoA, the product of the acetyl-CoA carboxylase reaction which is the first step in fatty acid synthesis. Malonyl CoA appears to inhibit the activity of carnitine acyltransferase I, the enzyme which catalyses fatty acyl carnitine formation. However, Baird (1982) suggested that malonyl CoA is unlikely to be important in the bovine liver, where the rate of lipogenesis is low (Ballard et al., 1969). It is unclear whether, as in the rat (McGarry, Robles-Valdes & Foster, 1975), the concentration of carnitine is important in the regulation of ruminant ketogenesis. As discussed by Bell (1980), the availability of carnitine may influence the relative proportions in which the liver releases ketones and acetate; the latter has been suggested by Costa, McIntosh and Snoswell (1976) to be an alternative product of intramitochondrial acetyl CoA utilisation. Certainly the

concentrations of free carnitine and particularly acetyl carnitine have been shown to increase during fasting in sheep (Snoswell & Henderson, 1970).

Baird (1982) also postulated that ketogenesis could be regulated by a TCA cycle intermediate, the level of which would reflect carbohydrate status and would link the level of turnover in the TCA cycle with the rate of ketogenesis by regulating the activity of carnitine acyltransferase II, which catalyses the release of fatty acyl CoA from fatty acylcarnitine esters. Bell (1980) has suggested that other factors including the redox state of the mitochondria and the availability of coenzyme A may be important, in that they influence the supply of acetyl CoA.

Whatever the mechanisms involved, the hyperketonaemia observed during the development of ketosis and/or the concurrent hypoglycaemia may lead to the development of a degree of hypophagia, which in turn precipitates a rapid decline in milk yield (Kronfeld, 1971). In animals in which food intake is unimpaired remission of ketosis may occur if the decline in milk yield is sufficient to restore energy balance, whereas more severe cases require therapeutic treatment to increase glucose supply (see Schultz, 1974).

Although clinical ketosis occurs in only about 2% of the UK dairy herd (Payne, 1966), Baird (1982) suggested that all cows in early lactation are likely to experience the metabolic changes characteristic of ketosis and that in some cases subclinical ketosis, which may go undetected and untreated, may have adverse effects on milk production.

THE POTENTIAL FOR DIETARY MANIPULATION OF PRODUCTION EFFICIENCY DURING LACTATION

The efficiency of utilisation of dietary energy for milk production. Within the limits imposed by genetic potential, previous nutritional history and stage of lactation, the gross efficiency of milk production is improved by increasing the supply of energy to the animal; there is an associated curvilinear increase in milk yield and consequent reduction in the proportion of energy expenditure represented by the energy cost of maintenance (see Moe & Tyrrell, 1974).

The value to the animal of the gross energy (GE) consumed is dependent upon the magnitude of the energy losses which are inevitably associated with digestion and metabolism. Of the energy supplied in rations typically given to dairy cows 70 to 75% is apparently digested, and the remainder excreted in the faeces (Flatt, Moe, Munson & Cooper, 1969; Sutton et al., 1980). Digestibility tends to increase as the proportion of concentrates in the ration is increased (Broster et al., 1979), and is reduced for many rations at increased levels of intake, on average by about 4% for each increase in intake equivalent to maintenance (Tyrrell & Moe, 1975; Broster et al., 1979). The effect of level of feeding on digestibility of high-concentrate diets is variable however. Whereas Tyrrell and Moe (1975) concluded that for hay- and silage-based diets the rate of depression of digestibility is greater for diets containing higher proportions of concentrates, Broster et al. (1979) failed to show a depressive effect of increased intake levels when feeding a 90:10 concentrate:forage diet. He suggested the relatively high crude

protein content in the diet which was used to be a possible explanation for this discrepancy.

Of the energy which is digested (DE), that disappearing from the rumen and caecum and colon does so as waste-products of microbial fermentation. Of these only VFA are absorbed and utilised, while methane and heat of fermentation constitute energy losses to the host. Ruminal production of VFA, methane and heat has been calculated to represent 75-88%, 9-18% and 3-7% respectively of the energy disappearing from the rumen (Thomas & Clapperton, 1972). Methane losses are generally reduced when feeding level (Blaxter & Clapperton, 1965) or the proportion of concentrate in the ration (Flatt, Moe, Munson & Cooper, 1969) is increased. The availability of energy to the animal is enhanced when dietary conditions are such that an increased proportion of the dietary energy is absorbed from the small intestine, thereby reducing the proportion of the DE represented by losses associated with fermentation.

Energy excreted in urine constitutes a further loss, which together with losses in faeces and methane, can be accurately measured and subtracted from the GE of the diet to determine the metabolisable energy (ME) supplied. As a result of the energy losses inevitably associated with the biochemical pathways involved, the metabolic uses to which the ME is put determine the efficiency with which ME is utilised. For example, acetate and propionate have been shown to be utilised by lactating cows for milk and tissue synthesis with an efficiency of about 60% (Orskov, Flatt, Moe, Munson, Hemken & Katz, 1969). However, when ME is used for body tissue synthesis and subsequently mobilised the overall efficiency of ME utilisation is reduced to about 48% (van Es, 1976) because the conversion of tissue

energy into milk energy is about 80% efficient (Moe, Tyrrell & Flatt, 1971; Moe, Flatt & Tyrrell, 1972).

The partitioning of ME between milk synthesis and tissue deposition is influenced by substrate availability and the associated hormonal status and therefore by the nature of the source of dietary energy. Thus the efficiency with which a given supply of dietary energy is utilised for milk production cannot be predicted accurately from ME intake, but requires a knowledge of the form of digestion products in which this energy becomes available to the animal (Flatt, Moe, Hooven et al., 1969; Sutton et al., 1980). Unfortunately, the relationships between diet and end-products of digestion are difficult to quantify, particularly for high-concentrate diets, where the situation is complicated by uncertainties regarding the relationship between molar concentrations of VFA in the rumen and their production rates (Sutton & Morant, 1978) and the difficulties associated with the measurement of the capacity of the small intestine for glucose absorption. Furthermore, the metabolic and production responses to specific digestion products have yet to be satisfactorily quantified. In spite of these problems it is evident that dietary manipulation of energy supply has important consequences on animal performance. To maximise efficiency over the whole lactation it is particularly important that dietary conditions during early lactation, when energy partitioning is more easily manipulated (Strickland & Broster, 1981), favour milk output while minimising the risk of metabolic disorders associated with high levels of production - milk fat depression and ketosis, for example. This is because the peak milk yield achieved appears to have a residual effect on total lactation yield (Broster, Broster & Smith, 1969).

Dietary manipulation of energy supply for milk synthesis. As energy availability is a major limitation to milk production during the first 8-10 weeks post-partum, feeding strategy at this time should encourage high energy intake, the partitioning of this energy towards milk synthesis and the mobilisation of energy reserves. The ration offered should therefore be highly digestible and energy-dense, while possessing digestion characteristics which give rise to a pattern of end-products of digestion with metabolic effects conducive to increased energy availability for milk synthesis.

High inputs of dietary energy are frequently achieved by the provision, at a high level, of a diet containing a relatively low ratio of forage to concentrate. Thus during early lactation the amount and type of concentrate offered can have an important impact on milk production. The level at which concentrate sources of readily-fermentable carbohydrate can be advantageously included in the diet is limited. It is necessary to avoid a 'high-propionate' type of rumen fermentation, as the associated decrease in the population of cellulolytic bacteria in the rumen may cause a depression in forage intake, and the reduced acetate:propionate ratio favour tissue deposition rather than milk production. These problems were clearly demonstrated in studies with cows in early lactation by Lamb, Prescott and Armstrong (1976): as the percentage of rolled barley in a dried-grass based diet was increased from 42% to 59% dry matter intake, digestibility of organic matter and cellulose, ruminal acetate:propionate ratio, milk production, and the proportion of C_{18} acids in the milk fatty acids were reduced. Broster et al. (1979) have suggested that these problems may be avoided when feeding

hay/concentrate diets to high-yielding dairy cows if the proportion of concentrate does not exceed 75% of the ration. Increasing feeding frequency of high-concentrate diets appears to be an effective method of avoiding high rates of fermentation and the associated high proportion of propionate in the digestion products and suppression of energy secretion in milk (Sutton, Hart & Broster, 1982). Orskov (1981) has suggested that sodium hydroxide treatment of grain, by slowing the release of starch may offer similar advantages.

Dietary inclusion of starch sources which are less readily fermented in the rumen and increase the availability in the small intestine of α-linked glucose polymers, for example ground raw maize (Karr et al., 1966; Watson et al., 1972; Sutton et al., 1980) or ground or micronised sorghum (McNeill, Potter & Riggs, 1971), might be expected to avoid suppression of cellulose digestibility and high ruminal propionate production, while increasing the efficiency of utilisation of dietary energy. Indeed, Lamb et al. (1976), in a comparison of diets containing approximately 40% rolled barley or ground maize, found that the digestibility of organic matter in the maize diet was higher and that this was associated with a higher yield of fat-corrected milk. In a comparison of these cereals in 60% and 90% concentrate diets (Sutton et al., 1980) starch flow to the duodenum at the lower level of concentrate inclusion was significantly increased and milk yield was slightly higher for the maize diet. However, the inclusion of maize rather than barley in the 90% concentrate diets was associated with reduced ruminal propionate production, increased acetate production and a more than three-fold increase in flow of starch to the duodenum but a significantly depressed milk yield. This may indicate that, whereas

during periods of energy stress a moderate increase in starch availability for post-ruminal absorption may offer the advantage of supplying energy in an efficiently utilised form, at higher levels glucose-yielding substrates, whether propionate absorbed from the rumen or glucose from the small intestine, elicit hormonal changes which favour tissue deposition.

Dietary addition of protected fat may provide an alternative method of increasing the energy density of the ration while avoiding the risk of rumen acidosis, reduced forage digestibility and milk fat depression. However, as Storry (1981) explained, a beneficial effect of supplementation with protected lipid on energy intake or milk yield has yet to be demonstrated clearly.

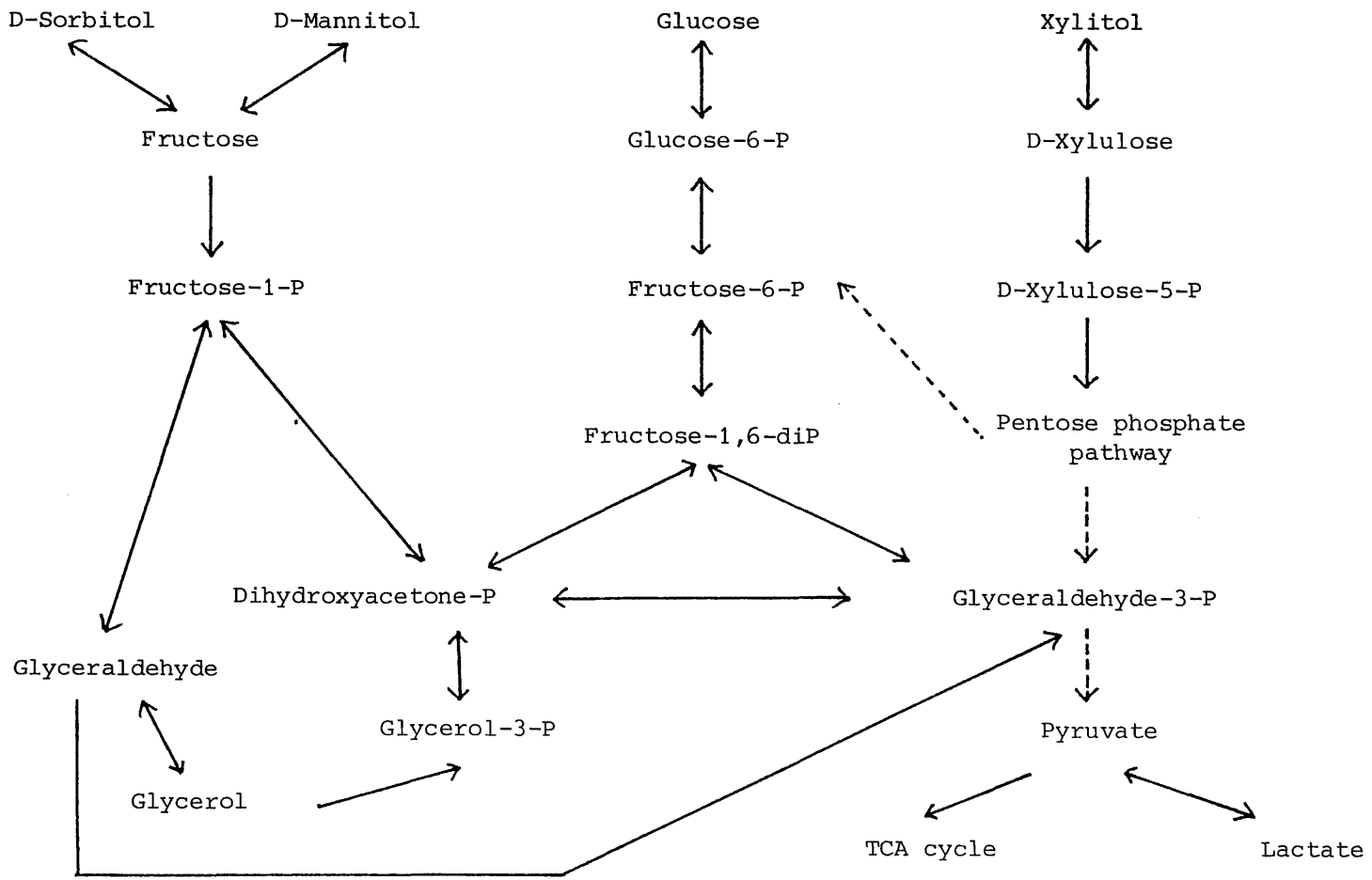
Interest has recently arisen in the potential of dietary inclusion of polyols as a means of manipulating the supply of glucogenic substrates to the ruminant. This followed the demonstration by Poutiainen, Tuori and Sirvio (1976) that some polyols, particularly xylitol and arabinitol, were resistent to fermentation during in vitro incubation with rumen liquor. Much of the present knowledge concerning the absorption from the digestive tract and subsequent metabolism of polyols has been learned from studies performed in non-ruminant species to investigate possible applications for xylitol in the prevention of dental caries (Scheinin & Makinen, 1975), the nutrition of diabetics (Yamagata, Goto, Ohneda, Anzai, Kawashima, Chiba, Maruhama & Yamauchi, 1965) and post-operative parenteral nutrition (Wretlind, 1975) in man.

Dietary xylitol is absorbed from the gastrointestinal tract of the rat by passive diffusion and passes to the liver, which accounts for 80-90% of total xylitol turnover (Bassler, 1969). Xylitol is

rapidly taken up by the liver, independently of insulin (Bassler, 1969) and metabolised via the pentose phosphate pathway, as outlined in Fig. 1.05, to produce primarily glucose (McCormick & Touster, 1957; Jakob, Williamson & Asakura, 1971; Woods and Krebs, 1973), the subsequent utilisation of which is insulin-dependent (Keller & Froesch, 1971; Froesch, 1976). Oral consumption of xylitol by rats and man is frequently accompanied in the initial stages by transient osmotic diarrhoea. It appears that increased xylitol tolerance is associated with adaptation both within the caecal microflora, leading to their increased ability to metabolise xylitol (Krishnan, Wilkinson, Joyce, Rofe, Bais, Conyers & Edwards, 1980) and in the liver, where increased activity of cytoplasmic polyol dehydrogenase leads to a more rapid uptake and metabolism of xylitol (Bassler, 1969).

Interpretation of data concerning the metabolic effects of xylitol is made difficult by the variation between studies in the level and route of xylitol administration, the apparent species differences in xylitol metabolism and the problems inherent in extrapolating from effects seen in isolated tissues to responses in the whole animal. Thus in dogs (Asano, Greenberg, Wittmers & Goetz, 1977), baboons (Jourdan, MacDonald & Henderson, 1972) and lambs (Eskeland & Pfander, 1973) intravenous administration of xylitol reduced plasma glucose concentration, while in man (Yamagata et al., 1965; Spitz, Rubenstein, Bersohn & Bassler, 1970) and rats (Forster, 1974) the concentration of glucose was unchanged or slightly increased following xylitol administration. Similarly, although xylitol, whether administered orally or intravenously, is a potent stimulator of insulin secretion in dogs (Kosaka, 1969; Asano et al.,

Fig. 1.05 Outlined pathways of the metabolism of Xylitol, D-Sorbitol and D-Mannitol in the liver. (After Wang & Van Eys, 1981)



1977) the insulin response to intravenous xylitol in cows and goats is comparable with that to glucose (Kuzuya, 1969) and the response in man and horses smaller and more variable (Kosaka, 1969; Kuzuya, 1969; Hirata, Fujisawa & Ogushi, 1969).

Certainly there appears to be a case for the use of xylitol in the nutritional management of carbohydrate intolerance in man, especially as intravenous xylitol administration has been shown to reduce plasma concentrations of FFA and ketone bodies in patients suffering from diabetic ketosis (Yamagata et al., 1969). The antiketogenic action of xylitol may, in addition to being a result of reduced adipose tissue mobilisation, reflect a direct effect on the liver; Haydon (1961) demonstrated that those polyols most readily oxidised in rat liver slices, namely sorbitol and xylitol, reduced ketone body production. Jakob et al. (1971) subsequently found that xylitol metabolism in perfused rat liver caused a large increase in the concentration of glycerol-3-phosphate and was associated with inhibition of ketone body production from endogenous fatty acids, possibly as a consequence of diversion of fatty acids from β-oxidation towards triglyceride synthesis. However, as xylitol had only a small effect on ketogenesis from exogenous oleate these authors suggested insulin-mediated suppression of adipose tissue mobilisation to be the important factor in reducing ketone body production during xylitol administration.

Interestingly, intravenously administered xylitol is apparently effective in reducing the concentration of ketone bodies and increasing that of glucose in the plasma of spontaneously ketotic cows (Hamada, Ishii & Taguchi, 1982). However, relatively little is known concerning the digestion and metabolism of xylitol in

ruminants. Bain (1980) confirmed the resistance of xylitol to fermentation during in vitro incubation with rumen liquor and suggested that if included in a diet offered to dairy cows almost 70% of this polyol was likely to be directly available to the host. These studies also showed xylitol to be fermented more rapidly when incubated with 'adapted' rumen liquor from sheep which had received intraruminally infused polyol mixture for a 2-week period. Enhanced xylitol fermentation was associated with an increased molar proportion of propionate in the VFA produced in the incubation mixture. Similarly Poutiainen et al. (1976) found incubation of xylitol, arabinitol, galactitol, mannitol, sorbitol or a mixture of polyols with 'adapted' rumen liquor to generally increase the molar proportions of propionate, butyrate and valerate at the expense of acetate in the VFA present compared to the mixtures of VFA in incubations with 'unadapted' rumen liquor. The increase in butyrate production was particularly marked for galactitol, mannitol and sorbitol, the more readily fermented polyols. Dietary supplementation with a mixture of polyols has also been shown to decrease ruminal acetate and increase butyrate and valerate proportions in sheep (Slee, 1980), and to increase the proportion of butyrate in the rumen in dairy cows (Tuori & Poutiainen, 1977). In neither case was the change in fermentation pattern reflected in significant changes in blood metabolites or in the latter case to milk yield, although plasma concentrations of glucose, insulin and β-hydroxybutyrate tended to be higher in the sheep receiving the polyol supplemented rather than the control diet.

AIMS AND OBJECTIVES

The nutritional and physiological states which characterise the early lactation period place the energy and glucose metabolism of the dairy cow under severe stress. Rations offered at this time are commonly of high energy density and contain a relatively large proportion of carbohydrate in the form of starchy concentrate. In mid-lactation yields of milk and milk constituents can be markedly affected by including in isoenergetic high-concentrate diets carbohydrate-rich sources which possess differing digestion characteristics. The mechanisms responsible are as yet unclear. It seems likely that the prevailing nutrient and endocrine status of early-lactation cows would increase their sensitivity to changes in glucose supply. As a consequence, comparatively moderate increases in the amount of glucose or glucogenic precursor supplied at a given dietary energy intake might be expected to enhance milk production, although the effects which the changes in the mixture of absorbed nutrients would have on the endocrine regulation of nutrient metabolism must also be considered.

On the basis of the properties of xylitol discussed in this section and the success with which commercial products containing it and other polyols are used in the prophylactic treatment of ketosis it would seem that xylitol might be a useful alternative to increasing intestinal digestion of starch as a means of enhancing glucose supply to the lactating cow.

The aims of the experiments presented in this thesis are:

(a) to assess the resistance of xylitol to fermentation in the rumen and the glucogenic potential of absorbed xylitol in the cow;

(b) to compare the metabolic and production effects of diets intended to increase glucose availability either by increasing the proportion of starch digested in the small intestine or by increasing the amount of directly absorbed glucogenic substrate (in the form of xylitol) with diets providing an equivalent amount of energy in the form of readily-fermented carbohydrate, and

(c) to examine the effects of a polyol mixture containing xylitol on metabolism and milk production when included in the ration offered to cows during the early stages of lactation.

SECTION II

MATERIALS AND METHODS

PREPARATION AND MANAGEMENT OF SURGICALLY PREPARED SHEEP

Rumen cannulation. A solid nylon cannula of a type similar to that described by Jarret (1948) was used. The cannula was mushroom-shaped with a hollow stem, which was threaded on its external surface. The head of the cannula was introduced into the rumen and the stem was exteriorised through the body wall and held in position by a nylon ring which was screwed down the stem of the cannula until it rested on the body wall. The cannula was closed with a screw cap.

The animal was fasted and denied access to water for 24 hrs prior to the operation. Wool was removed from the area of operation by clipping and shaving. Immediately prior to the operation the animal was weighed and anaesthesia was induced by intravenous injection of an appropriate amount of sodium pentobarbitone. Anaesthesia was maintained throughout the operation by the administration of halothane with a mixture of nitrous oxide and oxygen through a cuffed endotracheal tube. The animal was placed, on its right side, on an operating table and the area of operation was scrubbed with an antiseptic solution. For the insertion of the cannula an incision about 10cm long was made 5-8cm below the transverse processes of the lumbar vertebrae and 10cm posterior to the last rib. The muscle layers were blunt-dissected and the peritoneum opened to expose the rumen. A pouch of rumen was exteriorised and an incision about 10cm long made in it, into which the base of the cannula was inserted. The incision was sealed up to the neck of the cannula with a continuous suture. A purse-string suture was made around the stem of the cannula, making as tight a

seal as possible to prevent leakage of digesta. A nylon ring was passed over the stem of the cannula, which was then plugged with swabs and returned to the body cavity to be exteriorised through a small circular incision anterior to the initial incision. The peritoneum and muscle layers were closed using continuous sutures and the skin was closed with single stay sutures. A nylon retaining ring was screwed into position over the stem of the cannula, the swabs removed and the cannula was sealed with a screw cap.

Duodenal and ileal T-piece cannulation. The cannulas used were similar to those described by Weller, Pilgrim and Gray (1971). They were constructed in rigid nylon and consisted of a threaded stem attached to a gutter-shaped base, the size and shape of which were designed to fit closely within the lumen of the duodenum or ileum. When in position the cannulated gut was separated from the body wall by a smooth-edged ring, in which holes 6mm in diameter had been drilled 6mm apart, which fitted around the stem of the cannula. A second knurled-edged ring without holes was screwed down the stem of the cannula until it rested on the outside of the body wall. The cannula was closed with a screw cap.

The duodenal and ileal cannulas were inserted during a single operation in animals which had been cannulated in the rumen on a previous occasion. The animal was fasted for 24 hours prior to the day of operation and wool was clipped and shaved on the right side for an area extending from the last rib to the thigh. On the day of operation the rumen contents were removed and stored at 39^{o}C. Anaesthesia was induced with an intravenous injection of sodium pentobarbitone and maintained throughout the operation by the

administration of halothane in a mixture of nitrous oxide and oxygen through a cuffed endotracheal tube. The animal was placed on an operating table and the shaved area of its right side was scrubbed with antiseptic solution.

An incision approximately 20cm long was made in the right flank extending ventrally from a point 10cm posterior to the tip of the last rib. The muscle layers were split by blunt-dissection and the peritoneum opened to expose the pyloric region of the abomasum, from where the duodenum was located. A longitudinal incision was made in the duodenum 4-5cm from the phyloric sphincter. The gutter of the duodenal cannula was inserted and the incision closed to the stem of the cannula with a continuous suture. A purse-string suture was made around the stem of the cannula and closed firmly to prevent leakage of digesta. The stem of the cannula was plugged with a swab and returned to the body cavity anterior to the line of incision.

The ileum was located by first finding the ileocaecal valve. A longitudinal incision was made in the ileum about 15cm anterior to the valve and the ileal cannula was inserted in a way similar to that described for the duodenal cannula. The cannula was plugged with a swab.

A holed ring was placed over the stem of each cannula. These rings lay on the intestinal tissue so that the tissue would grow through the holes in them to form an adhesion to the body wall. The duodenal cannula was then exteriorised anterior to and the ileal cannula posterior to the initial incision through knife stabs. The peritoneum and muscle layers were closed using continuous sutures and the skin incision was closed using single stay sutures. A knurled-edged ring was screwed over the stem of each cannula until it rested

firmly on the body wall and the plugs in the cannulas were replaced with screw caps. When the animal had regained consciousness the rumen contents were replaced via the rumen cannula.

Management of prepared animals. All animals were given an intramuscular injection of a broad spectrum antibiotic and the incised areas were dressed with antibacterial powder at the time of surgery.

Animals were allowed to recover from anaesthesia in a heated, padded room and usually did so within 1-2 hours of the operation. At this time they were offered hay and about 1 l water. Rations and water were reintroduced and on the day following the operation the animals frequently consumed their full rations of food. Complete recovery of appetite occasionally required a further 2-3 days. The skin stitches were removed 7-10 days after the operation and the cannula(s) were subsequently washed at 3-5 day intervals and the wool was clipped from the area.

In general the animals took slightly longer to recover from surgery to introduce duodenal and ileal cannulas, and commonly full appetite did not return for 3-4 days. Occasionally animals developed anorexia and if significant amounts of food were not being consumed 24 hours after the operation these animals were given an intraruminal infusion of 200g glucose in 500ml water twice daily. Appetite usually returned to normal within 3-4 doses. The knurled rings were removed from the intestinal cannulas to facilitate washing.

THE PREPARATION OF CrEDTA SOLUTION

Chromium-ethylenediaminotetraacetic acid (CrEDTA) complex was prepared by the method described by Binnerts, van't Klooster and Frens (1968).

Reagents for 1 l CrEDTA solution

1. Chromium trichloride, $CrCl_3.6H_2O$, (14.2g) was dissolved in 200ml distilled water in an 800ml beaker.
2. Disodium salt of EDTA (20g) was dissolved in 300ml distilled water.

Calcium chloride solution (1M).

Solution 2 was added to solution 1 and the mixture was boiled gently for 1 hour, covering the beaker to prevent excessive evaporation. The solution became a deep violet colour as the 1:1 complex of Cr and EDTA was formed. The solution was cooled and the small excess of EDTA was neutralised by the addition of 4ml calcium chloride. The pH of the mixture was brought to 6-7 by the addition of a few drops of sodium hydroxide and the final volume made up to 1 l with distilled water in a volumetric flask. The solution was stored in a dark place until required.

COLLECTION AND PREPARATION OF SAMPLES

Feedstuffs. Feedstuffs included in the rations were sampled at least once during each treatment period. Subsamples were dried in triplicate at 60°C in a forced-draught oven for dry matter

determination and ground through a 1.0mm screen and stored for analysis. Additionally in the case of silage, approximately 1500g of wet material was minced through a 100mm dye (Crypto Ltd, London) and stored at -20°C until analysed.

Milk. Approximately 300ml of milk was collected into bottles containing 280mg potassium dichromate (Thompson and Capper Ltd, Runcorn, Cheshire), mixed thoroughly to dissolve the preservative and stored at 4°C. Samples to be bulked were warmed slowly to 40°C, the fat globules were dispersed by gentle shaking and subsamples were combined in proportion to the respective milk yield. A 50ml aliquot was stored at -20°C for analysis of milk fatty acid composition and the remainder was stored at 4°C.

Blood. Samples of blood were withdrawn from the jugular vein. When a series of samples was to be taken this was done via an indwelling Polythene cannula (I.D. 1.00mm, Dural Plastics, Australia) inserted a minimum of 18 hours before sampling commenced. Patency of the cannula was maintained by flushing and filling it with a sterile solution of 0.9% sodium chloride (w/v) and 0.5% trisodium citrate (w/v) between sampling. Blood samples were taken into syringes which had been rinsed with heparin solution (250 units/ml; Evans Medical Ltd., Middlesex) and dried before use. The heparinised blood was transferred to heparin-treated McCartney bottles. When required for glucagon analysis 1ml of blood was added to 0.05ml of the protease inhibitor Aprotinin (Sigma Chemical Company Ltd, Poole) in a 1.5ml capacity micro test tube and centrifuged at 8800g for 3 minutes in a

bench top centrifuge (Eppendorf, Hamburg, Germany) and the plasma removed and stored at -20°C. The remainder of the blood was centrifuged at 1300g for 15 minutes in a refrigerated centrifuge (MSE Scientific Instruments, Crawley) and the plasma stored at -20°C until analysed.

On occasions when single blood samples were to be taken these were withdrawn directly into evacuated heparin-treated glass tubes through small bore (20G) needles (Vacutainer; Becton-Dickinson (UK) Ltd, Wembley) while the animals were restrained in their stalls. Samples were centrifuged at 1300g for 15 minutes and the plasma stored at -20°C.

Digesta. Samples of rumen liquor were obtained under suction by inserting a 30cm long metal tube, along the length of which a series of holes had been bored, through the rumen cannula into the posterior ventral sac of the rumen. The pH of the digesta was determined using a Pye Universal pH meter (Pye Unicam Ltd, Cambridge) and large particles of food material were removed by squeezing the digesta through a double layer of muslin. The digesta was then centrifuged at 2500 rpm for 20 minutes and the supernatant stored at -20°C prior to analysis.

For the collection of duodenal and ileal digesta the appropriate cannula was opened and a plastic bag attached over it for approximately 15 minutes to collect a sufficiently large volume of digesta. Samples were centrifuged at 2500 rpm for 20 minutes and the supernatant kept at 4°C until bulked for a single sampling day and then stored at -20°C.

Faeces. For cows the faeces were collected from the clean concrete floor behind the animals. Subsamples (10%) of the total faecal material excreted during each of 5 consecutive days were taken and stored at 4°C until bulked at the end of the collection period. A 500g subsample of the bulked material was mixed with water (1:1) and macerated in a high-speed mixer-emulsifier and the slurry stored at 4°C. Subsamples of the remainder were taken in triplicate for dry matter determination and subsequently ground through a 1.0mm screen prior to analysis.

Total faecal collections from sheep were made over a 7-day period. The daily collection was weighed and stored at 4°C. A 400g subsample of the total output was taken and added to 1200g water and mixed into a slurry which was stored at 4°C prior to analysis. Triplicate subsamples of the bulked faeces were taken for dry matter determination and were ground for further analysis.

ANALYTICAL METHODS

Dry matter and ash. These were determined by standard methods. The dry matter contents of all samples of feedstuffs with the exception of silage, were determined by drying at 100°C a known weight of fresh sample to a constant weight. Dry matter in silage was determined using the Dean and Starke method (Dewar & McDonald, 1961) whereby the silage was refluxed in toluene and the volatile acid content of the water distillate was determined. Dry matter content was expressed as a percentage of the fresh weight. Ash content was determined by

burning the retained dry matter samples in a muffle furnace at 550°C for at least 3 hrs, and expressed as a percentage of the dry sample.

Total nitrogen. The nitrogen content of food and faeces samples was measured by a macro-Kjeldahl method using the Kjeltec apparatus (Tecator Ltd., Thornbury, Bristol). Sample containing 1-2mg nitrogen was digested with 98% sulphuric acid (nitrogen free) and catalyst tablets containing 2g potassium sulphate and 0.02g selenium. The digested sample was distilled with water and sodium hydroxide. Distillate (150ml) was collected into a conical flask containing 25ml boric acid solution (40g/l). This ammonia was titrated with 0.01N HCl (0.04N HCl for silage samples) after the addition of methylene blue/methylene red indicator solution. The development of a lilac colour marked the end-point. Crude protein content was calculated by multiplying the total nitrogen content of the sample by 6.25.

True protein and non-protein nitrogen (NPN) in silage. The true protein content of silage was determined by Kjeldahl analysis of the material precipitated by tannic acid (van Roth, 1939). NPN content was calculated by subtracting the true protein content from the crude protein content.

Reagent. Tannic acid (4.45g) was dissolved in distilled water. Concentrated sulphuric acid (0.1ml) was added and the mixture was made up to 100ml with distilled water. This was allowed to stand for 24 hours and then filtered through Whatman No. 42 filter paper.

Procedure. Wet minced silage (1g) was weighed accurately into a centrifuge tube and 20ml boiling tannic acid solution was added. The tube was placed in a boiling water bath for 15 minutes, cooled for 15 minutes and then centrifuged at 2500 rpm for 10 minutes. The supernatant was removed by suction through a tube covered at the end with a layer of fine, washed muslin. Any particles of sample were washed from the muslin back into the tube and the volume made up to 25ml with distilled water. The residue was resuspended and the tube centrifuged as before. The washing and centrifuging was repeated twice more and the residue was analysed for nitrogen content as described previously.

Ammonia nitrogen (NH_3-N) content of silage. This was determined on a water extract of the sample. The extract was prepared by placing 20g wet, minced silage and 200ml distilled water in a beaker in water bath at 40°C for 30 minutes, stirring intermittently. The extract was filtered by squeezing the silage juice through muslin, and was centrifuged at 3000 rpm for 20 minutes and the supernatant was retained and stored at -20°C.

Procedure. Ten ml of 10M sodium hydroxide was added to 10ml of silage extract and the ammonia was distilled over into 25ml boric acid solution (40g/l) using the Kjeltec apparatus (Tecator Ltd., Thornbury, Bristol) and the distillate was titrated with 0.01N HCl, as described for the determination of total nitrogen.

Lactic acid in silage. This was determined by the method of Conway (1957).

Reagents. Phosphate buffer, pH7 was prepared by dissolving 9.3606g sodium di-hydrogen phosphate, 19.8758g di-sodium hydrogen orthophosphate and 0.7473g semicabazide hydrochloride in 1 l distilled water.

Saturated ceric sulphate in 6N H_2SO_4.

Procedure. Conway dishes were wiped with silicone grease. Phosphate buffer (2ml) was pipetted into the centre of the dish. Silage extract was prepared as described on page 68 and 2ml of this and 0.4ml saturated ceric sulphate was pipetted into the outer ring of the dish, which was then covered with a glass slide. The solutions in the outer ring were gently mixed by rotating the dish on the bench and then incubated at 37°C in an oven for 2 hours. Of the solution in the centre ring 1ml was taken and diluted to 10ml with distilled water in a volumetric flask and the absorbance of this solution at 224nm was read on a spectrophotometer. The concentration of lactic acid in the samples was determined by reference to a graph of absorbance readings for standard solutions of lithium lactate containing 25 to 70mg/l.

Acid-detergent fibre. This was determined in feedstuffs and faeces by the method of Goering and Van Soest (1970).

Reagents. Acid detergent solution. Cetyltrimethylammonium bromide (20g) was added to 49.04g sulphuric acid diluted with distilled water. This was dissolved by heating. When cooled the volume was made up to 1 l with distilled water.

Decahydronaphthalene (Dekalin).

Acetone.

Procedure. Dried sample (0.5-1g) was weighed into a 500ml conical flask. Acid detergent solution (100ml) and 2ml Dekalin were added. A reflux condenser was attached and the mixture was boiled for 1 hour from the onset of boiling. The contents of the flask were completely transferred to a preweighed sintered glass crucible (porosity 1), washing out the flask with hot water. The solid material was washed under suction twice with almost boiling water, followed by a washing with acetone. The crucible was dried at 100°C for 8 hours and weighed. Acid-detergent fibre content was calculated as follows:

$$\text{ADF \%} = \frac{\text{weight of dried sample in crucible}}{\text{weight of sample dry matter}} \times 100$$

Cellulose. The method used for the determination of the cellulose content of feedstuffs was that of Crampton and Maynard (1938).

Reagents. Acetic acid, 80% (v/v).

Ethanol, 95% (v/v).

Procedure. Dried sample (1g) was added, with 15ml of 80% acetic acid and 1.5ml concentrated nitric acid, to a 150ml round-bottomed flask fitted with a reflux condenser. After boiling gently for 20 minutes the contents were transferred to a 50ml tube, 20ml ethanol was added and the mixture centrifuged at 1500 rpm for 10 minutes. The

supernatant was discarded and the residue transferred to a Gouch crucible and washed with hot benzene, hot ethanol and diethyl ether. The contents were dried, weighed, ashed in a muffle furnace at 580°C and re-weighed. The cellulose content was calculated from the weight loss on ignition.

Digestible organic matter. The digestible organic matter contained in the dry matter (DOMD) of dried hay samples was measured using the method of Morrison (1972) based on the lignin content of the food. The method involved the removal of the interfering phenolic materials, lipids, waxes and colouring matter and the acetylation of the lignin hydroxyl groups, which were then brought into solution with acetyl bromide and acetic acid. Hydroxylammonium chloride solution was added to remove the excess reagents, bromine and polybromide. The proteinaceous sediment was removed and lignin was estimated as an 'A' value from the optical density of the filtrate measured spectrophotometrically at 280nm.

Reagents. Acetone.

Diethyl ether.

Ethanol, absolute, 99.7-100%.

Glacial acetic acid (Analar).

Acetyl bromide/acetic acid solution. This was prepared by mixing 25ml acetyl bromide (99%) with glacial acetic acid in a 100ml volumetric flask and making this mixture up to volume with additional acetic acid.

Sodium hydroxide solution (2.0M).

Hydroxylammonium chloride solution (0.5M).

Procedure. A 50mg sample of food was weighed into a 25ml glass stoppered tube and 20ml water added. The tube was stoppered, mixed and heated at 70°C in a waterbath for 30 minutes. The tube was shaken at 10 minute intervals. The sample was then filtered through a GF/A filter paper in a nylon filter unit and the residue was washed in order with four 5ml portions of each of water, ethanol, acetone and diethyl ether. The filter paper was returned to the test tube and heated at 100°C for 5 minutes to remove all traces of organic solvents. Five ml acetyl bromide/acetic acid solution was added to the residue and the tube was stoppered, shaken and heated in a water bath at 70°C for 30 minutes with shaking as before. The mixture was cooled in a water bath at 20°C for 30 minutes and 20ml glacial acetic acid was added and the contents mixed. A 5ml aliquot of the supernatant was transferred to a 50ml volumetric flask containing 1ml sodium hydroxide solution and 7.5ml glacial acetic acid. Absolute ethanol was added to bring the volume up to approximately 45ml and 1.5ml hydroxylammonium chloride was added. The flask was shaken the volume made up to 50ml with absolute ethanol. The mixture was shaken thoroughly again and allowed to stand for 1 hour before the contents were filtered through a 9cm GF/A filter paper. A blank was prepared as above but without the addition of sample. The optical density of the filtrate was measured spectrophotometrically at 280nm.

Calculation. The lignin content was reported as an 'A' value from the following formula:

$$'A' = \frac{OD_s - OD_b}{C} \quad l/g/cm$$

where OD_s = optical density of the sample

OD_b = optical density of the blank

C = concentration of sample dry matter in the solution used for optical density measurement (g/l).

The DOMD value of the hay was calculated from the regression equation:

$$DOMD = 94.178 - 13.069A$$

Total and individual short-chain fatty acids in rumen liquor and silage. This was determined by the method of Cottyn and Boucque (1968).

Reagents. Preservative mixture. This contained 30ml metaphosphoric acid (25% w/v), 10ml formic acid (90%, Analar) and 10ml distilled water.

Internal standard. Hexanoic acid (2g) dissolved in 1l distilled water.

Procedure. A 2ml sample of strained and centrifuged rumen liquor or of silage extract (prepared as described previously) was pipetted into a test tube and 2ml hexanoic acid solution and 1ml preservative

mixture were added. The tube was shaken and allowed to stand for 20 minutes and then centrifuged at 2500rpm for 20 minutes. The supernatant was analysed by gas chromatography. The sample was injected onto a glass column packed with 5% Carbowax 20M/TPA on Chromosorb G 80/100 mesh. The oven temperature was 135°C, the carrier gas (N_2) flow rate was 60ml/min and the flame of the ionisation detector was maintained with hydrogen and air at pressures of 1.8kg/cm^2.

Molar concentration was calculated for each acid from the peak area on the chromatograph relative to that of hexanoic acid. Corrections were made for the differences in the response of the detector to each acid using factors derived from the analysis of a standard mixture containing known amounts of acetic, propionic, butyric, iso-butyric, valeric and iso-valeric acids.

Chromium-EDTA in digesta. The amount of chromium in samples of rumen liquor and duodenal and ileal digesta was determined as chromate by the method of Stevenson and De Langan (1960).

Reagents. Acid mixture. This contained 5 parts concentrated H_2SO_4, 5 parts H_3PO_4 (sp. gr. 1.75), 10 parts distilled water and 1 part $MnSO_4.4H_2O$, 10% (w/v).

$KBrO_3$ solution, 4.5% (w/v).

$KBrO_3$ solution, 0.45% (w/v).

Stock solution. This contained 2.555g $K_2Cr_2O_4$/l (1ml ≡ 1mg Cr_2O_3).

Procedure. Samples containing chromium in amounts within the range equivalent to 1-5mg Cr_2O_3 were ashed at 580°C in a muffle furnace

overnight. The ashed sample was brushed quantitatively into a 100ml conical flask and 6ml acid mixture was added. The mixture was brought to the boil on a hot plate and 3ml of 4.5% $KBrO_3$ solution was added. Boiling was continued for 30 to 60 seconds after the appearance of SO_3 fumes. The mixture was then allowed to cool before 20ml of 0.45% $KBrO_3$ solution was added and the mixture was boiled for a further 3-4 minutes. The contents of the flask were then transferred to a 100ml volumetric flask and made up to volume with distilled water. 10ml of this was pipetted into a boiling tube with 5ml of 10% NaOH solution and heated in a boiling water bath for a few minutes to allow the MnO_2 precipitate to settle. The solution was cooled, 10ml distilled water were added and the tube inverted gently once, taking care not to shake the tube as the precipitate is readily broken down. The solution was filtered through a Whatman No. 40 filter paper and the absorbance of the filtrate was read at 400nm on a spectrophotometer. A blank determination was performed on CrEDTA-free digesta and reagents. A calibration curve was drawn from determinations on standards containing 1-5mg Cr_2O_3.

Xylitol in digesta

Reagents. Internal standard. This contained 40g pentaerythritol in 1 l distilled water.

Pyridine.

Acetic anhydride.

Procedure. A 15ml sample of digesta, which had been processed as described on p.65 prior to storage, was pipetted into a centrifuge tube and 1.25ml pentaerythritol solution was added. The mixture was

shaken and then centrifuged at 15000rpm for 20 minutes. The supernatant was removed into a 50ml Quickfit tube and was frozen as evenly as possible around the inner surface of the tube by rotating the tube in a bath of dry ice and acetone. The tube was then immediately attached to a freeze-drier (Modulyo, Edwards High Vacuum, Crawley, Sussex) fitted with a drum manifold accessory, and freeze-dried overnight. The freeze-dried material was transferred to a 15ml glass-stoppered conical test-tube and 1ml pyridine and 1ml acetic anhydride were added. The tube was stoppered, sealed with tape and rolled to mix the contents. The tube was then placed in an oven at 100°C for 30 minutes. After cooling slightly the tube was cooled rapidly in an ice-bath. The supernatant was removed and stored at 4°C until analysed by gas chromatography.

The sample was injected onto a glass column containing 5% OV-225 on Chromosorb W AW DMCS 100/120 mesh. The operational temperature was 210°C, the carrier gas was nitrogen with a flow rate of 50ml/min and the flame of the ionisation detector was maintained with hydrogen and air. Xylitol concentration in the sample was calculated by comparing the peak area ratios in the sample with those of a standard containing known amounts of xylitol and pentaerythritol.

Milk total solids. Total solids content of milk was determined gravimetrically according to British Standard 1741 (1963). A known weight of milk was dried, initially by evaporation over a boiling water bath and then in an oven at 100°C.

Milk fat. This was determined by the 'Gerber' method according to British Standard 696 (1969). Fat was extracted by the addition of concentrated H_2SO_4 and measured directly using a Gerber butyrometer.

Milk protein. Total nitrogen was determined by a macro-Kjeldahl method (Association of Official Agricultural Chemists, 1975). This value was multiplied by 6.38 to obtain protein content.

Milk lactose. Milk lactose content was determined polarimetrically using the method of Grimbley (1956).

Fatty acid composition of milk fat. Fat was extracted from milk by a method based on that of Bligh and Dyer (1959) and methyl esters were formed by a procedure modified from that of Christopherson and Glass (1969).

Procedure. The sample of milk was heated slowly to 40°C in a water bath and shaken to disperse the fat globules. One ml of milk was pipetted into a glass-stoppered 50ml test tube and 3ml distilled water, 10ml CH_3OH (Analar) and 5ml $CHCl_3$ (Analar) was added and mixed well. A further 5ml $CHCl_3$ was added and mixed again. Five ml of 0.88% KCl was added and, after mixing, the mixture was centrifuged at 3000rpm for 15 minutes. The lower layer was transferred, using a pasteur pipette into a 50ml round-bottomed flask and evaporated to dryness at 50-60°C using a rotary evaporator. The extracted lipid was washed with acetone and redried. The lipid was taken up in 3ml hexane and transferred to a 15ml conical test tube. The flask was

washed with another 3ml hexane and the washings were added to the test tube. The hexane was evaporated from the sample at 50-60^{o}C under nitrogen and 0.5ml hexane and 0.02ml of 2M sodium methoxide in dry methanol was added to methylate the lipid. The tube was stoppered and shaken at room temperature for 5 minutes. Hexane (0.5ml) and a little dry calcium chloride powder was added. The sample was allowed to stand for 1 hour and was then centrifuged at 2000rpm for 2-3 minutes. The supernatant was poured off into a stoppered glass tube and stored at 4^{o}C until analysed by gas chromatography.

The methyl esters of the fatty acids were separated on a glass column packed with 15% EGSS-X on Gas Chrom P 100/120 mesh (Pierce and Warriner Ltd, Chester) using a temperature-programmed run. The initial temperature of 80^{o}C was held for 4 minutes after which the temperature was increased at a rate of 6^{o}C/minute up to 180^{o}C. The nitrogen carrier gas flow rate was 40ml/min and the detection flame was maintained with hydrogen and air at 1.5kg/cm^{2} and 1.0kg/cm^{2} respectively.

The proportion of each fatty acid in the mixture was calculated from the relevant peak area relative to that of palmitic acid. Corrections were made for differences in the response of the detector to each acid using factors derived from the analysis of a standard mixture of known amounts of caproic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acids.

Plasma glucose. The concentration of glucose in blood plasma was analysed using an enzyme/colour reagent from a commercial kit (Boehringer Corporation Ltd., Lewes). The glucose was oxidised by glucose oxidase and, in the presence of peroxidase, the hydrogen peroxide formed oxidised the chromogen, Perid (2,2 Azino-di[3 ethyl-benzthiazoline sulphonate]). The colour intensity of the dye was proportional to the glucose concentration.

Reagents. Protein precipitating solution. This contained 1.6g/l uranyl acetate and 9g/l NaCl.
GOD-Perid reagent. This contained no less than 10U/ml peroxidase and 1.0mg chromogen/ml in 100mM phosphate buffer, pH 7.0.

Procedure. Plasma (0.5ml) was added to 5ml uranyl acetate solution, mixed and centrifuged at 3000rpm for 10 minutes. Supernatant (0.2ml) was incubated in a test tube with 5ml of GOD-Perid reagent at room temperature for 45 minutes. Blank determinations were performed on distilled water. Absorbance at 640nm was read on a spectrophotometer (Pye Unicam Ltd., Cambridge) and glucose concentration determined by reference to a standard curve for solutions containing 2-10mg/100ml glucose, correcting for the dilution involved in the deproteinisation stage.

Plasma β-Hydroxybutyrate. The enzymatic method used was based on that of Williamson and Mellanby (1974). The determination depended on the increase in extinction by the reaction mixture at 340nm caused by the formation of NADH during the following reaction:

$$\beta\text{-Hydroxybutyrate} + NAD^{+} \underset{\text{dehydrogenase}}{\overset{\beta\text{-Hydroxybutyrate}}{\rightleftharpoons}} \text{Acetoacetate} + NADH + H^{+}$$

The hydrazine present in the mixture trapped the acetoacetate in the form of the hydrazone, allowing the procedure to proceed quantitatively.

Reagents. Hydrazine hydrate glycine buffer. This contained 2.5g glycine in 100ml distilled water and 5ml hydrazine hydrate. The pH was adjusted to 8.5 using 5N HCl.

Perchloric acid solution, 10% (w/v).

Potassium hydroxide, 20% (w/v).

NAD (10mg/ml distilled water).

β-Hydroxybutyrate dehydrogenase. Commercially prepared in 3.2M ammonium sulphate solution from Rhodopseudomonas spheriodes (Boehringer Corporation Ltd., Lewes).

Procedure. A sample of plasma was deproteinised by the addition of an equal volume of ice-cold perchloric acid. The mixture was shaken and centrifuged at 3000rpm for 15 minutes. The supernatant was taken and the pH adjusted to 7-8 with 20% KOH. The potassium chlorate precipitate was removed by centrifugation at 3000rpm for 10 minutes and the deproteinised plasma was used for analysis. Of this 0.15ml was pipetted into a quartz cuvette, together with 0.5ml hydrazine hydrate glycine buffer, 0.1ml NAD solution and 0.25ml distilled water. After mixing, the absorbance at 340nm was read (A_1) on a spectrophotometer (Gilford, Teddington) by reference to a distilled

water blank. β-Hydroxybutyrate dehydrogenase (0.01ml) was then added with stirring and the absorbance read until a steady reading was reached (A_2).

$$\text{Plasma β-Hydroxybutyrate concentration (mmol/l)} = \frac{(A_2-A_1) \times V \times D}{\varepsilon \times v \times d}$$

where (A_2-A_1) = change in absorbance at 340nm

V = final assay volume (ml)

ε = absorption coefficient of NADH at 340nm

d = light path (cm)

v = sample volume (ml)

D = dilution factor

Plasma acetoacetate. The concentration of acetoacetate in blood plasma was determined by the method of Mellanby and Williamson (1976), using β-hydroxybutyrate dehydrogenase. At pH 7, with an excess of NADH this enzyme catalyses the reduction of acetoacetate:

$$\beta\text{-Hydroxybutyrate} + NAD^+ \underset{\text{dehydrogenase}}{\overset{\beta\text{-Hydroxybutyrate}}{\rightleftharpoons}} \text{Acetoacetate} + NADH + H^+$$

The decrease in absorbance at 340nm due to oxidation of NADH is proportional to the amount of acetoacetate present.

Reagents. Phosphate buffer (0.1M). This was prepared by adding KH_2PO_4 (0.1M) to 100ml K_2HPO_4 (0.1M) to obtain a pH of 6.8.

Perchloric acid solution, 10% (w/v).

Potassium hydroxide solution, 20% (w/v).

NADH (5mg NADH-Na_2/ml distilled water).

β-Hydroxybutyrate dehydrogenase. Commercially prepared in 3.2M ammonium sulphate solution from Rhodopseudomonas spheroides (Boehringer Corporation Ltd., Lewes).

Procedure. Plasma was prepared for analysis as described for the determination of β-hydroxybutyrate concentration except that for this assay the pH of the deproteinised plasma was adjusted to pH 6-7. Of this, 0.45ml was pipetted into a quartz cuvette and 0.5ml phosphate buffer and 0.05ml NADH was added. After mixing, the absorbance at 340nm was read (A_1) on a spectrophotometer (Gilford, Teddington) by reference to a distilled water blank. β-Hydroxybutyrate dehydrogenase (0.01ml) was added and mixed. The absorbance was read until a steady reading was reached (A_2).

$$\text{Plasma Acetoacetate concentration (mmol/l)} = \frac{(A_2-A_1) \times V \times D}{\varepsilon \times v \times d}$$

where (A_2-A_1) = change in absorbance at 340nm.

V = final assay volume (ml)

ε = absorption coefficient of NADH at 340nm.

v = sample volume (ml)

D = dilution factor.

Plasma lactate. The concentration of L-lactate in blood plasma was determined using the enzyme lactate dehydrogenase which catalyses the oxidation of lactate as follows:

$$\text{L-Lactate} + NAD^+ \xrightleftharpoons[\text{dehydrogenase}]{\text{Lactate}} \text{Pyruvate} + NADH + H^+$$

Under the alkaline conditions of the assay and in the presence of hydrazine the reaction proceeds quantitatively from left to right:

$$\text{L-Lactate+Hydrazine+}NAD^+ \xrightarrow[\text{dehydrogenase}\ \text{pH 9.0}]{\text{Lactate}} \text{Pyruvate hydrazone+NADH+}H_2O$$

The increase in absorbance at 340nm due to the formation of NADH is proportional to the amount of L-lactate present.

Reagents. Hydrazine hydrate glycine buffer. This contained 2.5g glycine in 100ml distilled water and 5ml hydrazine hydrate. The pH of the buffer was adjusted to 8.5 by the addition of 5N HCl.

Perchloric acid solution, 10% (w/v).

Potassium hydroxide solution, 20% (w/v).

NAD (10mg/ml in distilled water).

Lactate dehydrogenase. Commercially prepared in 3.2M ammonium sulphate solution from beef heart (Sigma, Poole, Dorset).

Procedure. Plasma was deproteinised and the supernatant was adjusted to pH 7-8 as described for the determination of β-hydroxybutyrate. For the assay 0.5ml hydrazine hydrate buffer, 0.1ml NAD, 0.05ml prepared plasma and 0.35ml distilled water were pipetted into a quartz cuvette and mixed. The absorbance (A_1) was measured at 340nm on a spectrophotometer (Gilford, Teddington) by reference to a distilled water blank. Lactate dehydrogenase (0.01ml) was added and the contents of the cuvette were mixed. The absorbance was measured until a steady reading was reached (A_2).

$$\text{Plasma L-Lactate concentration (mmol/l)} = \frac{(A_2-A_1) \times V \times D}{\varepsilon \times v \times d}$$

where (A_2-A_1) = change in absorbance at 340nm.

V = final assay volume (ml)

ε = absorption coefficient of NADH at 340nm.

v = sample volume (ml)

D = dilution factor.

Plasma free fatty acids (FFA). The concentration of free fatty acids (FFA) in blood plasma was determined enzymatically (Wako Pure Chemical Industries, Japan). In the presence of CoA, ATP and Acyl-CoA synthetase (ACS) FFA are converted to acyl CoA. This is oxidised to 2,3-trans-enoyl-CoA and hydrogen peroxide in a reaction

catalysed by Acyl-CoA oxidase (ACOD). In the presence of hydrogen peroxide and peroxidase the oxidative condensation of 3-methyl-N-ethyl-N-(6-hydroxyethyl)aniline (MEHA) with 4-amino antipyrine forms a red quinone complex. The optical density of the coloured complex was used to determine the FFA concentration.

Reagents. Solution A contained ACS, CoA, ATP, 4-amino antipyrine, phosphate buffer solution pH 6.9, magnesium salt and Triton X-100. Solution B contained ACOD, peroxidase, MEHA and Triton X-100. FFA stock solution, 1mM/l oleic acid.

Procedure. The following incubation mixtures were prepared:

(a) Blank. One ml of solution A was added to 0.05ml distilled water.

(b) Sample blank. One ml of solution A was pipetted into a test tube.

(c) Standard. One ml of solution A was added to 0.05ml FFA standard solution.

(d) Sample. One ml of solution A was added to 0.05ml of blood plasma.

The mixtures were incubated at 37^{o}C for 10 minutes prior to the addition of 2ml of solution B to each tube. The mixtures were incubated for a further 10 minutes. At the end of the incubation period 0.05ml blood plasma was added to the sample blank tube. The optical densities were measured spectrophotometrically at 550nm and the FFA concentration in blood plasma was calculated by reference to the optical densities of a series of standard oleic acid solutions containing 0.50-1.97mM/l.

Plasma hormones. Radioimmunoassay (RIA) techniques were used to measure the concentration of hormones in blood plasma. These assays depend on the reaction between an antiserum containing specific antibodies to the hormone (antigen) to be measured, the hormone itself in the standard or sample, and a fixed concentration of ^{125}Iodine-labelled antigen. A fixed quantity of antiserum which is insufficient to react with all the antigen present is used so that a constant amount of bound product is produced. Because there is competition between the labelled and unlabelled antigen for the limited amount of antiserum which is available the activity of the bound product depends on the proportion of labelled antigen in the mixture. There are several methods available for separating the bound product from the free materials. These involve the addition of a second antiserum containing antibodies to the first antiserum, the addition of polyethylene glycol (PEG) or a combination of these two. The bound product is then separated as a pellet by centrifugation and hormone concentration is calculated from the activity of this pellet by reference to those for a series of standards containing known amounts of unlabelled antigen.

Plasma insulin. The concentration of insulin was determined by double-antibody RIA by the method of Vernon et al. (1981) using iodinated rat insulin prepared by the technique described by Madon, Finley and Flint, 1984). The iodination procedure used Enzymobeads impregnated with lactoperoxidase (Bio-Rad Laboratories, Richmond, California). To 25µl Enzymobeads was added 10µl of 0.5M phosphate buffer (pH 7.2), 5µg hormone, 500µCi ^{125}Iodine and 15µl glucose

solution (2% w/v). After 15 minutes the reaction was stopped by the addition of 200µl sodium azide (1mg/ml) in phosphate-buffered saline (PBS) and the mixture was centrifuged at 1000rpm for 2 minutes. The supernatant was transferred to a Sephacryl S-200/Sephadex G-10 column. The beads were then washed by the addition of 200µl KI (10mg/ml) in PBS containing 1% bovine serum albumin (BSA) and re-centrifuged and the supernatant added to the column. The column was eluted with 0.5% BSA:PBS containing 0.1% sodium azide at a flow rate of 6ml/hour and 0.5ml aliquots were collected. Samples (10µl) from the 6 aliquots containing the highest levels of gamma-counts per minute (cpm) were individually mixed with 25µl trichloroacetic acid (40% w/v) to precipitate labelled hormone. Free ^{125} Iodine was removed by decantation and the counts remaining were measured and from this the % iodination of the preparation was calculated.

^{125}I-labelled hormone was used under the following RIA conditions. Insulin standards ranging from 0-5ng insulin/ml were prepared by diluting proprietary insulin stock solution with RIA buffer (0.5% BSA:PBS at pH 7.4). Each standard and sample was assayed in duplicate. The volume taken for analysis was 100µl. To this was added 100µl 'first antibody'. This was antiserum to bovine insulin-GP-3, which cross reacts with rat insulin, diluted 1:20,000 in RIA buffer. After addition of the antiserum the contents of the tubes were mixed thoroughly and incubated overnight at 4^{o}C, together with duplicate 'blank' tubes containing 200µl RIA buffer. On the next day ^{125}I-insulin was diluted with RIA buffer so that 100µl of this mixture produced approximately 12,000cpm. Iodinated hormone

(100µl) was added to all tubes and also to an additional 2 'total counts' tubes. The tubes were incubated at 4°C overnight and then the second antibody, anti-guinea pig precipitating serum, was added. The 'second antibody' mixture was prepared by dissolving 560mg EDTA in 19ml RIA buffer, adjusting the pH to 7.4 and adding 160µl normal guinea pig serum (1:10) and 240µl anti-guinea pig precipitating serum. This was mixed thoroughly and 19ml of 25% PEG was added and mixed. With the exception of the total counts tubes all tubes received 100µl 'second antibody' mixture and were incubated for 2 hours at room temperature. One ml of 4% PEG was added to all but the 'total counts' tubes, after which the blanks, standards and samples were centrifuged at 2500rpm for 30 minutes at 4°C. The supernatant was decanted and the activity of the pellets was counted. The concentration of insulin in the samples was calculated from the blank- and background-corrected activity by reference to that of the standards.

Plasma growth hormone. This hormone was assayed by double-antibody RIA. Iodinated growth hormone was prepared by the method described previously for insulin. Growth hormone standards ranging from 0-20ng/ml were prepared by diluting a proprietary growth hormone preparation with RIA buffer (0.5% BSA:PBS, pH 7.4). The volume of standards and samples taken for analysis was 200µl. 'Blank' tubes contained 300µl RIA buffer. First antibody (100µl), antiserum to ovine growth hormone raised in rabbits, was added to all but the 'blank' tubes at a dilution of 1:20,000 in RIA buffer. All tubes were incubated overnight at room temperature. The following day

10,000cpm ^{125}I-growth hormone was added to each tube (in 100µl RIA buffer) and to 2 'total counts' tubes. The tubes were incubated overnight at 4°C. 'Second antibody' mixture was prepared by dissolving 140mg EDTA in 15ml RIA buffer, adjusting the pH to 7.4 and adding 5µl normal rabbit serum, 125µl anti-rabbit precipitating serum and 15ml of 16% PEG. With the exception of the 'total counts' tubes each tube received 300µl 'second antibody' mixture. The contents of the tubes were mixed thoroughly and incubated at room temperature for 2 hours before being centrifuged at 2500rpm for 30 minutes at 4°C. The supernatant was decanted and the activities in the 'total counts' tubes and the pellets in the remaining tubes were counted. The concentration of growth hormone in the samples was calculated by reference to the standard curve.

Plasma glucagon. Glucagon was assayed by double-antibody RIA. Commercially prepared ^{125}I porcine glucagon (I.R.E. UK Ltd, Bucks), diluted to working strength with 'FAM' - 0.04M phosphate buffer, pH 7.4, containing human serum albumin (0.5% w/v) and sodium azide (1mg/ml), was used. A series of standards containing 0-10ng glucagon/ml were prepared from a proprietary glucagon preparation by dilution with FAM to which had been added NaCl (1.5mg/ml) and trypsin inhibitor (3.45ml Aprotinin/100ml). Assays were performed in duplicate. Standard and sample volumes were 100µl. 'Blank' tubes contained 100µl of the buffer used to dilute the standards and 50µl FAM. The latter buffer was used to dilute the glucagon antiserum (rabbit) 1:1000 and 50µl of this was added to all but the 'blank' tubes. All tubes were incubated overnight at 4°C. ^{125}I-glucagon was

added to all tubes to produce 10,000cpm/tubes and to 2 'total counts' tubes. The mixtures were incubated overnight at 4°C and 100µl 'second antibody' mixture was added to all but the 'total counts' tubes. This mixture comprised 612.5mg EDTA in 15.75ml FAM, pH 7.4 containing 35µl normal rabbit serum, 875µl anti-rabbit precipitating serum and 17.5ml of 25% PEG. After the addition of second antibody the mixtures were incubated at 4°C for 4 hours, 0.5ml of 4% PEG was added and the tubes centrifuged at 2500rpm for 30 minutes at 4°C. The supernatant was decanted and the activity remaining in the pellets and the 'total counts' tubes was measured and glucagon concentration in the plasma samples was calculated by reference to the standard curve.

Plasma prolactin. Plasma prolactin concentration was measured by double-antibody RIA by the method of Vernon et al. (1981). Prolactin was iodinated by the same method as that previously described for insulin. A proprietary prolactin standard was diluted with RIA buffer to give a series of standards ranging from 0-40ng prolactin/ml. Assays were performed in duplicate. Standard and sample volumes were 100µl. 'Blank' tubes contained 200µl RIA buffer. First antibody and ^{125}I-labelled prolactin were added simultaneously to all but the 'blank' tubes as 200µl of a 1:1 mixture of antiserum to bovine prolactin diluted 1:60,000 in RIA buffer and ^{125}I ovine

prolactin diluted in RIA buffer so that 100µl produced 20,000cpm. ^{125}I-labelled prolactin (100µl) was added to the 'blank' tubes and to 2 'total counts' tubes.

All tubes were incubated overnight at room temperature. The 'second antibody' mixture was the same as that described previously for the insulin RIA. All tubes except the 'total counts' tubes received 100µl of this mixture and were incubated at room temperature for 2 hours before 1ml of 4% PEG was added, the tubes centrifuged at 2500rpm for 30 minutes at 4°C and the supernatant decanted. The activity in the pellets and the total counts tubes was measured and prolactin concentration in the samples was calculated from the counts after correction for the blank and background readings by reference to those for the standards.

Plasma free triiodothyronine (FT_3). The concentration of FT_3 in blood plasma was measured by RIA using a commercial kit (Gruppo Lepetit, Milan, Italy). The assay procedure involved a chromatographic separation of thyroid hormones using Sephadex LH-20 columns and RIA of the absorbed FT_3 using LISO-PHASE columns. FT_3 standards (500µl) ranging from 0-20pg/ml or samples were added to Sephadex LH-20 columns and allowed to drain. The columns were incubated for 15 minutes at room temperature, drained and washed with 2ml buffer solution to remove the protein fraction. A protein solution (200µl) was added and allowed to drain and after 15 minutes incubation the hormones were eluted with 500µl buffer solution. The eluate was collected into LISO-PHASE columns which were capped at the bottom and contained, below a porous polythene disc, a second

antibody covalently linked to Sepharose gel. Above this disc the eluted FT_3 was incubated for 1 hour at room temperature with 100µl ^{125}I-T_3 and 100µl antiserum. The caps were then removed from the bottom of the columns to allow the reaction mixture to pass through the porous disc to reach the immunosorbent, which retained the antigen-antibody complex. The unbound hormone was eluted by washing the columns with 2ml buffer. After this had been allowed to drain the radioactivity in the columns was counted and FT_3 concentration in the samples was determined from the standard curve.

SECTION III

THE DIGESTION CHARACTERISTICS AND METABOLIC EFFECTS OF XYLITOL WHEN INFUSED INTO THE DIGESTIVE TRACT

EXPERIMENT 1

Mixtures of polyols are apparently digested by ruminants (Poutiainen et al., 1976). The majority of these polyols are fermented in the rumen but if, as evidence from in vitro studies suggests, xylitol is particularly resistant to fermentation by rumen microorganisms (Poutiainen et al., 1976; Bain, 1980) the possibility exists that some of this polyol may be absorbed directly and enter metabolism. Xylitol is glucogenic in non-ruminant hepatic tissue (Woods & Krebs, 1973) and is associated, when given intravenously, with a tendency for glucose concentration to be increased and for ketone body concentrations to be reduced in previously ketotic cows (Hamada et al., 1982). These properties of xylitol indicate that this polyol may be potentially useful as a source of glucogenic substrate when included in diets given to ruminant animals. However, as few studies relating to polyol digestion in the ruminant have been reported and in none of these has the fate of xylitol in the rumen been measured directly, the amount of xylitol available for metabolism in such a situation is unknown.

The following experiment, consisting of 3 parts, was therefore designed with the aim of quantifying in vivo the rate of disappearance of xylitol from the rumen, the passage of xylitol to the duodenum and the absorptive capacity of the small intestine with respect to xylitol.

Experimental

Animals and their management. Three Finnish Landrace x Dorset Horn wethers weighing approximately 50kg and fitted with rumen, duodenal

and ileal cannulas were used. The animals were housed in metabolism cages and were offered the diet in hourly meals via automatic feeders. Water and mineral blocks were freely available.

Experimental diets and infusion solutions. The animals received a diet of 1200g/d (fresh weight) of a commercially-prepared cubed feedstuff ('AA6'; Seafield Mill, Midlothian) throughout the experiment. CrEDTA solution was prepared as described in Section II and xylitol (Sokerikermia Oy, Kotka, Finland) was dissolved in this as appropriate.

Experimental plan and procedure. The animals received the diet for an adaptation period of 10 days prior to the start of the experiment. Three experimental treatments were then applied in the same sequence to all animals. The treatment periods were separated by an interval of 8 and 5 days respectively.

The first treatment consisted of a period of 12 hours during which xylitol disappearance relative to the rate of outflow of the liquid phase of digesta from the rumen was measured. Rumen liquor was sampled as described in Section II at 09.45 hrs and immediately after this 0.5 l CrEDTA (2.77g Cr/l) solution containing 60g xylitol was given as a single dose dispersed at several sites into the rumen. Samples of rumen fluid were removed 60, 120, 180, 240, 375, 555 and 705 min after dosing.

The second treatment involved continuous intraruminal infusion of xylitol and measurement of the recovery of xylitol at the duodenum. During consecutive periods of 6, 4 and 4 days 2 l/d of diluted CrEDTA solution (0.69g Cr/l) supplying 0, 30 and

60g xylitol/d during the respective periods was infused. Infusions were made via polythene tubing (I.D. 1.5mm; Portex, France) using a peristaltic pump (Quickfit Ltd.). Samples of duodenal digesta were taken on the final day of each period at 10.00, 12.00, 14.00 and 16.00 hrs. The level of xylitol inclusion was increased immediately after sampling was completed.

The final experimental treatment consisted of a series of 5 periods, each 3 days in length. Throughout this time a measured volume approximating to 0.5 l/d of CrEDTA solution (2.77g Cr/l) was infused continuously into the duodenum using a peristaltic pump (Technicon Ltd.). Successive infusates supplied 0g and approximately 15, 30, 45 and 60g xylitol/d. Samples of ileal digesta were obtained on the final day of each period at 10.00, 12.00, 14.00 and 16.00 hrs.

Chemical analysis. Samples of digesta were processed, stored and analysed as described in Section II. Digesta were analysed for chromium (Cr) and xylitol content.

Calculation of results. Rumen volume and the rate of outflow of liquid phase from the rumen were calculated from Cr disappearance as outlined by Weston and Hogan (1967). The degree to which the disappearance of xylitol exceeded that attributable to loss in the liquid phase flowing from the rumen was assessed by the following mathematical treatment. The absolute concentrations of Cr and xylitol at each sampling time were expressed as a percentage of the respective initial concentration as determined from the intercepts of

semilogarithmic plots of concentration against time. Logarithm$_{10}$ of these normalised values were regressed against time and the areas under the regression lines compared for individual animals.

The flow of intraruminally administered xylitol to the duodenum was calculated by reference to the duodenal flow of Cr assuming a 100% recovery of the CrEDTA infused. Similarly, the recovery of xylitol at the ileum when infused into the duodenum was related to the Cr content of the liquid phase of the ileal digesta. These data were subjected to a 2-way analysis of variance.

Results

Animal health and food intake. There was no evidence of digestive disturbance or ill-health in animals receiving xylitol at any level of administration. Rations were consumed completely throughout the experiment.

Flow of liquid phase of digesta in the rumen, duodenum and ileum. Values for rumen volume and outflow of liquid phase derived from disappearance of CrEDTA introduced as a single dose, together with rates of flow of liquid digesta at the duodenum are shown in Table 3.01. Duodenal flows, which were generally high, may have been slightly elevated in all animals by the volumes concurrently infused into the rumen. No correction was made for this. Duodenal flows were significantly increased when xylitol was infused whether at 30g/d ($P < 0.01$) or, to a significantly ($P < 0.05$) lesser extent, 60g/d ($P < 0.05$). The mean duodenal flow was greater than the rumen outflow, apparently reflecting the secretion of fluids in the abomasum. This effect was particularly marked in one of the animals.

Table 3.01 Rumen volume (l) and the flow of liquid phase of digesta from the rumen, at the duodenum and at the ileum (l/d) during xylitol administration in 3 sheep during periods 1, 2 and 3 of Experiment 1.

	Experimental period	Level of xylitol administration (g/d)					
		0	15	30	45	60	SEM
Rumen volume	1	-	-	-	-	4.70	0.81
Rumen outflow	1	-	-	-	-	9.95	0.69
Duodenal flow	2	10.31	-	13.68	-	12.04	0.35**
Ileal flow	3	6.47	5.70	6.20	5.92	5.95	0.31

** $P < 0.01$

Ileal flow measurements were not corrected for volumes infused intraduodenally. Values (Table 3.01) were similar for all animals and did not vary ($P > 0.05$) with treatment.

Disappearance of xylitol from the rumen. As shown in Fig. 3.01 the rate at which xylitol disappeared from the rumen was greater than that attributable to loss of xylitol in the liquid phase of digesta flowing from the rumen. However, this difference was small and the proportion of the xylitol dose which disappeared during 12 hours as the result of breakdown and/or direct absorption through the rumen wall was less than 5% (Table 3.02).

Passage of xylitol to the duodenum. As Table 3.02 shows, even at the higher level (60g/d) of xylitol infusion into the rumen, less than 1% of the daily dose was detected at the duodenum. Had, therefore, the high duodenal flow rate of liquid phase recorded in one sheep been an overestimation of the true rate this would not have altered significantly the result in terms of the amount of xylitol reaching the small intestine. The tendency for xylitol concentration at the duodenum to be lowest in this animal suggests that the flow marker data were in fact correct.

Absorption of xylitol from the small intestine. Irrespective of dose level a considerable amount of the xylitol infused intraduodenally was recovered at the ileum (Table 3.02). Xylitol was absorbed from the small intestine in significantly greater amounts as the dose level was increased and under these conditions the relationship was linear (Fig. 3.02); approximately 35% of the xylitol

Fig. 3.01 The proportions of CrEDTA (●) and xylitol (▲) remaining in the rumen fluid of 3 sheep which had received a single intraruminal dose of 60g xylitol in 1ℓ CrEDTA solution.

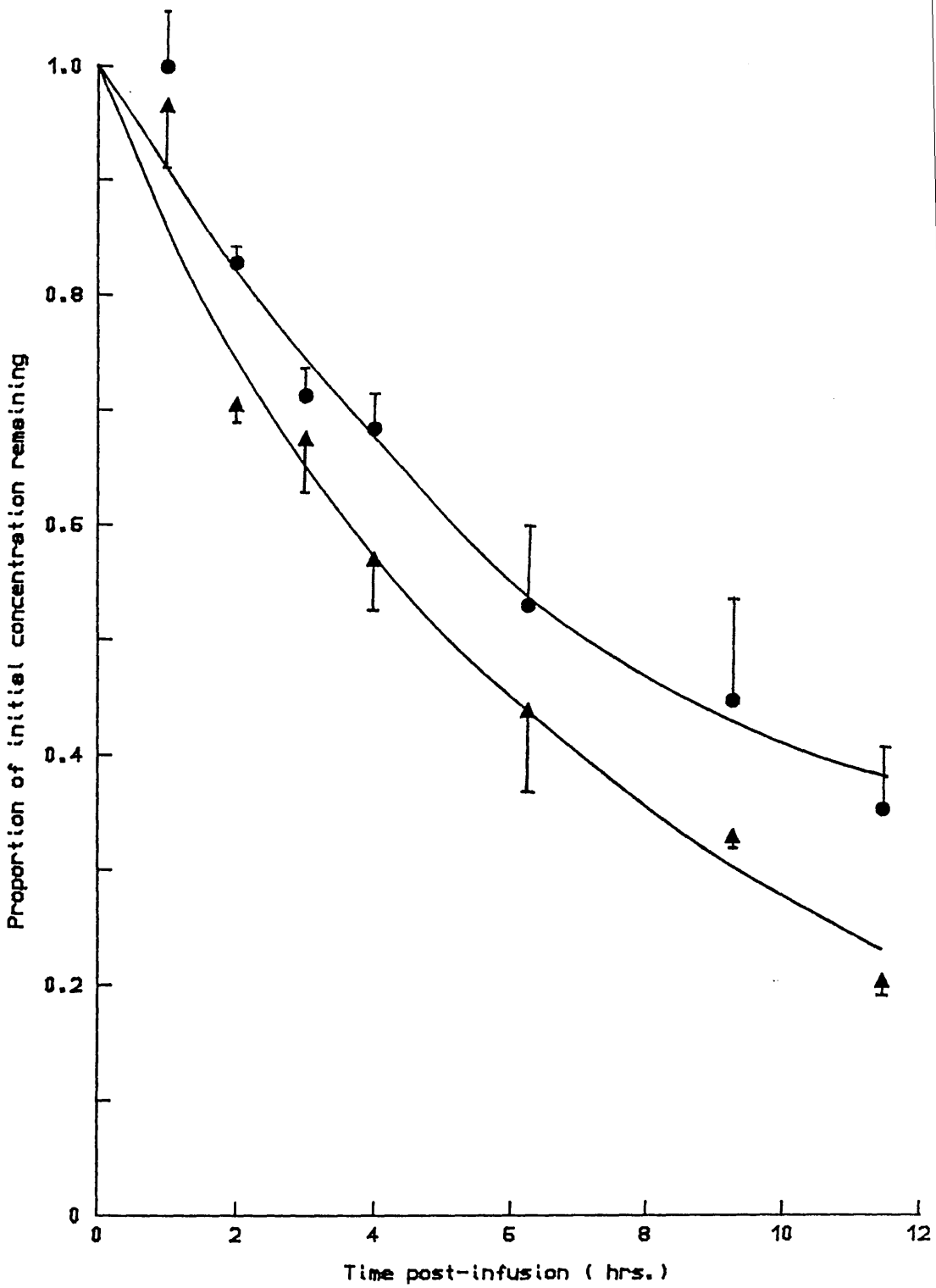


Table 3.02 The disappearance of xylitol, corrected for that flowing from the rumen, during 12 hrs after intraruminal administration, the passage to the duodenum of intraruminally administered xylitol and the absorption from the small intestine of intraduodenally administered xylitol in 3 sheep during periods 1, 2 and 3 of Experiment 1.

	Experimental period	Level of xylitol administration (g/d)				
		15	30	45	60	SEM
Breakdown and/or absorption from the rumen	1					
% of dose†		-	-	-	4.86	1.47
Passage to the duodenum	2					
% of dose††		-	0.53	-	0.86	0.50
g/d		-	0.16	-	0.52	0.27
Absorption from the small intestine	3					
% of dose††		37.1	30.9	37.2	36.2	6.23
g/d		5.57	9.27	16.73	21.72	0.94***

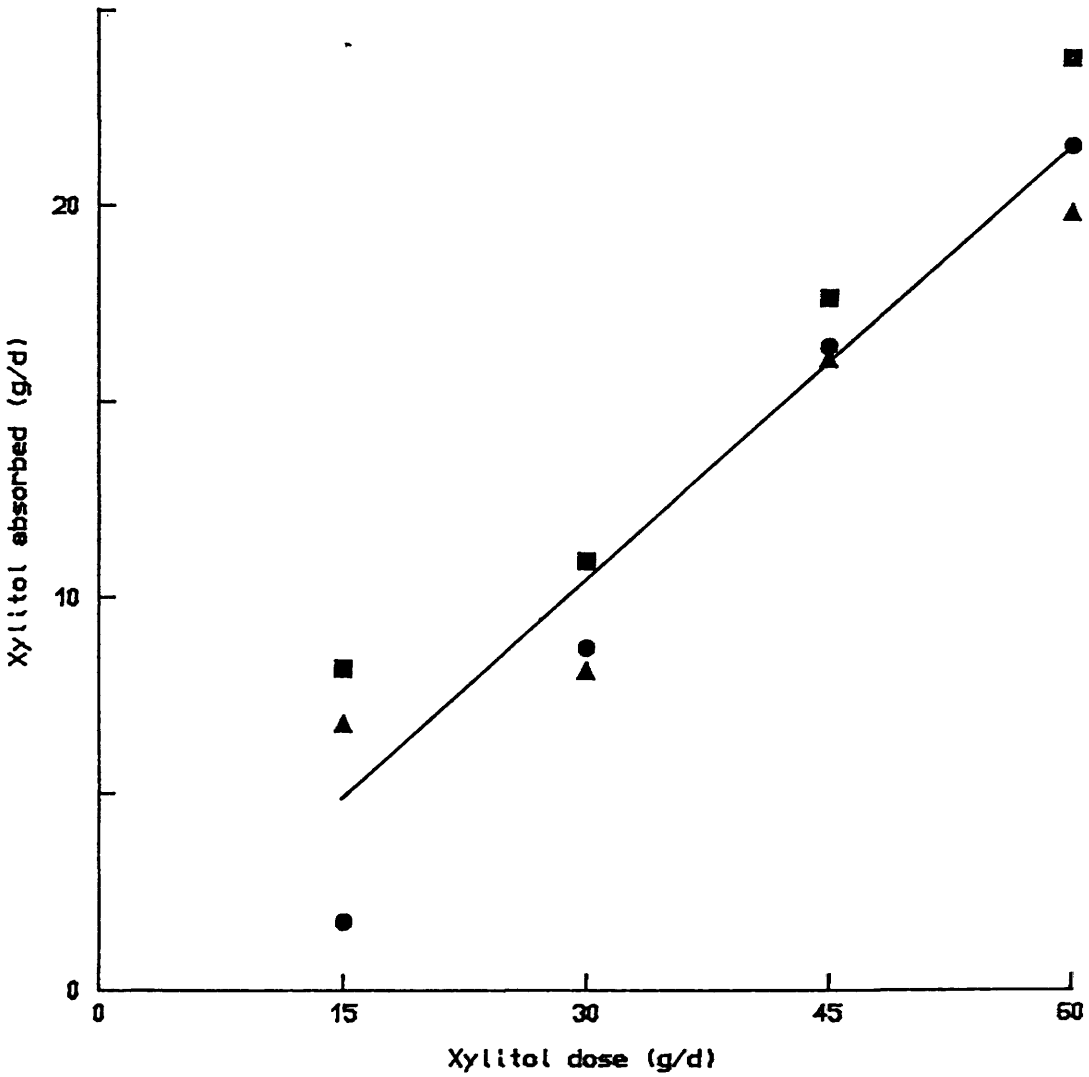
†Xylitol dose given as a single injection
††Xylitol dose given as a continuous infusion
*** $P < 0.001$

infused was absorbed from the small intestine. The amount of xylitol absorbed was highly significantly increased ($P < 0.001$) when 60g rather than 15g xylitol/d was infused.

Discussion

Values for rumen volume and outflow rate were comparable with those reported by Weston and Hogan (1967). The slow rate at which xylitol disappeared from the rumen confirms the resistance of this polyol to microbial fermentation *in vivo*. Furthermore, these results may be an overestimation of fermentation; as Tuori and Poutiainen (1977) suggest some xylitol may have been absorbed directly through the rumen wall. The possibility exists, however, that during prolonged exposure to xylitol adaptation within the rumen microbial population may increase the ability of the population to ferment this polyol. *In vitro* studies have found evidence of adaptive changes within the rumen populations of sheep which had received polyol mixtures intraruminally for periods of up to 3 weeks (Bain, 1980; Lister & Smithard, 1984). As a consequence of these changes xylitol was fermented more rapidly when incubated with rumen liquor from these animals but the extent to which xylitol was fermented during a period equivalent to rumen turnover time was not significantly altered. No studies of the occurrence of adaptation within rumen microbial population to xylitol in the absence of other, more readily-fermented polyols have been reported, but it would seem reasonable to assume that the effects of this polyol on the composition and metabolism of the microbial population would be relatively small. Studies in rats (Krishnan, James *et al.*, 1980; Krishnan, Wilkinson *et al.*, 1980) have, however, shown that the cessation of the osmotic diarrhoea

Fig. 3.02 The intestinal absorption of xylitol infused into the duodenum in 3 sheep.



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which frequently accompanies the introduction of large amounts of xylitol to diets given to non-ruminant species (Makinen & Scheinin, 1975; Forster, 1978) is associated with an increased ability of the caecal flora to metabolise xylitol. This would suggest that some adaptation within the rumen microbial population during prolonged exposure to xylitol is likely. Krishnan, Wilkinson et al. (1980) suggest the adaptive mechanisms to involve mutation, selection of microorganisms capable of metabolising polyols and the induction of enzymes involved in polyol metabolism.

The demonstration that, while as much as 95% of a xylitol dose escapes fermentation in the rumen, less than 1% of xylitol infused intraruminally is detected at the duodenum indicates that xylitol is readily absorbed from the abomasum or more likely the omasum. This is consistent with the observation of Lister and Smithard (1984), who found duodenal flow of xylitol in sheep which had received a mixture of polyols supplying 0.65g xylitol/kg liveweight $^{0.75}$/d intraruminally for a period of 11 weeks to be equivalent to only 1.8% of the daily dose. The results of the present experiment demonstrate that the capacity for absorption is not exceeded when as much as 3.19g xylitol/kg liveweight $^{0.75}$/d is administered, and that this capacity is possessed within a relatively short period of time after the introduction of xylitol to the digestive tract.

Evidently the possibility of xylitol reaching the small intestine exists only if it were to be included in the diet at the level of a major nutrient. The results of the final part of this experiment show that any xylitol reaching the small intestine would be absorbed with limited efficiency. Xylitol disappearance from the small intestine increased significantly as the amounts infused were

progressively increased. Because of the experimental design used here the effects of increasing the amount of xylitol given on xylitol absorption are confounded with time and possibly with the development of an adaptive increase in xylitol absorption. However, in view of the close correlation between uptake and infusion rate (Fig. 3.02) it seems likely that the changes in absorption were principally related to the dose rate of xylitol infused.

For the reasons already discussed adaptation within the gut microflora is unlikely to account for the observed increases in xylitol disappearance, especially as microbial numbers in the small intestine are relatively low (Harfoot, 1981). Thus changes in xylitol disappearance appear to reflect changes in the rate at which the polyol is absorbed from the intestinal lumen. In experiments with rats, Krishnan, Wilkinson et al. (1980) found no evidence for changes in gut wall function which might facilitate xylitol absorption in rats receiving xylitol. Increased xylitol absorption does not appear to be a consequence of adaptive mechanisms at the level of the liver. Bassler (1969) suggested that xylitol absorption, being a passive process, is enhanced by an increase in the gradient of xylitol concentration across the gut wall as a consequence of an increased rate of xylitol extraction by the liver, where the activity of enzymes involved in xylitol metabolism is increased in 'adapted' animals (Bassler, 1969; Hosoya & Iitoyo, 1969). However, Forster (1978) disputes the impact which these changes are likely to have on xylitol absorption from the gut on the basis of the wide margin between polyol concentrations in the intestinal lumen and in portal blood; these range in rats receiving

polyols from 2000 to 4000mg/100ml and only 20 to 30mg/100ml respectively.

Dose level would therefore appear to be the major determinant of the rate, and through this the extent, of xylitol absorption. Certainly from the linearity with which xylitol absorption from the small intestine increased with dose rate in the present experiment there would appear to be, as in monogastrics (Forster, 1978), a direct relationship between the concentration of xylitol in this region of the ruminant digestive tract and the rate at which it is absorbed.

EXPERIMENT 2

The resistance of xylitol to fermentation in the rumen was confirmed *in vivo* in Experiment 1, as was the ability of the ruminant to absorb xylitol from the post-ruminal digestive tract. The following experiment was designed to quantify, by comparison with glucose, the potential of xylitol to contribute to glucose supply when introduced into the digestive tract of the cow.

Experimental

Animals and their management. Three non-lactating Ayrshire cows fitted with rumen and duodenal cannulas were used. The animals were housed in individual stalls in a small byre and received their rations individually in 2 equal meals/day at 09.00 and 16.00 hrs.

Experimental diet and infusion solutions. The animals received a diet of 8kg/d (fresh weight) of a moderate quality hay.

Three experimental treatments, single doses of 200g xylitol into the rumen or duodenum or of 236g glucose into the duodenum, were given as solutions in 1 l water warmed to 37^{o}C. A control dose of 1 l of solution containing 9g sodium chloride was administered intraduodenally.

Experimental plan and procedure. The experimental treatments were applied to each cow in a sequence arranged as a 3x3 Latin Square design. A period of 6 days separated each infusion. This treatment sequence was followed in 2 cows by control infusions.

When infusions were to be given the meals at 16.00 hrs on the previous day and at 09.00 hrs on the day of infusion were withheld and the total ration for that day was given at 16.00 hrs.

Blood sampling commenced at 10.45 hrs and was continued at 15-minute intervals. Immediately after collection of a sample at 11.15 hrs the appropriate solution was infused and sampling was continued until 15.30 hrs. Solutions were infused at a rate of 100ml/min using a peristaltic pump (MRE 200, Watson Marlow Ltd., Falmouth). When infusing intraruminally the rubber tubing (8mm ID) passing from the pump was perforated throughout the last 15cm and attached to a metal pipe such that it extended for about 25cm inside the rumen and could be moved to optimise the mixing of infusate with rumen contents. Intraduodenal infusions were made through polythene tubing (5mm ID) which was connected to the rubber tubing from the pump and which passed through the rubber bung fitted into the duodenal cannula. Blood samples were removed via an indwelling jugular catheter.

Chemical analysis. Blood plasma was prepared and analysed for glucose concentration as described in Section II.

Statistical analysis. Mean changes in plasma glucose concentration at each sampling time post-infusion relative to mean pre-infusion concentration were expressed graphically for each treatment and the area under each response curve was calculated using Simpson's rule (Cornish-Bowden, 1981). Responses in plasma glucose concentration to the experimental and control treatments were tested for significant differences from the respective pre-infusion values by paired t-test.

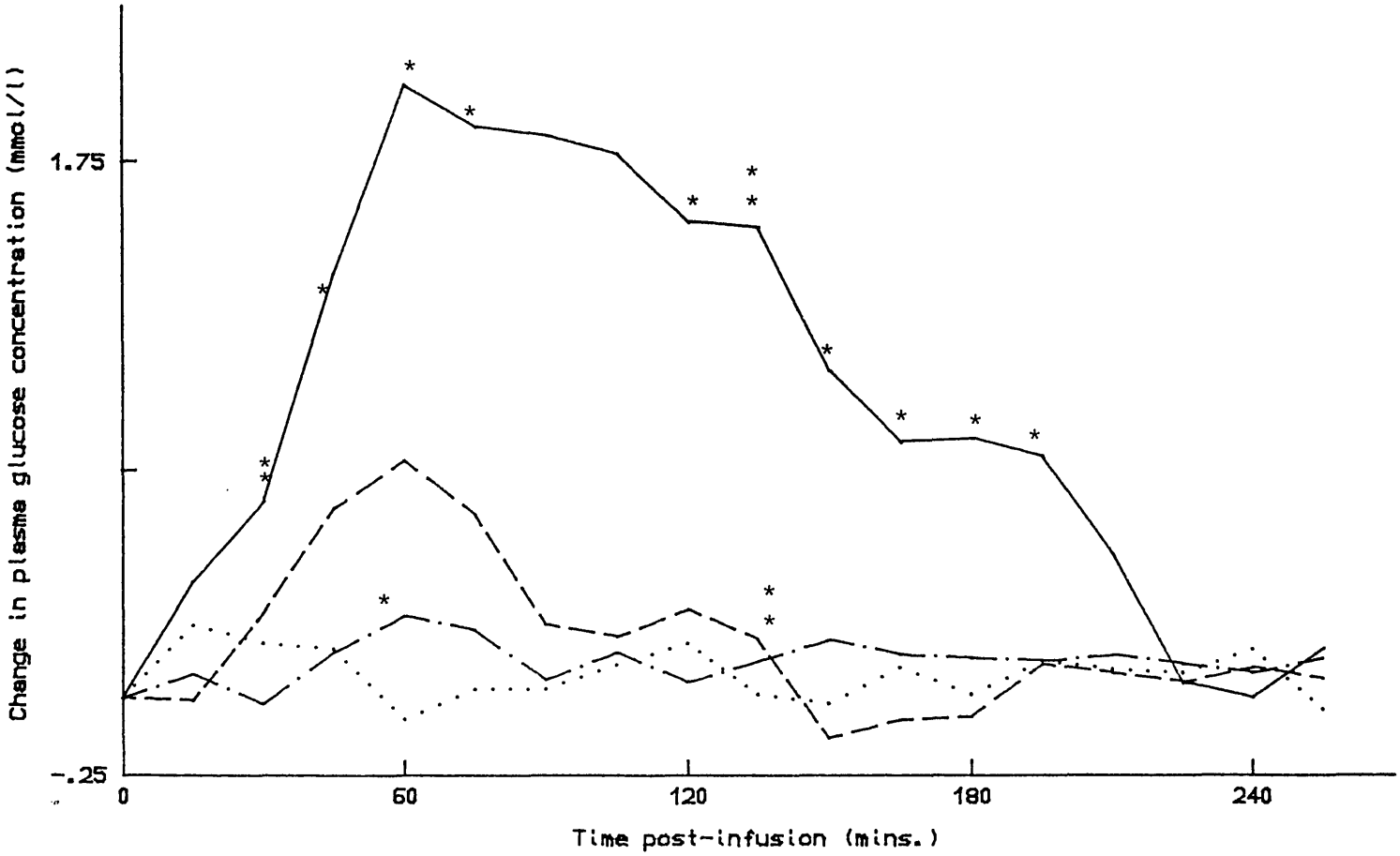
Results

Pre-infusion plasma glucose concentrations were similar for all treatments. Prior to infusion of glucose into the duodenum, xylitol into the rumen and xylitol into the duodenum mean glucose concentrations were 3.98 $\pm$ 0.15, 3.90 $\pm$ 0.19 and 4.00 $\pm$ 0.20mM respectively. For all experimental treatments plasma glucose reached maximal concentrations between 45 and 90 minutes after the administration of the test substance and returned to pre-infusion levels within the period during which samples were taken (Fig. 3.03). Plasma glucose concentration was not altered significantly ($P > 0.05$) by the control treatment. Glucose given into the duodenum was the most effective treatment, both in terms of maximal plasma glucose concentration achieved and the duration of the response. Plasma glucose concentration was increased by a highly significant amount ($P < 0.01$) within 30 minutes of dosing. A corresponding or higher glucose level was maintained for a further 165 minutes. However, because the time-course of the changes for individual animals varied, the differences between 45-195 minute values and the pre-infusion value were generally only significant at the $P < 0.05$ level.

The response to xylitol administered intraruminally, although less marked, was significant ($P < 0.05$) 60 minutes post-infusion. The response was more protracted and amounted to 12% of that to intraduodenally administered glucose.

The response in plasma glucose concentration to intraduodenal administration of xylitol showed a similar pattern as that to glucose given intraduodenally, but the peak concentration of glucose was lower and more transient and the duration of the overall response was

Fig. 3.03 Post-infusion changes in blood plasma glucose concentrations with time in 3 cows given infusions of glucose intraduodenally (——), xylitol intraduodenally (— —), xylitol intraruminally (·—·—) and saline intraduodenally (·····)†.



† Values are means for 2 animals only.

Asterisks denote level of significance of difference from pre-infusion glucose concentration

shorter. There was again variation between individuals in the timing of the response such that mean glucose concentration was only increased significantly ($P < 0.01$) 135 minutes after xylitol was given. As judged by the areas under the respective response curves the response to intraduodenal xylitol was 19% of that to glucose.

Discussion

Food was withheld for a 24 hour period prior to the administration of glucose or xylitol to induce a mild shortage of energy in the animals to encourage maximal utilisation of infused substrates for glucose metabolism. In this situation the response to the provision of additional glucose served as an index against which the response to xylitol could be assessed. As would be expected in these circumstances intraduodenal dosing with glucose produced a significant increase in plasma glucose concentration. The responses to equivalent amounts of xylitol were considerably smaller.

On the basis of Experiment 1 the fraction of the xylitol dose given intraruminally which would be fermented during the time for which plasma glucose concentration was subsequently monitored was very small, approximately 2%. It would also appear from the results of Experiment 1 that virtually 100% of the xylitol escaping fermentation would be absorbed prior to the duodenum. Thus, the pattern of response to xylitol given intraruminally is likely to be a reflection of the availability of xylitol for absorption from the omasum and hence of the limitations imposed by dilution in the rumen and the rate of rumen emptying. Taking these factors into consideration it is likely that approximately only 70g of xylitol administered was absorbed during the experimental period.

If, again on the basis of the results of Experiment 1, it is assumed that 35% of xylitol given intraduodenally was absorbed from the small intestine the intraduodenal administration of xylitol in the present experiment would also have resulted in the absorption of approximately 70g xylitol. As 1.2 moles of xylitol are converted to 1.0 mole glucose, the maximum possible response to this amount of xylitol would be expected to be approximately 30% of that to the glucose infusion, making the further assumption that the glucose was absorbed completely from the small intestine (Janes et al., 1985a). Evidently the responses in plasma glucose to xylitol were smaller than this and indicated that under these conditions at most 40-60% of this xylitol was utilised for gluconeogenesis. This range is lower than that which might be anticipated from evidence of 60-70% conversion of xylitol to glucose in isolated perfused rat liver (Forster, 1974) and 80% conversion, again in rats, measured using ^{14}C-labelled xylitol (Froesch, 1976).

While these comparisons involve a number of assumptions, at a metabolic and endocrine level as well as those already discussed, the results of the experiment demonstrate that xylitol absorbed from the gut can contribute to glucose availability in the ruminant.

SECTION IV

THE EFFECTS OF DIETARY CARBOHYDRATE SOURCES WITH DIFFERING DIGESTION CHARACTERISTICS ON METABOLISM AND MILK PRODUCTION IN COWS

EXPERIMENT 3

The observed differences between the responses in milk production to diets containing barley-based concentrates and those containing maize-based concentrates (Sutton et al., 1980) illustrate the impact that, at a fixed energy intake, the digestion characteristics of the major starch component of the diet can have on milk synthesis. These effects apparently derived from the influence of the different starchy feeds on the products of digestion and from the consequent responses in hormonal secretion and nutrient utilisation. The digestion characteristics of these cereals are such that less than 10% of starch in barley escapes rumen fermentation while as much as 30% of that in ground raw maize passes intact to the duodenum (Watson et al., 1972; Waldo, 1973). While the proportion of starch disappearing from the small intestine which enters the portal blood stream as glucose is uncertain (see Armstrong & Smithard, 1979), recent evidence from sheep receiving a maize-based diet suggests that a major part of the starch disappearing is absorbed as glucose and that only a small proportion is metabolised during absorption (Janes et al., 1985a). From the evidence discussed in Section I, differences in the amount and nature of the glucogenic substrate present in the mixtures of digestion end-products absorbed from barley- and maize-based diets are likely to elicit differences in the hormonal responses to these diets. However, it is apparent that differences in the responses in the yields of individual milk constituents to such diets are not invariably observed (Sutton et al., 1980).

Experiment 3 was designed to investigate the impact on energy metabolism and milk production of dietary energy sources possessing different digestion characteristics. A comparison was made of the responses by dairy cows in early lactation to supplementation of a basal diet with 3 different energy sources. Barley was chosen as a source of carbohydrate which would be readily degraded in the rumen and ground raw maize as a carbohydrate source of which a considerable proportion would pass as starch to the small intestine. The third supplement contained xylitol. The results of Experiments 1 and 2 indicated that when included in the diet some of this polyol would escape fermentation in the rumen to be absorbed post-ruminally and contribute to glucose supply.

Experimental

Animals and their management. Eight intact Ayrshire cows weighing approximately 470kg were used. The animals were in the second week of lactation when the experiment commenced. The animals were housed in individual stalls in a small byre and were milked in the stalls at 06.00 hrs and 16.00 hrs daily. Concentrate allowances were divided into 8 equal meals and given at 3-hourly intervals using automatic feeders. Hay was given in 4 meals, at 6.00 hrs, 10.00 hrs, 16.00 hrs and 22.00 hrs.

Experimental diets. The 4 experimental diets consisted of a basal ration of 7kg/d (fresh weight) hay and 8kg/d of a cubed barley/soya bean meal-based dairy concentrate given either alone or with 2kg/d of one of 3 isoenergetic and isonitrogenous supplements. The roughage:concentrate ratio of the basal ration was 45:55 on a DM

basis. The supplements were rolled barley, ground raw maize and a mixture of xylitol (Sokerikermia Oy, Kotka, Finland) and rolled barley (25:75 on a fresh weight basis). The composition of the dietary constituents is given in Table 4.01. ME contents were derived as follows: for hay using 0.15 x DOMD% (Ministry of Agriculture, Fisheries and Food, 1975), for barley using 12.9MJ/kgDM (Department of Agriculture and Fisheries for Scotland, 1975) and for the remaining constituents from standard tables, using the figure for sugar cane molasses for xylitol (Ministry of Agriculture, Fisheries and Food, 1975).

Experimental plan and procedure. The treatments were applied in sequence according to a duplicated 4x4 Latin Square design. Each treatment period was two weeks long and the diets were changed abruptly at the end of each period. Food intake and milk yield were recorded daily. Samples of feedstuffs were taken fortnightly. Milk samples were taken at the last 4 milkings of each period. Blood samples were taken at approximately 10.00 hrs on day 2 and subsequent alternate days throughout the period from an indwelling jugular catheter as described in Section II.

Chemical analysis. Samples were prepared and analysed as described in Section II. Food samples were analysed for dry matter, ash, total nitrogen and cellulose. The amount of digestible organic matter in the dry matter (DOMD) of the hay was determined *in vitro*. Milk samples were analysed for total solids, fat, crude protein, lactose and fatty acid composition of the milk fat. Blood plasma was

Table 4.01 The chemical composition of feedstuffs given in Experiment 3

	Hay	Dairy concentrate	Rolled barley	Maize	Xylitol/barley mixture
Dry matter (DM)†	832.7	865.5	857.6	853.0	892.7
Crude protein (g/kg DM)	66.6	285.7	115.6	97.2	83.3
Cellulose (g/kg DM)	342.3	80.5	64.5	33.3	46.5
Ash (g/kg DM)	58.2	102.0	23.0	13.2	16.6
DOMD (%)	54.9	-	-	-	-
Estimated ME†† (MJ/kg DM)	8.2	13.1	12.9	14.2	12.9

†g/kg fresh weight
††see text for estimation of ME values

analysed for glucose, β-hydroxybutyrate, acetoacetate, lactate and FFA.

Statistical analysis. Milk yields for days 7-14 were meaned for each period. The mean concentration in the 7 plasma samples taken during each period was calculated for each metabolite. These data, together with those for milk composition, were subjected to an analysis of variance and the statistical significance of the treatment effects was determined by F-test. Level of significance is indicated in the tables by asterisks accompanying the SED values (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Between treatment differences were examined for statistical significance by t-test. Such statistically significant differences are indicated in the tables by superscripts.

Results

Food intake. Intakes of feedstuffs are given for each ration in Table 4.02. Intake of hay was significantly lower than for the other diets when maize was offered ($P < 0.01$), and small amounts of the supplement were also refused. Thus whilst total DM intake was, as intended, highly significantly greater for all supplemented diets ($P < 0.001$), DM intake for the maize diet was significantly lower ($P < 0.01$) than when the other supplements were offered. However, intakes of ME and crude protein did not differ significantly between the supplemented diets, and were highly significantly greater ($P < 0.001$) in all cases than for the basal diet.

Table 4.02 Feed consumption (kg DM/d) and nutrient intake by cows offered diets of hay and dairy concentrate alone or supplemented with rolled barley, ground maize or a xylitol/rolled barley mixture

	Supplement				
	None	Barley	Maize	Xylitol/barley	SED
Hay	5.82	5.76	5.29	5.73	0.13**
Dairy concentrate	6.81	6.82	6.92	6.92	0.09
Barley	-	1.72	-	-	
Maize	-	-	1.60	-	
Xylitol/barley	-	-	-	1.79	
Total DM	12.64	14.30	13.80	14.44	0.17***
Estimated ME (MJ/d)	137.3	159.5	157.7	161.3	1.70***
Crude protein (g/d)	2333	2532	2485	2508	27.8***

Milk yield and composition. Milk yield increased significantly in response to all supplements (Table 4.03). Although differences between the supplemented diets were not significant ($P > 0.05$) the increases in milk yields when the diets supplemented with barley or maize were given were both greater than for the diet supplemented with xylitol/barley. Milk composition was not significantly ($P > 0.05$) altered by dietary supplementation. As Table 4.04 shows, increases in the yields of milk constituents in response to the supplements therefore reflected the responses in milk yield and were consistently greater for the barley and maize supplements than for the barley/xylitol mixture. All supplements significantly increased the yields of all but fat of the major milk constituents. Yields of total solids and of solids-not-fat for the maize treatment were significantly greater than those for the xylitol/barley treatment ($P < 0.05$).

Supplementation of the basal diet had significant effects on milk fat composition (Table 4.05). All supplemented diets tended to be associated with an increase in the proportion of short- and medium-chain (C_6-C_{16}) fatty acids and a reduction in the proportion of C_{18} fatty acids in the milk fat in comparison with the proportions present when the basal diet was offered. These trends were particularly pronounced in animals receiving the xylitol/barley supplement, to the extent that the increase in the proportion of C_{16} for this treatment was significant at the $P < 0.01$ level, as was the reduction in that of $C_{18:1}$. Changes associated with supplementation of the basal diet in the yields of individual fatty acids were similar for all supplements; yields of all but C_{12} of the short and medium-chain fatty acids were significantly increased

Table 4.03 The yield (kg/d) and composition (g/kg) of milk produced by cows offered diets of hay and dairy concentrate alone, or supplemented with rolled barley, ground maize, or a xylitol/rolled barley mixture

	Supplement				
	None	Barley	Maize	Xylitol/barley	SED
Milk yield	22.1^{abc}	24.3^{a}	24.6^{b}	23.5^{c}	0.55**
Total solids	128.3	127.1	126.8	128.2	1.98
Fat	39.9	39.4	38.7	40.2	1.82
SNF	88.5	87.8	88.1	88.1	0.64
Protein	31.5	31.2	31.2	31.4	0.69
Lactose	49.0	49.3	49.5	49.3	0.45

Values in the same row with the same superscript differ significantly (a and b, $P < 0.01$; c, $P < 0.05$).

Table 4.04 The yields of milk constituents (g/d) by cows offered diets of hay and dairy concentrate alone or supplemented with rolled barley, ground maize or a xylitol/rolled barley mixture.

	Supplement				
	None	Barley	Maize	Xylitol/barley	SED
Total solids	2825^{abc}	3089^{a}	3123^{bd}	2995^{cd}	59.9**
Fat	876	957	953	929	37.5
SNF	1949^{abc}	2131^{a}	2169^{bd}	2066^{cd}	48.0**
Protein	693^{cde}	757^{c}	769^{d}	735^{e}	19.1*
Lactose	1087^{cde}	1198^{c}	1211^{d}	1168^{e}	32.9*

Values in the same row with the same superscript differ significantly (a and b, $P < 0.01$; c, d and e, $P < 0.05$).

Table 4.05 The fatty acid composition (weight %) of the milk fat produced by cows offered diets of hay and dairy concentrates alone or supplemented with rolled barley, ground maize or a xylitol/rolled barley mixture.

	Supplement				
Fatty acid	None	Barley	Maize	Xylitol/barley	SED
6:0	2.5	2.7	2.9	3.0	0.17
8:0	1.3	1.5	1.6	1.7	0.19
10:0	2.3^{cd}	2.9^{c}	2.8	3.1^{d}	0.25*
12:0	3.0	3.4	3.1	3.5	0.36
14:0	10.6^{cde}	12.3^{c}	12.0^{d}	12.6^{e}	0.61*
16:0	33.3^{ac}	34.9^{c}	34.8^{b}	37.1^{b}	0.77**
18:0	13.1^{cd}	11.7^{c}	12.7	11.5^{d}	0.44*
18:1	31.5^{acd}	27.8^{c}	27.5^{d}	24.9^{a}	1.46**
18:2	1.8	1.8	1.8	1.7	0.07
18:3	1.0	0.9	0.8	0.8	0.05*

Values in the same row with the same superscript differ significantly (a and b, $P < 0.01$; c, d and e, $P < 0.05$).

(Table 4.06) while those of long-chain fatty acids tended to be reduced. The changes in the yields of fatty acids were consistently largest in response to dietary supplementation of the basal diet with the xylitol/barley mixture.

Blood plasma composition. Blood plasma metabolite concentrations are shown in Table 4.07. Barley and, to a smaller degree, maize supplements each significantly increased plasma glucose concentration and these supplements were equally effective in decreasing plasma β-hydroxybutyrate concentration. Concentrations of these metabolites when the xylitol/barley mixture was added to the diet were, however, similar to those in animals receiving the basal diet. The differences between the effects of the xylitol/barley supplement and the other supplements were such that glucose concentration in animals receiving this supplement was significantly lower ($P < 0.01$) than when barley was offered and β-hydroxybutyrate concentration was significantly higher ($P < 0.05$) than when the animals received the maize-supplemented diet. Barley and maize also tended to be more effective than the xylitol/barley mixture in reducing acetoacetate concentration. All supplements tended to increase lactate and decrease FFA concentrations in comparison with those for the unsupplemented diet. These effects were not, however, significant ($P > 0.05$).

Energy balance. On the basis of calculated ME intake and requirements for ME for maintenance and production estimated from initial liveweight and from milk yield and composition (Table 4.08) the animals were in negative energy balance throughout the

Table 4.06 The yields (g/d) of individual fatty acids in the milk fat produced by cows offered diets of hay and dairy concentrate alone or supplemented with rolled barley, ground maize or a xylitol/ rolled barley mixture.

	Supplement				
Fatty acid	None	Barley	Maize	Xylitol/barley	SED
6:0	21.8^{abd}	25.7^{d}	27.5^{a}	28.1^{b}	1.4**
8:0	11.5^{abc}	15.1^{a}	15.2^{b}	15.7^{c}	1.0**
10:0	19.4^{abc}	27.0^{a}	26.8^{b}	28.6^{c}	2.1**
12:0	26.7	31.2	29.7	32.1	3.3
14:0	90.7^{abc}	115.9^{a}	113.3^{b}	116.8^{c}	6.0**
16:0	290.4^{def}	335.4^{d}	329.6^{e}	344.4^{f}	16.1*
18:0	115.7	112.8	121.8	107.0	6.5
18:1	279.9	269.1	264.5	233.0	20.8
18:2	15.2	16.9	15.7	15.8	0.9
18:3	8.4	8.5	8.0	7.5	0.5

Values in the same row with the same superscript differ significantly (a, b and c, $P < 0.01$; d, e and f, $P < 0.05$).

Table 4.07 The concentrations of some blood plasma constituents (mmol/l) in cows offered diets of hay and dairy concentrate alone or supplemented with rolled barley, ground maize or a xylitol/rolled barley mixture

	Supplement				
	None	Barley	Maize	Xylitol/Barley	SED
Glucose	2.71^{ab}	3.11^{ac}	2.98^{b}	2.83^{c}	0.09**
β-Hydroxybutyrate	2.58^{ab}	1.94^{a}	1.80^{bd}	2.47^{d}	0.21**
Acetoacetate	0.60	0.45	0.38	0.51	0.10
Lactate	0.78	0.88	0.84	0.93	0.08
FFA	0.38	0.29	0.28	0.28	0.05

Values in the same row with the same superscript differ significantly (a, b and c, $P < 0.01$; d, $P < 0.05$).

Table 4.08 Estimated energy balance (MJ/d) of cows offered diets of hay and dairy concentrate alone or supplemented with rolled barley ground maize or a xylitol/barley mixture.

	Supplement				
	None	Barley	Maize	Xylitol/barley	SED
ME intake[1]	137.3	159.5	157.7	161.3	1.70***
ME required for maintenance[2]	49.1	49.1	49.1	49.1	
Milk energy[3]	68.2	74.6	75.1	72.6	1.45***
ME required for milk production[4]	110.0	120.3	121.1	117.1	
Energy balance	-21.8	-9.9	-12.5	-4.9	2.94***

[1]See text for calculations.

[2]$0.55 MJ/kgW^{0.73}$, assuming no change in liveweight (Ministry of Agriculture, Fisheries & Food, 1975)

[3]Equation 1 in Tyrrell and Reid (1965).

[4]Assuming constant liveweight and an efficiency of utilisation of ME for lactation, $K_1 = 0.62$.

experiment, though to a significantly lesser extent ($P > 0.001$) when receiving the supplemented diets. The poorer production performance in response to the xylitol/barley supplement was reflected in a tendency for the imbalance between energy consumed and requirements to be smallest when the diet was offered, so much so that energy balance differed significantly ($P < 0.05$) from that observed when maize was given.

Discussion

The results indicate that as is commonly the case in early lactation, the animals were in negative energy balance, particularly when receiving the unsupplemented diet. The response in milk secretion to supplementary energy showed the classical curvilinearity (Broster, 1974) associated with a partial suppression of tissue mobilisation, which appeared to be greatest when the diet was supplemented with xylitol/barley. Plasma metabolite concentrations, while within normal ranges (Baird, 1977), were consistent with the animals being in varying degrees of negative energy balance; glucose concentration was generally low and ketone body and FFA concentrations tended to be high.

In these circumstances the changes in energy metabolism and the output of major milk constituents in response to additional dietary carbohydrate, whether as barley or maize, were similar. This is despite the fact that in comparison with the barley supplement a larger proportion of the energy supplied by the maize supplement would have been in the form of glucogenic substrate. Although evidence from studies of the effects of exogenous glucose on glucose metabolism in lactating cows (Bartley & Black, 1966; Thompson *et*

al., 1975; Clark et al., 1977; Baird et al., 1980) have shown that endogenous glucose production is likely to be reduced when glucose absorption from the small intestine is increased, recent evidence in sheep receiving maize-based diets indicates that the suppression of gluconeogenesis does not fully compensate for the increased contribution from directly absorbed glucose (Janes et al., 1985a).

The effects of the barley and maize supplements with respect to the yields of milk fat in the present experiment resemble those observed by Sutton et al. (1980) in that fat yields were similar for barley- and maize-containing diets at the same level of energy intake. The lack of diet-induced differences in in vitro rates of lipogenesis or in the specific activities of lipogenic enzymes in adipose tissue isolated from sheep receiving an 80% barley diet or an 80% maize diet (Piperova & Pearce, 1982) is in accordance with this. The similarities in the responses in lactose and in protein synthesis to the barley and maize supplements in the present experiment contrast with the findings of Sutton et al. (1980). However, the dietary treatments used by Sutton et al. (1980) were more extreme than those used here. The responses observed to the supplements offered in Experiment 3 indicate that changes in nutrient availability which are achieved by moderate changes in the dietary content of carbohydrate-rich sources which possess different digestion characteristics are unlikely to have major effects on milk production.

This is despite the likely marked distinctions between the patterns of end-products of digestion absorbed from these supplementary carbohydrate sources. The proportion of energy digestion and hence the amount of VFA produced in the rumen is likely

to have been lower when maize rather than barley was offered. As VFA proportions are apparently unchanged when maize replaces barley as almost 60% of a diet (Sutton et al., 1980) it seems likely that the production of all VFA would have been proportionately lower when maize was offered, such that the balance of VFA absorbed was unchanged. However, the passage of starch to the duodenum when the barley and maize supplements were given would have, according to the assumptions of Armstrong and Smithard (1979), increased the amount of glucose absorbed from the small intestine by 70g/d and 265g/d respectively. Thus, the amount of additional glucose which the barley supplement could have supplied as glucose absorbed from the small intestine represents an increase in glucose availability equivalent to approximately only 6% of the glucose required for the lactose synthesis with the control unsupplemented diet. The corresponding value for the maize supplement was approximately 23% of this requirement. It is, however, possible that the similar responses to barley and maize supplements by the animals may in part reflect homeorhetic modification of the mechanisms controlling nutrient partitioning between milk production and body tissue synthesis (see Bauman & Currie, 1980; Bauman & Elliot, 1983).

The responses in plasma metabolite concentrations and in the synthesis of milk constituents to the addition of the xylitol/barley supplement, while they showed the same pattern as those to the other supplements, were particularly small. In fact they were less than would have been expected on the basis of the barley content of this supplement alone. This result is apparently inconsistent with the results of Section III, from which it would be expected that approximately 475g xylitol per day would have been absorbed by

animals receiving the xylitol/barley supplement, and that this xylitol would have contributed in the region of 200g glucose per day. However, the effects of this polyol, in resulting in persistent high rates of ketogenesis, a limited response in plasma glucose concentration and a poor response in milk synthesis, are not those that might have been anticipated from the results of short-term (see Section III) or parenteral (Hamada et al., 1982) administration of xylitol to ruminants. Nor are they in accord with results of studies of xylitol metabolism in rat hepatic tissues (Jakob et al., 1971; Woods & Krebs, 1973).

It is possible that the effects of xylitol observed in the present experiment arose at least in part from changes in rumen metabolism during prolonged exposure to xylitol. Presumably these changes would depend on (a) extensive fermentation of xylitol in the rumen with the production of a distinctly different mixture of VFA and/or (b) changes in the patterns of fermentation of other dietary components as a result of associated modification of the microbial population. The likely importance of such changes is unclear from the information available in the literature. However, the results of this experiment would seem to indicate that adaptation within the rumen microbial population during periods of xylitol consumption may be greater than indicated by in vitro studies (Poutiainen et al., 1976; Bain, 1980) and that a considerable amount of the xylitol ingested is fermented in the rumen. Whether adaptation would significantly alter VFA production is unclear, although it may have promoted the production of ketogenic rather than glucogenic VFA in animals receiving the xylitol/barley supplement. This is suggested by the persistence in these animals of high plasma ketone body

concentrations in the absence of elevated FFA concentrations, as this is consistent with increased ketogenesis from butyrate in the rumen wall and liver (Pennington, 1952) rather than from FFA liberated during tissue mobilisation. Also, FFA concentrations in animals receiving xylitol were similar to those observed when the other supplements were offered and the contribution of long-chain fatty acids in the milk fat produced was if anything slightly smaller. However, the implication from this that the xylitol/barley supplement was equally as effective as barley and maize in suppressing adipose tissue mobilisation is particularly surprising in view of the way in which absorbed xylitol would have been metabolised. As xylitol is rapidly taken up and utilised for gluconeogenesis by hepatic tissue (Forster, 1978) it is unlikely to reach the peripheral circulation and consequently could stimulate insulin secretion only indirectly by contributing to glucose production. It would seem likely that the production and utilisation of glucose would be more closely matched in this situation and therefore that insulin-mediated suppression of adipose tissue mobilisation would be small. Certainly plasma glucose concentration was not greatly increased during supplementation of the basal diet with xylitol/barley mixture but the small production responses suggest that this supplement may not be as effective as the others in contributing to glucose supply.

As hepatic extraction of FFA is regulated by plasma concentration (see Bell, 1980) the similarities in plasma FFA concentrations for the supplemented diets indicates that FFA uptake by the liver in animals receiving the xylitol/barley supplement was comparable with that in animals receiving the other supplemented diets. If so, any contribution of hepatic ketogenesis to the

relatively high rate of ketone body production in animals receiving xylitol would appear to be possible only as the result of an increase in the proportion of FFA which entered β-oxidation and was incompletely oxidised. However, while Jakob et al. (1971) showed in in vitro studies that xylitol does not suppress ketogenesis in perfused rat liver slices supplied with exogenous FFA, they did not find ketogenesis to be enhanced by xylitol administration. The relatively low plasma glucose concentration and generally poor production response in animals receiving this supplement indicates that such a hepatic effect, if it does indeed occur, is most likely to be a consequence of carbohydrate insufficiency (Baird, 1981).

EXPERIMENT 4

The similarities in the responses in metabolism and in milk production to supplements of barley or maize in Experiment 3 may have been in part a result of the comparatively small contribution which, as supplements, these cereals made to the overall diet. In view of this the following experiment was designed so that carbohydrate sources possessing different digestion characteristics comprised a larger proportion of the diets offered. These diets were silage-based and again one of the energy sources included in the comparison was barley. As in the previous experiment, the effects of this source of readily-degradable starchy carbohydrate on metabolism and milk production were compared with those of feedstuffs which were expected to produce different patterns of end-products of digestion. In a second diet barley was replaced with barley treated with an acid-formalin preservative to reduce the rate and extent of starch degradation in the rumen. Recent studies conducted by M. Kassem (personal communication) at this Institute have confirmed the efficacy of this treatment in reducing the degradation of starch in barley suspended in the rumen in Dacron bags and have shown the performance of lactating cows to be improved when receiving preservative-treated grain.

In a third experimental ration a xylitol-enriched polyol mixture was substituted for some of the barley in the diet. Other polyols present in the mixtures available as industrial by-products are fermented more rapidly than xylitol *in vitro* (Poutiainen *et al.*, 1976; Lister & Smithard, 1984). This being so, and assuming that the effects of xylitol observed in Experiment 3 were at least partly a

consequence of changes in VFA production, it might be expected that a polyol mixture would exert relatively greater effects on VFA production. The likely nature of these effects is unclear from in vitro studies. Incubation of polyols with unadapted rumen liquor has little impact on the pattern of VFA produced, whether they are included in large amounts (Poutiainen et al., 1976) or in addition to a milled feedstuff at a level equivalent to that likely to be used as a dietary inclusion (Lister & Smithard, 1984). However, when incubated with rumen liquor from animals previously infused intraruminally with polyol mixtures, an increased fermentation of the polyols is accompanied by changes in VFA production. Poutiainen et al. (1976) found an increased proportion of butyric acid and to a lesser extent propionic acid in the VFA mixtures produced when either a polyol mixture or individual polyols were incubated with adapted rumen liquor, whilst when polyol mixture was included at a lower level in incubations Lister and Smithard (1984) found adaptation to be associated with increased acetic acid production and a reduced propionic acid production. Although dietary polyol mixtures have been reported to be utilised without detrimental effects on energy metabolism in ruminants, the formation of butyric acid tended to be increased in lactating cows (Tuori & Poutiainen, 1977) and in sheep (Slee, 1980) receiving these mixtures. In the latter case rumen acetate production was reduced.

The present experiment included an assessment of the nature of hormonal responses during early lactation to energy presented in different forms. As the results observed by Sutton et al. (1980) demonstrate, the relationships between end-products of digestion, endocrine status and nutrient utilisation for milk production are

unclear. This is particularly so for early lactation, when these relationships appear to be modified by homeorhetic influences (Bauman & Currie, 1980; Bauman & Elliot, 1983).

Experimental

Animals and their management. Four Ayrshire cows weighing approximately 510kg and four Friesian cows weighing approximately 530kg were used. The experiment spanned weeks 3-12 of lactation. The animals were housed in individual stalls in a small byre and were milked in the stalls at 06.00 hrs and 16.00 hrs daily. Concentrate allowances were given in 8 equal meals/d at 3-hourly intervals via automatic feeders. Silage was given in 4 meals, at 06.00 hrs, 10.00 hrs, 16.00 hrs and 22.00 hrs.

Experimental diets. Three silage-based diets with different concentrate components were compared. Each diet contained 40kg/d (fresh weight) silage given with 7.5kg/d barley or treated barley or with 6.5kg/d barley, 1kg/d polyol mixture absorbed on palm kernel meal and an additional 0.25kg/d xylitol. Soyabean meal (1 kg/d) was included in each ration to ensure that the calculated requirements for rumen degradable and undegradable protein requirements were satisfied (Agricultural Research Council, 1980). A commercial mineral mixture (120 g/d) was also given.

The silage was made without wilting and was precision-chopped to an average length of 20mm and treated with an additive containing 20% formalin, 20% sulphuric acid and formic acid (Farmline, West Bromwich) at a rate of 2.2 l/tonne, prior to ensilage in a clamp silo, which was sealed with a weighted polythene sheet.

Whole barley was taken for treatment from the same batch as that included, untreated and rolled, in the other rations and was prepared fortnightly by mixing with an acidified formalin grain additive (Farmline, West Bromwich), the composition of which is given in Table 4.09, at a rate of 8.3 l/tonne. The treated material was rolled the following day and was not offered to the animals until a minimum of 3 days later, to avoid problems of low palatability.

The composition of the polyol mixture is given in Table 4.10. This mixture was given as a solution containing 49.2% polyol absorbed onto palm kernel meal in the ratio 2:1 (on a fresh weight basis). A single batch, sufficient for this and Experiments 5 and 6, was prepared commercially (Farmline, West Bromwich). The additional xylitol (Sokerikermia Oy, Kotka, Finland) was included when the rations were weighed out each day. The composition of feedstuffs is shown in Table 4.11.

Experimental plan and procedure. The 3 dietary treatments were applied to each animal in the sequence dictated by a Youden Square design with 8 replicates and 3 periods (Cochran & Cox, 1950). Each experimental period of 21 days consisted of an adaptation period of 10 days followed by 11 days during which samples were taken. Rations were changed abruptly at the end of each experimental period.

Daily food intakes and milk yields were recorded throughout the experiment. Liveweights were measured weekly at approximately 11.00 hrs. Feedstuffs were sampled fortnightly. Total outputs of faeces during the last 5 days of each period were collected. Milk samples were taken at a.m. and p.m. milkings on days 15 and 16 and days 20 and 21 and bulked to provide 2 samples for analysis. Hourly

Table 4.09 The chemical composition (% w/w) of the additive used to treat barley offered in Experiment 4.

Constituent	%
Formalin[1]	41
Isobutyric acid	22
Acetic acid	21
Lignone sulphonate[2]	7.5
Stabiliser[3]	7.5

[1] A 37% w/w solution of formaldehyde containing 6.8% w/w methanol.

[2] Aqueous solution containing 12 to 55% by weight of lignosulphite, 20 to 60% of lignose, 18 to 25% by weight of hemicellulose and sugar acids e.g. lignoceric acid, and 4 to 12% of organic acids mainly C_1 to C_6 aliphatic carboxylic acids) and minor amounts of other compounds e.g. sodium sulphite and/or dioxan.

[3] 30% w/w solution in water of urea and utropine in a ratio of 10:1 (U.K. Patent Application GB2 078 080 A).

Table 4.10 The dry matter content of the polyol solution (% w/v) and the proportion of individual polyols in the polyol mixture (%) absorbed onto palm kernel meal and offered in Experiment 4.

Dry Matter	49.2
Xylitol	24
Sorbitol	23
Arabinitol	21
Mannitol	16
Rhammitol	8
Galactitol	8

(Farmos Group Ltd, West Bromwich, personal communication)

Polyol mixtures are by-products of wood processing produced during the commercial preparation of xylitol from xylans.

Table 4.11 The chemical composition (g/kg DM) of feedstuffs offered in Experiment 4.

	Silage	Barley	Treated barley	Soyabean meal	Polyol/palm kernel meal
Dry matter (DM)†	207.8	815.9	825.4	861.0	563.4
Organic matter	906.9	973.4	975.7	930.9	894.4
Crude protein	132.3	100.4	98.6	477.1	156.7
ADF	319.0	66.2	71.7	-	-
Cellulose	-	-	-	137.2	178.8
True protein	50.6	-	-	-	-
NPN as % TN‡	61.6	-	-	-	-
NH_3 as % TN	9.5	-	-	-	-
pH	4.05	-	-	-	-
Lactic acid	104.1	-	-	-	-
VFA	25.0	-	-	-	-

† g/kg fresh material.

‡ Total nitrogen

samples of blood was taken via an indwelling jugular catheter for a 24 hr period commencing on day 14 at 06.00 hrs before the animals had been milked or received silage. Subsamples of plasma to be analysed for glucagon were preserved by the addition of a protease inhibitor as described in Section II.

Chemical analysis. Minced wet silage was analysed for toluene dry matter, total nitrogen, true protein, ammonia, lactic acid, VFA and pH. Dried samples of silage were analysed for ash and acid detergent fibre (ADF). Concentrate samples, dried faeces samples and faecal slurries were analysed as appropriate for dry matter, total nitrogen, ash and ADF. Soyabean meal and the polyol solution on palm kernel meal proved unsuitable for the analytical method for ADF determination and were analysed for cellulose content. Milk samples were analysed for total solids, fat, lactose, crude protein and fatty acid composition. Blood plasma samples taken at 08.00, 11.00, 15.00 and 17.00 hrs were analysed for glucose, β-hydroxybutyrate, acetoacetate and FFA. All 24 samples taken in each period were assayed individually for insulin, growth hormone and prolactin. Subsamples of these were combined in four 6-hourly bulks which were assayed for free triiodothyronine (FT_3). Samples for glucagon analysis were bulked in the same way.

Statistical analysis. Feedstuff intakes for days 11-21 were meaned for each period. The data for nutrient digestibilities included three missing values and were therefore expressed simply as means with standard errors for each treatment. Average milk yield during the last 7 days of each period was analysed. Milk composition data

for the 2 bulked samples in each period were meaned. The concentrations for each plasma metabolite and hormone were meaned to provide a single concentration for each treatment period. The results were subjected to an analysis of variance and the treatment means adjusted to compensate for the unequal replication of the treatments within each period of the experiment. Plasma insulin, GH and prolactin concentrations were also meaned across treatments for each sampling time. Differences in mean concentrations of these hormones before and after milking and/or giving silage were assessed for statistical significance by paired t-tests.

Results

Food intake and nutrient digestibilities. As shown in Table 4.12 intakes of silage were similar for all diets and refusals of concentrates were small and infrequent. Intakes of dry matter, and crude protein were therefore similar for all dietary treatments. The digestibilities of the dry matter, organic matter and nitrogen and the amount of digestible organic matter in the dry matter (DOMD) supplied by the experimental diets were closely similar for all diets (Table 4.13). ME intake, which was calculated by taking ME content for each diet to be 0.16 x DOMD% (Ministry of Agriculture, Fisheries and Food, 1975), was significantly lower for the diet containing the xylitol-enriched polyol mixture than for the barley diet ($P < 0.05$).

Milk yield and composition. Milk yield and composition was very similar for all treatments (Table 4.14). Consequently, as Table 4.15 shows, the yields of milk constituents were unaffected by the diets. As Tables 4.16 and 4.17 show, no treatment effects on the proportions

Table 4.12 Feed consumption (kg DM/d) and nutrient intake by cows offered silage-based diets containing rolled barley, rolled treated barley, or rolled barley with a xylitol-enriched polyol mixture.

	Diet			
	Barley	Treated barley	Barley + xylitol/polyol	SED
Silage	7.30	7.14	7.25	0.12
Barley	6.12	-	5.17	
Treated barley	-	6.15	-	
Soyabean meal	0.86	0.86	0.86	
Polyol/palm kernel meal	-	-	0.56	
Xylitol	-	-	0.25	
Total DM	14.27	14.16	14.09	0.16
Crude protein (g/d)	1988	1980	1973	18.9
Estimated ME† (MJ/d)	172.0	170.7	166.8	1.9*

†Where ME content (MJ/kg DM) of diet = 0.16 x DOMD% (see Table 4.13) (Ministry of Agriculture, Fisheries & Food, 1975).

Table 4.13 The intakes, faecal outputs and apparent digestibilities (%) of dry matter, organic matter and nitrogen and the amount of digestible organic matter in the dry matter (DOMD) in diets given to cows in Experiment 4.

Numbers in parentheses refer to the number of observations contributing to each mean.

	Diet		
	Barley	Treated barley	Barley + xylitol/polyol
	(8)	(7)	(6)
Dry matter (kg/d)			
Intake	14.27±0.17	14.32±0.21	14.42±0.29
Faecal loss	3.06±0.09	3.08±0.09	3.25±0.11
Digestibility	78.4 ±0.65	78.5±0.47	77.4±0.64
Organic matter (kg/d)			
Intake	13.37±0.16	13.43±0.19	13.46±0.26
Faecal loss	2.62±0.08	2.64±0.08	2.80±0.10
Digestibility	80.4±0.56	80.3±0.47	79.2±0.75
Nitrogen (g/d)			
Intake	317.8±3.71	317.1±4.37	321.9±6.02
Faecal loss	90.6±3.74	94.8±0.90	97.2±2.27
Digestibility	71.5±1.03	70.1±0.38	69.8±0.48
DOMD (%)	75.3±0.52	75.4±0.44	74.0±0.69

Table 4.14 The yield (kg/d) and composition (g/kg) of milk produced by cows offered silage-based diets containing rolled barley, rolled treated barley, or rolled barley with xylitol-enriched polyol mixture.

	Diet			
	Barley	Treated barley	Barley + xylitol/polyol	SED
Milk yield	21.82	21.98	21.15	0.49
Total solids	123.8	125.0	126.4	1.52
Fat	39.6	40.1	42.0	1.41
SNF	84.2	84.9	84.5	0.29
Protein	27.1	27.7	27.1	0.41
Lactose	49.8	49.8	49.8	0.31

Table 4.15 The yields of milk constituents (g/d) by cows offered silage-based diets containing rolled barley, rolled treated barley, or rolled barley with a xylitol-enriched polyol mixture.

	Diet			
	Barley	Treated barley	Barley + xylitol/polyol	SED
Total solids	2699	2743	2669	55.0
Fat	864	880	884	27.9
SNF	1835	1863	1786	40.1
Protein	590	606	573	14.8
Lactose	1086	1095	1046	25.0

Table 4.16 The fatty acid composition (weight %) of the milk fat produced by cows offered silage-based diets containing rolled barley, rolled treated barley or rolled barley with a xylitol-enriched polyol mixture.

Fatty acid	Diet			SED
	Barley	Treated barley	Barley + xylitol/polyol	
6:0	3.4	3.4	3.5	0.1
8:0	1.7	1.7	1.7	0.1
10:0	3.7	3.6	3.5	0.2
12:0	4.2	4.1	4.1	0.2
14:0	13.7	13.7	13.9	0.5
16:0	41.8	41.7	42.5	0.8
18:0	10.1	10.0	9.7	0.3
18:1	19.5	19.5	19.3	0.8
18:2	1.0	1.1	0.9	0.1
18:3	1.0	1.2	1.1	0.1

Table 4.17 The yields (g/d) of individual fatty acids in the milk fat produced by cows offered silage-based diets containing rolled barley, rolled treated barley or rolled barley with a xylitol-enriched polyol mixture.

Fatty acid	Diet			
	Barley	Treated barley	Barley + xylitol/polyol	SED
6:0	29.5	29.8	30.6	0.8
8:0	14.9	14.9	14.8	0.7
10:0	31.2	31.0	30.5	1.7
12:0	35.1	35.1	35.0	2.0
14:0	117.6	119.1	120.8	4.5
16:0	371.7	368.7	376.4	14.1
18:0	87.4	88.4	86.6	4.7
18:1	170.0	174.0	173.5	11.0
18:2	7.9	9.2	7.6	0.7
18:3	8.6	10.5	9.1	0.9

or yields of individual fatty acids in the milk fat were evident.

Blood plasma composition. Mean blood plasma concentrations of metabolites and hormones did not differ significantly ($P > 0.05$) between treatments (Table 4.18). Glucose concentration tended to be highest and β-hydroxybutyrate and acetoacetate concentrations to be lowest in animals receiving rolled barley while plasma FFA concentrations were unchanged by diet.

The largest differences in hormone concentrations, although small, were also between the barley diet and the other two diets. There was a tendency for insulin concentration and the ratio of this to glucagon concentration to be slightly elevated in the plasma of animals receiving barley. Concentrations of FT_3 were highest when this diet was given and growth hormone and prolactin concentrations were lowest. Hormone concentrations in the plasma of the animals receiving treated barley were virtually identical to those when the mixture of barley and xylitol-enriched polyol mixture was given.

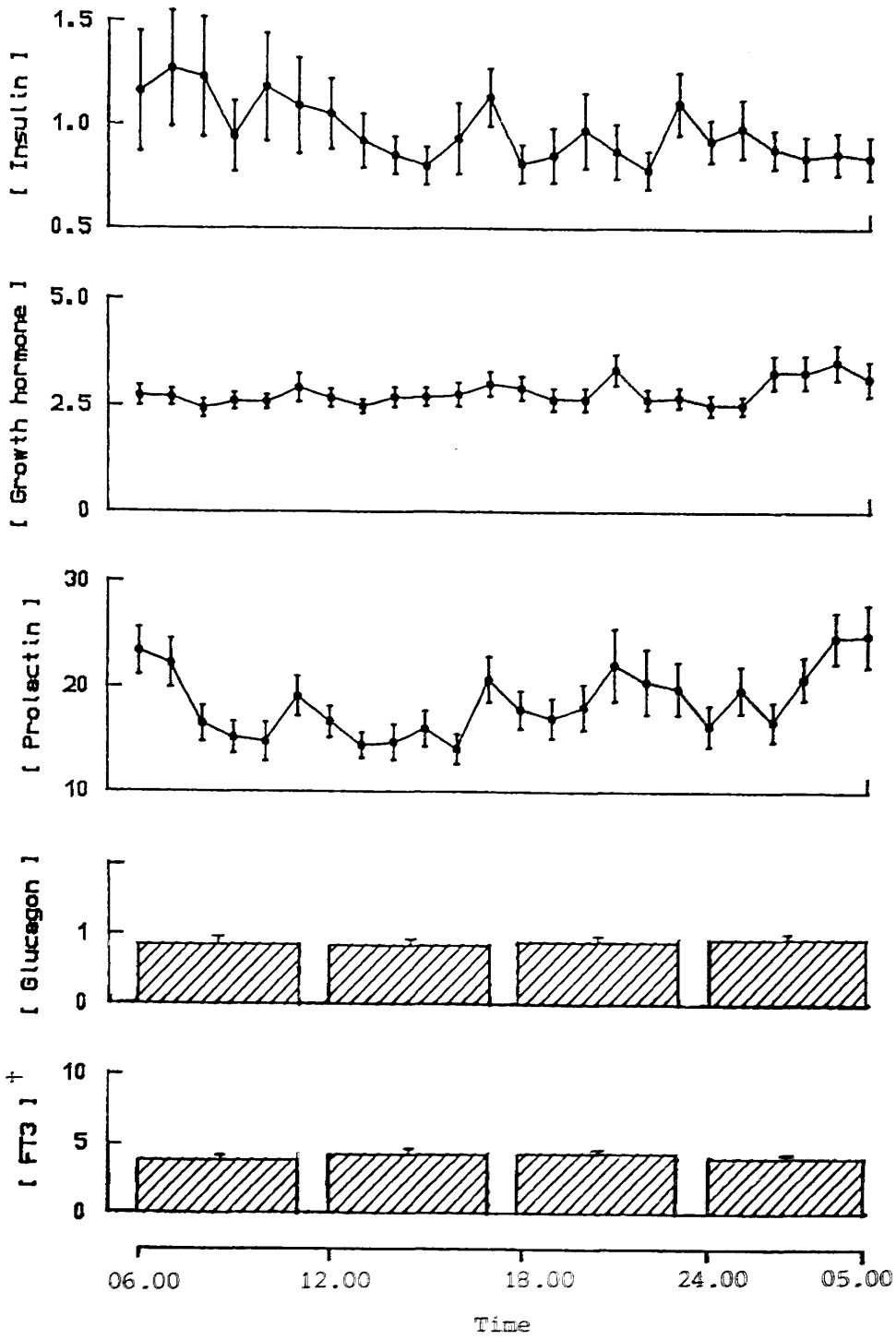
Diurnal patterns of hormone concentrations. As there were no significant differences between treatments in mean hormone concentrations the values for each sampling time or bulked sample for all treatments were combined. Hourly mean concentrations of insulin, growth hormone and prolactin for a 24 hr period are shown in Fig. 4.01, as are the mean values for glucagon and FT_3 in the bulked samples. Changes in hormone concentrations with time were generally small and did not show any clear pattern. Insulin, growth hormone and prolactin were apparently secreted in small pulses throughout the day, at different times for individual animals. There was a tendency

Table 4.18 The concentrations of some blood plasma metabolites (mmol/l) and hormones (ng/ml) in cows offered silage-based diets containing rolled barley, rolled treated barley, or rolled barley with a xylitol-enriched polyol mixture.

	Diet			
	Barley	Treated barley	Barley + xylitol/polyol	SED
Glucose	3.76	3.49	3.52	0.22
β-Hydroxybutyrate	1.05	1.54	1.76	0.48
Acetoacetate	0.21	0.34	0.35	0.08
FFA	0.15	0.18	0.15	0.03
Insulin	1.14	0.82	0.95	0.19
Glucagon	0.86	0.82	0.94	0.09
Growth hormone	2.45	2.82	3.08	0.25
Prolactin	18.24	18.48	18.90	1.73
FT_3 †	4.26	4.02	3.90	0.19

†Free triiodothyronine (pg/ml)

Fig. 4.01 Diurnal trends in mean plasma concentrations (ng/ml) of hormones. Each point is the mean of 24 observations.



† pg/ml

for insulin concentration to be increased in the first sample taken after milking and/or a silage meal. As this response did not occur in all instances and was variable in size this increase was significant only after the silage meal at 22.00 hrs. The silage meals at 10.00 hrs and again at 16.00 hrs, when the animals were also milked, were associated with subsequent significant increases in prolactin concentration. Changes in growth hormone concentration did not appear to be related to milking or silage meals. The effects, if any, of these events on glucagon and FT_3 concentrations cannot be assessed from these results, but as the ranges of concentrations of these hormones were very small their secretion does not appear to have varied greatly during the course of the day.

Energy balance and liveweight change. As Table 4.19 shows, there were no treatment differences in the estimated energy balance of the animals, which were in slight positive energy balance throughout the experiment. Consistent with this, liveweight changes during the experiment were generally small and variable.

Discussion

There was little difference in the responses to the diets compared in the present study despite likely differences in the digestion characteristics of the major carbohydrate components which they contained. Differences in responses were apparent only at a metabolic level, but these were small and evidently insufficient to produce differences in milk production. In comparison with Experiment 3 the performance of the animals in the present experiment was generally poorer. As the estimates of energy balance show, these

Table 4.19 Estimated energy balance (MJ/d) of 8 cows offered silage-based diets containing rolled barley, rolled treated barley or rolled barley with a xylitol-enriched polyol mixture.

	Diet			
	Barley	Treated barley	Barley + xylitol/polyol	SED
ME intake [1]	172.0	170.7	166.8	1.9*
ME required for maintenance [2]	52.9	52.9	52.9	
Milk energy [3]	65.5	66.6	65.3	1.4
ME required for milk production [4]	105.6	107.4	105.3	
Energy balance	+15.5	+9.8	+8.5	4.6
Liveweight change (kg/period)	-8.4	-5.1	+1.3	6.84

[1] See text for calculation

[2] $0.55 MJ/kg\ W^{0.73}$ (Ministry of Agriculture, Fisheries & Food, 1975)

[3] Equation 1 in Tyrrell and Reid (1965)

[4] Assuming no liveweight change and efficiency of utilisation of ME for lactation, $k_1 = 0.62$.

animals, although in early lactation, were in slight positive energy balance during the experiment and as such may have been less sensitive to differences in the form in which nutrients were available. This may be a reflection of the genetic potential of the animals used.

It is possible that the impact of the differences in carbohydrate composition on the pattern of nutrients absorbed was diminished because the concentrates were given with silage in this experiment; the pattern of VFA production in the rumen is less sensitive to alterations in concentrate input in animals receiving silage-based diets (Chalmers, 1979). As feeding frequency does not appear to alter responses to diets containing more than 200g ADF/kg DM (Sutton, Broster, Napper & Siviter, 1985), the high frequency with which concentrates were given in this experiment, where ADF contents of the rations were approximately 200g/kg DM, is unlikely to have influenced the overall results. Presumably the post-prandial peaks in nutrient absorption associated with this routine were smaller than those which would have occurred had the diets been given in fewer daily meals. Patterns of hormonal response may have been moderated as a consequence of this, but mean hormone concentrations are unlikely to have been affected at this level of concentrate inclusion (Sutton et al., 1982).

Certainly, the ranges of concentrations of insulin and growth hormone observed in the present experiment were narrower than those reported for cows producing similar amounts of milk but receiving their rations in 2 meals/d (Bines, Hart & Morant, 1983). The fact that the latter diet contained 90% concentrates is, however, likely to have contributed to the differences between these patterns of

hormonal response. Thus the absence of a clear diurnal trend in insulin secretion may be attributable to the frequency with which concentrates were given in this experiment and to the type of diet. The episodic pattern of growth hormone secretion, though less extreme in this experiment, is consistent with the observations of others (Vasilatas & Wangness, 1981; Bines et al., 1983). Interpretation of the results for glucagon and FT_3 are limited because the samples were bulked prior to analysis, but the ranges of concentrations of these hormones during 24 hrs were small. Very little information is available regarding plasma glucagon concentrations in lactating cows but de Boer, Trenkle and Young (1985) have recently made hourly measurements for a 24 hr period during early lactation. Although the concentrations which they reported were considerably lower than those observed in this experiment it is interesting to note that they found plasma glucagon concentration to be unaffected by feeding. Bines et al. (1983) found no consistent trends in thyroxine concentration over a 24 hr period. Thus the observations in the present experiment are consistent with those reported previously in that they suggest that short-term changes in nutrient availability are not reflected in immediate changes in hormone secretion, with the possible exception of insulin.

Dietary inclusion of readily-fermentable carbohydrate in the form of barley was associated with slightly higher plasma glucose concentrations and lower ketone body concentrations than those observed when the other diets were given. At the same time mean insulin concentration tended to be marginally higher and the concentration of growth hormone slightly lower than for the other diets. Given the respective anabolic and catabolic actions of these

hormones with respect to adipose tissue metabolism (Bauman, 1976; Smith et al., 1976) the relatively high ratio insulin:growth hormone concentrations in animals receiving this diet might be expected to have enhanced tissue deposition at the expense of milk synthesis. Indeed when the barley diet was given milk fat yield was lowest, though not significantly less than for the other treatments.

Comparison of the responses to the barley and treated barley diets show the effects of the treatment of barley on the metabolic and production responses to the cereal to be small. This is inconsistent with the improvements in milk production associated with grain treatment observed by other workers at this Institute (M. Kassem, personal communication). The reasons for this difference are not apparent, although the diets were offered ad lib and to cows in mid-lactation in studies where grain treatment proved to be advantageous. Nevertheless the results of this comparison were in agreement with those of Experiment 3, in that differences between the digestion characteristics of the carbohydrate in the concentrates given were not reflected in differences in milk production. This is consistent with the findings of Sutton (1984), based on the results of a series of experiments in which carbohydrate sources were compared in diets containing various proportions of concentrates and roughage, that differences in energy utilisation for diets of differing carbohydrate composition are apparent only when ADF concentration in the diets is less than about 200g/kg DM.

The similarity between the hormonal responses to the diet containing barley and xylitol-enriched polyol mixture and those to the other diets indicate the lack of specific metabolic effects of

xylitol. Interestingly, β-hydroxybutyrate concentration tended to be comparatively high in animals receiving the mixture of polyols, which suggests that xylitol, or some other polyol(s) in the mixture, was having an effect similar to that observed in the previous experiment.

SECTION V

SOME EFFECTS OF DIETARY INCLUSION OF A MIXTURE OF POLYOLS ON DIGESTION, METABOLISM AND MILK PRODUCTION

EXPERIMENTS 5, 6 AND 7

During early lactation appetite is frequently depressed while milk yield is rapidly increasing and in consequence glucose supply from the diet is rarely sufficient to meet requirements for milk production. In this situation, as Baird (1982) has recently discussed, the priority given to the lactating mammary gland for nutrients in the high-producing dairy cow may jeopardise the animal's metabolism to such an extent that clinical ketosis develops. In less extreme cases animals may well become sub-clinically ketotic to the detriment of peak milk yield. Evidently the ability of the diet to contribute to glucose supply and the nature of the metabolic and hormonal responses to the nutrients absorbed is particularly important in this situation.

Interest in the possible advantages of the inclusion of polyol mixture in diets given to cows during early lactation stems from the apparent success in the prophylactic treatment of ketosis of commercial products ('Acetona'; Farmline, West Bromwich) in which a mixture of polyols is included. In controlled experiments Tuori and Poutiainen (1977) found a polyol mixture to be utilised as effectively as molasses when included in a diet given to lactating cows, and in Experiment 4 responses to a xylitol-enriched polyol mixture were similar to those to conventional dietary sources of energy. Experiment 5 was conducted with the aim of assessing the usefulness of a polyol mixture in circumstances where the adequacy of dietary energy supply was likely to be marginal. For this reason the experiment was conducted during the first 8 weeks of lactation and

the cows used comprised a calving-group in which there was a previous history of ketosis.

Further experiments in conjunction with this study were conducted in sheep. In Experiment 6 the digestibilities of the nutrients in the rations given to the cows were measured. In Experiment 7 a similar basal diet was used to investigate the effects of polyol inclusion on VFA proportions in the rumen.

Experimental

EXPERIMENT 5

Animals and their management. Twenty intact spring-calving Ayrshire cows were used. The animals were housed and received their rations individually in stalls and were milked in an 8x16 herringbone milking parlour (Gasgoine, Gush & Dent, Reading), commencing at 06.00 hrs and 15.00 hrs daily. Rations were offered at 07.00 hrs and 16.00 hrs.

Experimental diets. The 2 experimental diets in this comparison were based on hay and a dairy concentrate mixture containing barley, sugar beet pulp, soyabean meal and minerals. The amounts of these constituents offered and the proportion of concentrate in the ration was increased weekly for the first 3 weeks of lactation, as shown in Table 5.01, and given at a fixed level thereafter. Throughout the experiment the 'control' diet was supplemented with 0.90kg fresh weight/d rolled barley and the 'polyol' diet was supplemented with an energetically equivalent amount (1.25kg fresh weight/d) of a 2:1 (on a fresh weight basis) mixture of a solution of a mixture of polyols

Table 5.01 The amounts of hay and dairy concentrate (kg fresh weight/d) offered in the basal diet in Experiment 5.

	Hay	Dairy concentrate
Pre-calving	6.0	4.0
Post-calving		
Week 1	6.0	6.0
2	7.0	8.0
3-8	7.0	10.0

(49.2% DM) absorbed onto palm kernel meal. The polyol supplement was from the same batch as was used in Experiment 4 and was prepared commercially (Farmline, West Bromwich). At the highest level of feeding the proportion of concentrate in these rations was 58% in the dry matter. The composition of the dietary constituents is given in Table 5.02 and the amounts of individual polyols supplied by the polyol diet is shown in Table 5.03.

Experimental plan and procedure. The animals were divided into 2 groups of 10 cows balanced as closely as possible for lactation number and previous lactation yield. Each group was allocated one of the 2 experimental treatments 2 weeks before the expected dates of calving. The animals continued to receive the experimental diet post-calving for 8 complete sampling weeks. Food intake and milk yield was recorded daily and the animals were weighed twice weekly. Samples were taken at the same time of the week throughout the experiment. Feedstuff samples were taken weekly. Samples of milk were taken for analysis at four consecutive milkings each week. Blood samples were taken into heparinised vacuum tubes at approximately 10.00 hrs once per week. At the same time a small sample of milk was withdrawn from the udder and Rothera's test for the presence of ketone bodies (Rothera, 1908) was performed. These procedures were followed in all healthy animals each week for the first 8 weeks of lactation, during which time the general health of the animals was monitored closely. Animals exhibiting signs of disorder (inappetance, nervousness and a reduction in milk yield) and giving a positive result to Rothera's test were diagnosed to be clinically ketotic. They were removed from the experiment and given

Table 5.02 The chemical composition (g/kg DM) of feedstuffs offered in Experiment 5.

	Hay	Dairy concentrate	Rolled barley	Polyol/palm kernel meal
Dry matter (DM)†	859.0	832.5	824.5	569.0
Organic matter	942.1	938.9	974.8	896.9
Crude protein	52.5	185.3	97.2	145.9
ADF	414.8	102.4	66.0	203.5

†g/kg fresh weight

Table 5.03 The amounts of individual polyols (g/d) consumed by animals receiving the 'polyol' diet in Experiment 5.

Total polyol	410
Xylitol	98.4
Sorbitol	94.3
Arabinitol	86.1
Mannitol	65.6
Rhamnitol	32.8
Galactitol	32.8

therapeutic treatment. When possible a venous blood sample was taken immediately before such animals were treated.

Chemical analysis. All samples were prepared for analysis, stored and analysed as described in Section II. Feedstuff samples were analysed for dry matter, ash, total nitrogen and ADF. Milk samples were analysed for total solids, fat, crude protein and lactose. Fatty acid composition of the milk fat was determined for milk samples taken during weeks 2, 5 and 8 of lactation. Blood plasma was analysed for glucose, β-hydroxybutyrate, acetoacetate and FFA.

Statistical analysis. Data from all 20 cows are included in the analyses, but are complete for only 4 animals in the polyol group because the remaining animals in this group became clinically ketotic and were removed from the experiment. Consequently, while data from the control group are derived from 10 animals for each week, data for the polyol group are derived from 10, 10, 9, 8, 7, 5, 4 and 4 animals for weeks 1, 2, 3, 4, 5, 6, 7 and 8 of lactation respectively. Treatment differences for each week were tested for statistical significance by t-test, where SE was calculated using a formula for samples of equal or unequal numbers as appropriate (Snedecor & Cochran, 1980). Statistically significant differences are indicated in tables by asterisks accompanying SED values (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

EXPERIMENT 6

Animals and their management. Nine intact crossbred wether sheep were used. The animals were housed in metabolism cages, received the experimental ration in equal meals at 10.00 hrs and 22.00 hrs and had free access to drinking water and mineral blocks.

Experimental diets. The sheep received one of three diets, of which the 'control' and 'polyol' diets contained the same feedstuffs, in the same proportions, as the corresponding diets given to the cows during weeks 3-8 of lactation in Experiment 5, with the exception that the hay was chopped. The third diet consisted of chopped hay alone. Each diet was given at a level calculated to meet the requirements for maintenance. Feedstuff composition is given in Table 5.04.

Experimental plan and procedure. The sheep were divided into 3 groups of 3 animals. Each group received a different diet. The animals were established on diet for 14 days prior to an experimental period of 21 days, during which food intake was recorded daily. Feedstuff samples were taken during the first week of the experimental period. Total faecal collections were made over the last 7 days of the period.

Chemical analysis. Feedstuff and faeces samples were analysed for dry matter, ash, total nitrogen and ADF by the methods described in Section II.

Table 5.04 The chemical composition (g/kg DM) of feedstuffs offered in Experiment 6

	Chopped hay	Dairy concentrate	Rolled barley	Polyol/palm kernel meal
Dry matter (DM)†	875.0	844.0	811.8	586.1
Organic matter	944.7	929.4	975.8	892.3
Crude protein	56.3	181.3	96.9	160.0
ADF	390.1	108.4	60.8	-

† g/kg fresh weight

Statistical analysis. The results were statistically analysed by one-way analysis of variance. Statistically significant differences are indicated in tables by asterisks accompanying SEM values (*, $P < 0.05$; **, $P < 0.01$; *** $P < 0.001$).

EXPERIMENT 7

Animals and their management. Four Finnish Landrace x Dorset Horn wethers weighing approximately 50kg and fitted with rumen cannulas were used. The animals were housed in metabolism cages and had free access to water and mineral blocks. Rations were given in equal meals at 10.00 and 22.00 hrs.

Experimental diets. The 2 rations offered were designed to be as similar as possible in composition to, and of the same roughage:concentrate ratio (42:58 in DM of the basal ration) as, those given to the cows during weeks 3-8 of Experiment 5. The basal diet consisted of 420g/d (fresh weight) chopped hay and 600g/d dairy concentrate. To this was added a further 30g/d dairy concentrate (control diet) or 50g/d polyol solution (polyol diet). Supplies of the commercially-prepared mixture of polyol solution on palm kernel meal used in Experiments 4, 5 and 6 had been exhausted and therefore the polyol solution, which was available (Farmline, West Bromwich), was incorporated directly into the concentrate portion of the ration in the polyol diet such that it supplied 2.7% of DM of the total ration. Feedstuff composition is given in Table 5.05.

Experimental plan and procedure. All animals received the dietary treatments in the same sequence. The animals were established on the control diet for 14 days, after which the experiment was commenced. The control diet was offered for a period of 2 weeks, the polyol diet for the following 3 weeks and finally the control diet for a further 2 weeks. Dietary changes were made abruptly. Food intake was recorded daily. Samples of feedstuffs were taken weekly for analysis. On day 14 of each control period and on days 7, 14 and 21 of the polyol period rumen liquor was sampled immediately before rations were given at 10.00 hrs and again 1, 2, 3, 4, 6 and 8 hours later.

Chemical analysis. Samples were prepared and analysed as described in Section II. Feedstuffs were analysed for dry matter, ash, nitrogen and ADF. Rumen liquor was analysed for VFA and pH.

Statistical analysis. Daily mean values for VFA content and pH were calculated from the values for each sampling time. These mean values were subjected to a 2-way analysis of variance. Significance was determined by F-test and is indicated in the tables by asterisks accompanying the SED values (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Statistically significant differences between the means for each sampling day are indicated in the tables by superscripts.

Results

EXPERIMENT 5

Animal health. The weekly cumulative occurrence of clinical ketosis during the experiment is shown in Fig. 5.01. All cases occurred in animals receiving polyol, reaching an incidence of 60% by week 7 of lactation. Examination of herd records showed that of the polyol-supplemented group 2 animals had received treatment for ketosis in a previous lactation. The corresponding figure for the control group was 4 animals.

Food intake. Food consumption and nutrient intake by healthy animals during the experiment is shown in Table 5.06. Feedstuff refusals by these animals were generally small and intakes were therefore close to those intended. ME intake was estimated using values for ME content of the hay, concentrates including barley supplement and concentrates including polyol/palm kernel calculated for hay as 0.15 x DOMD% and for concentrate as 0.16 x DOMD% (MAFF, 1975) using values for DOMD% measured in sheep in Experiment 6 (see Table 5.13). These ME contents were 7.44, 13.1 and 13.1MJ/kg DM respectively.

Milk yield and composition. Milk yields were persistently slightly higher for animals receiving the control diet (Fig. 5.02). Although total milk production over the course of the entire study was significantly greater for the control group of animals the weekly difference between the two groups was significant during week 5 of

Fig. 5.01 The cumulative occurrence of clinical ketosis in cows receiving a diet of hay and concentrates supplemented with either barley (●) or polyol mixture (◐).

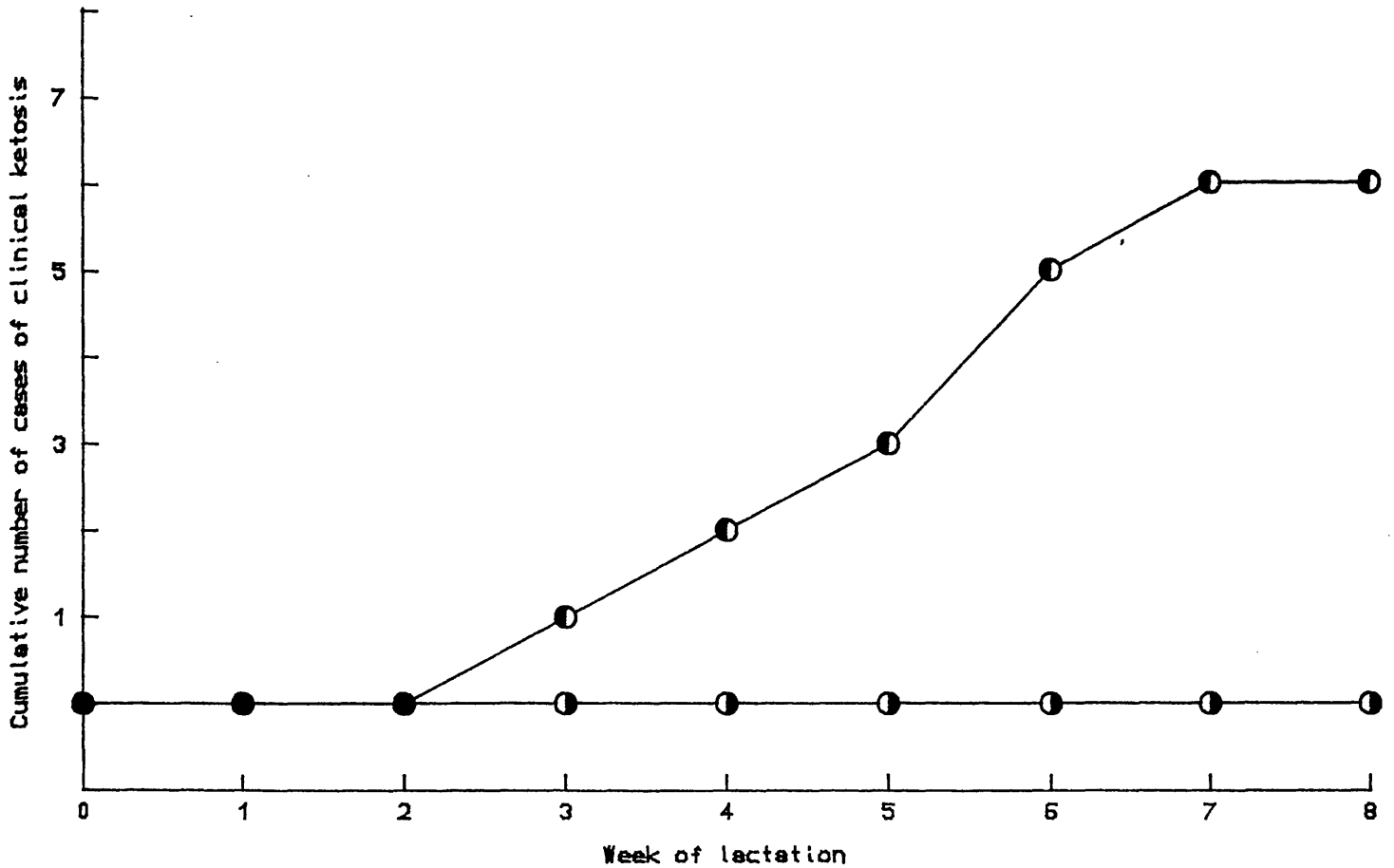


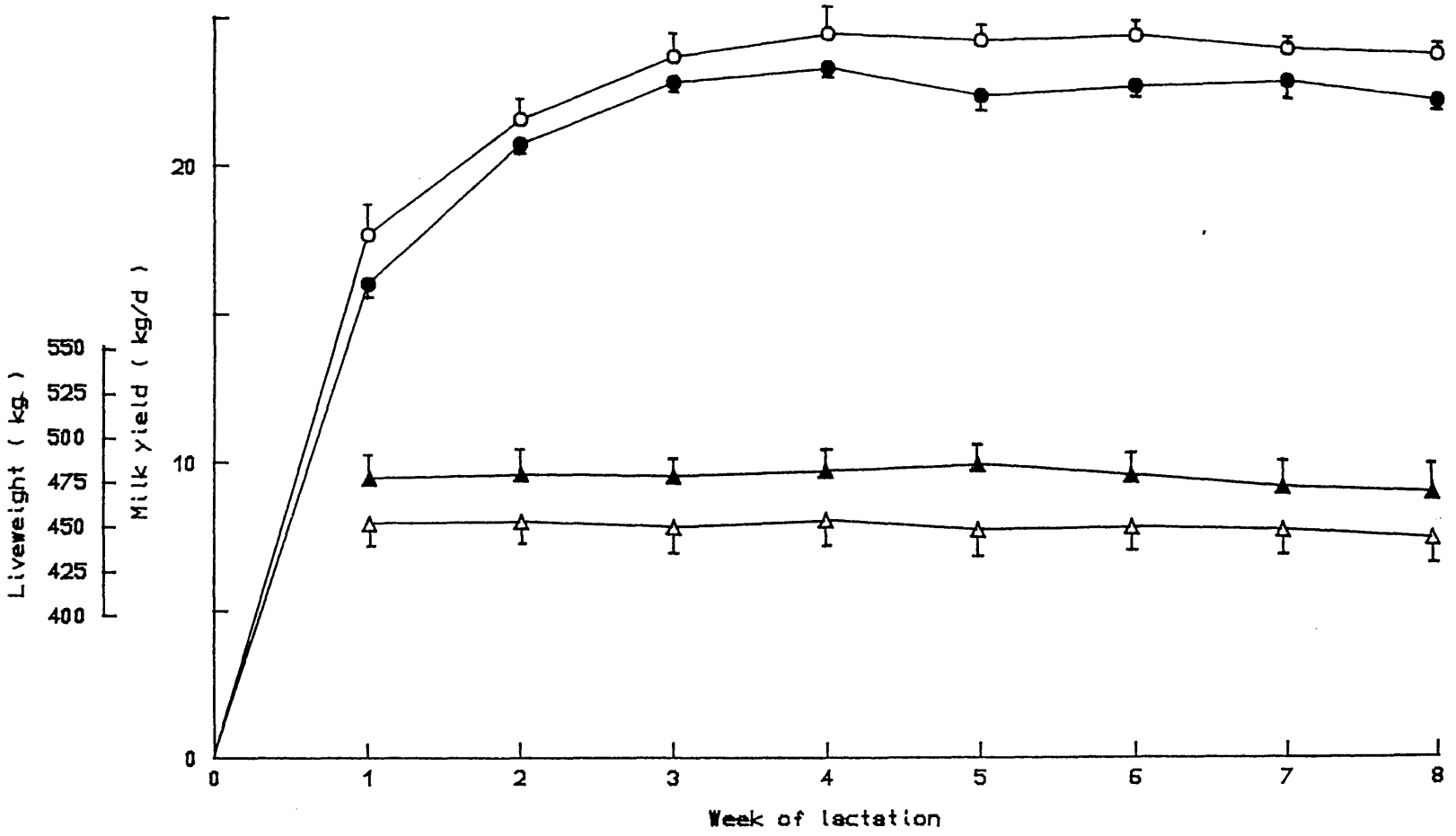
Table 5.06 Feed consumption (kg DM/d) and nutrient intake by cows receiving a diet of hay and concentrate supplemented with either barley (C) or polyol mixture (P). Numbers in parentheses refer to the number of cow-weeks contributing to the mean.

	Week of lactation								
	1			2			3-8		
	C (10)	P (10)	SED	C (10)	P (10)	SED	C (60)	P (37)	SED
Hay	4.69	4.78	0.32	5.38	5.72	0.21	5.73	5.91	0.07*
Dairy concentrate†	5.81	5.79	0.26	7.50	7.41	0.39	8.98	8.99	0.06
Total DM	10.50	10.57	0.45	12.88	13.13	0.36	14.71	14.92	0.11*
Estimated ME (MJ/d)††	109.3	109.7	4.4	136.0	137.4	3.7	157.5	159.2	1.05
Crude protein (g/d)	1259	1297	53.6	1600	1647	47.9	1900	1949	12.9***

†Total concentrate including supplement

††See text for calculation.

Fig. 5.02 The milk yields and liveweights of cows receiving a diet of hay and concentrates supplemented with either barley (open symbols) or polyol mixture (closed symbols).



lactation only. Similarly, weekly differences between the groups in milk composition (Table 5.07) were not significant ($P > 0.05$). However, the concentrations of constituents in milk produced by animals receiving the polyol diet were persistently slightly higher, and when the observations for all 8 weeks were combined milk content of total solids, protein and lactose, but not fat, was significantly higher for the animals receiving the polyol supplement. Yields of milk constituents were similar in both groups (Fig. 5.03). Differences between the groups in milk fat composition were generally small (Table 5.08), and the statistically significant differences between the groups in the contributions from $C_{10:0}$ and $C_{18:3}$ were unlikely to have been physiologically significant.

Blood plasma composition. The blood plasma concentrations of glucose, β-hydroxybutyrate, acetoacetate and FFA given in Table 5.09 exclude plasma metabolite concentrations in samples obtained from clinically ketotic animals immediately before therapeutic treatment as blood profile would doubtless have been confounded by the severe reduction in food intake which had occurred by this time. Such samples were taken from 3 of the 6 ketotic individuals and the metabolite concentrations in these plasma samples were consistent with extreme ketosis (Table 5.10). As Table 5.09 shows, the high incidence of clinical ketosis in animals receiving the diet supplemented with polyol mixture was reflected in the blood composition of animals in this group. Glucose concentration tended to be lower and β-hydroxybutyrate, acetoacetate and FFA concentrations tended to be higher than in animals receiving the control diet. These trends were, with one exception, not significant

Table 5.07 The yield (kg/d) and composition (g/kg) of milk produced by cows receiving a diet of hay and concentrate supplemented with either barley (C) or polyol mixture (P). Numbers in parentheses refer to the number of observations contributing to the control and polyol mean.

	Milk yield			Total solids			Fat			Protein			Lactose		
Week of lactation	C	P	SED	C	P	SED	C	P	SED	C	P	SED	C	P	SED
1(10,10)	17.67	16.01	1.12	134.9	135.2	3.4	43.9	42.5	2.8	36.2	36.0	1.7	47.7	48.9	0.9
2(10,10)	21.52	20.68	0.75	128.9	130.5	3.0	40.0	40.4	2.8	32.2	32.7	1.2	49.0	49.7	0.8
3(10,9)	23.62	22.76	0.85	126.1	126.1	2.7	38.3	37.4	2.8	30.5	31.9	1.0	49.3	49.5	0.7
4(10,8)	24.36	23.21	1.02	124.6	129.2	3.2	37.6	41.0	2.8	30.4	31.0	1.0	48.7	49.2	0.7
5(10,7)	24.11	22.24	0.85*	122.4	128.4	3.2	36.0	40.1	2.9	29.6	30.7	0.8	49.0	49.6	0.8
6(10,5)	24.28	22.56	0.92	122.4	126.9	3.6	36.3	38.8	3.3	29.4	30.3	0.8	48.9	50.4	0.8
7(10,4)	23.80	22.70	0.77	122.6	127.2	4.0	36.7	38.8	4.0	29.0	30.6	1.0	49.1	50.5	1.0
8(10,4)	23.59	22.05	0.80	123.8	130.3	3.7	38.6	42.2	3.2	28.9	30.3	1.3	48.6	50.1	0.8
$\bar{x}$(80,57)	22.86	21.14	0.51***	125.7	129.6	1.3**	38.4	40.2	1.1	30.8	32.1	0.5*	48.8	49.6	0.3**

Fig. 5.03 The yields of milk constituents (g/d) from cows receiving a diet of hay and concentrates supplemented with either barley (open symbols) or polyol mixture (closed symbols).

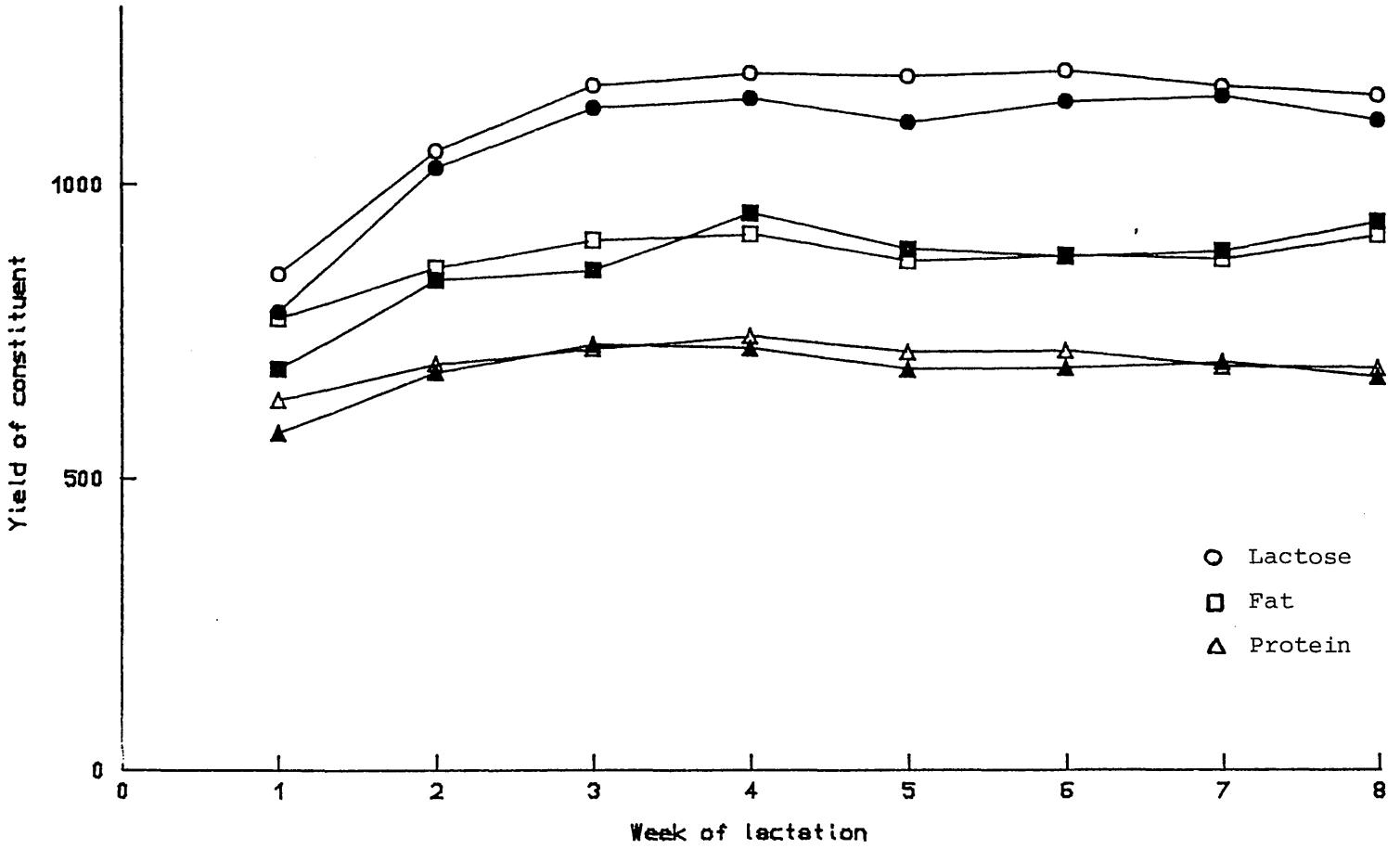


Table 5.08 Mean fatty acid composition (weight %) of the milk fat produced during weeks 2, 5 and 8 of lactation by cows receiving diets of hay and concentrate supplemented with either barley (C) or polyol mixture (P).

The numbers in parentheses refer to the number of observations contributing to each mean.

Fatty acid	Diet		SED
	C (30)	P (22)	
6:0	2.76	2.70	0.09
8:0	1.30	1.16	0.09
10:0	2.80	2.43	0.16*
12:0	3.08	2.71	0.19
14:0	12.49	12.11	0.47
16:0	36.88	37.16	0.78
18:0	9.20	9.12	0.07
18:1	27.70	29.07	1.28
18:2	2.77	2.75	0.23
18:3	0.89	0.73	0.08*

Table 5.08 Mean fatty acid composition (weight %) of the milk fat produced during weeks 2, 5 and 8 of lactation by cows receiving diets of hay and concentrate supplemented with either barley (C) or polyol mixture (P).

The numbers in parentheses refer to the number of observations contributing to each mean.

Fatty acid	Diet		SED
	C (30)	P (22)	
6:0	2.76	2.70	0.09
8:0	1.30	1.16	0.09
10:0	2.80	2.43	0.16*
12:0	3.08	2.71	0.19
14:0	12.49	12.11	0.47
16:0	36.88	37.16	0.78
18:0	9.20	9.12	0.07
18:1	27.70	29.07	1.28
18:2	2.77	2.75	0.23
18:3	0.89	0.73	0.08*

Table 5.10 The concentration of glucose, ketone bodies and FFA (mmol/l) in blood plasma of 3 untreated clinically ketotic cows

	Glucose	β-Hydroxybutyrate	Acetoacetate	FFA
1	1.15	5.84	0.51	0.53
2	2.42	3.42	0.38	1.05
3	2.08	5.31	0.38	0.24

on a weekly basis but they were evident throughout the experiment despite the exclusion of the clinically ketotic animals from the comparison. Plasma β-hydroxybutyrate concentration was significantly higher in animals receiving the polyol supplement than in those receiving the barley supplemented diet during week 5 of lactation, which reflects the impending development of clinical ketosis in 2 animals of this group during the subsequent week. β-Hydroxybutyrate concentration was also significantly greater in animals receiving the polyol when the comparison was made of the combined data for the entire experiment.

Energy balance and liveweight change. As anticipated, the animals in both groups were in negative energy balance, which was particularly severe during the first 2 weeks of post-calving (Table 5.11). At no stage of the experiment were differences in energy balance related to the dietary treatments evident. Mean liveweight of the animals in the polyol group tended to be slightly higher, but not significantly so ($P > 0.05$), than that of animals in the control group (Fig. 5.02). However, liveweight losses during the experiment were similar in both groups, and were surprisingly small in view of the energy status of the animals.

EXPERIMENT 6

Food intake and faecal output. The rations offered to the sheep are shown in Table 5.12. These rations were completely consumed

Table 5.11 Estimated energy balance (MJ/d) of cows receiving diets of hay and concentrates supplemented with either barley (c) or polyol mixture (P). Numbers in parentheses refer to the number of observations contributing to the control and polyol means.

Week of lactation	ME intake[1]			ME required for maintenance[2]			Milk energy[2]			Energy balance[2]		
	C	P	SED	C	P	SED	C	P	SED	C	P	SED
1(10,10)	109.3	109.7	4.4	49.4	51.3	1.4	58.5	52.8	3.8	-34.1	-25.1	6.7
2(10,10)	136.0	157.4	3.7	49.4	51.8	1.4	66.9	65.3	3.1	-21.3	-19.7	6.7
3(10,9)	154.2	156.8	3.5	49.2	51.3	1.4	71.2	68.7	3.2	- 9.8	- 5.4	6.5
4(10,8)	154.6	159.1	3.3	49.0	51.5	1.6	72.4	72.5	3.5	-11.1	- 9.3	5.3
5(10,7)	158.3	160.1	1.1	48.4	51.7	1.4*	69.9	68.8	3.4	- 3.1	- 2.7	6.1
6(10,5)	159.5	160.4	0.9	49.1	51.3	1.6	70.5	68.9	3.1	- 3.2	- 2.0	5.8
7(10,4)	159.2	160.4	1.4	48.9	50.7	1.9	69.5	59.6	4.4	- 1.7	- 2.5	8.0
8(10,4)	159.3	160.4	1.4	48.6	50.5	1.9	70.2	70.1	3.6	- 2.8	- 3.2	6.6

[1] See text for calculations
[2] Calculated as for Table 4.19.

Table 5.12 Feed consumption (g DM/d) by sheep receiving diets of hay alone or with dairy concentrate (control) or with dairy concentrate supplemented with polyol mixture (polyol) in Experiment 6

	Diet		
	Hay	Control	Polyol
Chopped hay	744	210	210
Dairy concentrate	-	291	291
Rolled barley	-	32.5	-
Polyol/palm kernel	-	-	23.4

throughout the experiment. Intakes and faecal excretion of dry matter, organic matter and nitrogen are given in Table 5.13.

Digestibility of nutrients. Nutrient digestibilities and the amount of digestible organic matter in the dry matter in the diets and, by difference from the hay-only diet, in the concentrate portions of the control and polyol diets are given in Table 5.13. The hay offered in the diet was of poor quality, with a dry matter digestibility of only 51.3%. The apparent digestibilities of the nutrients supplied by the control and the polyol diet were closely similar.

EXPERIMENT 7

Food intake. The rations offered were completely consumed throughout the experiment. Feedstuff and nutrient intakes, as shown in Table 5.14, were similar for both diets.

VFA concentration and pH in the rumen. Dietary inclusion of polyol mixture had distinct, yet apparently unsustained effects on rumen metabolism. That is to say the effects of polyol inclusion were generally largest 14 days after the introduction of polyols and were partially reversed after a further 7 days. As Table 5.15 shows, total VFA concentration was significantly reduced from the control levels when the polyol diet was offered. Significant reductions ($P < 0.01$) in the concentrations of acetic and propionic acid, together with a slight, non-significant ($P > 0.05$) decrease in butyric acid concentration, 14 days after polyol introduction contributed to the

Table 5.13 The intakes, faecal outputs, apparent digestibilities (%) of dry matter, organic matter and nitrogen and the amount of digestible organic matter in the dry matter (DOMD) in diets given to 3 sheep in Experiment 6.

	Diet			
	Hay	Control	Polyol	SEM
Dry matter (g/d)				
Intake	744	534	525	
Faecal loss	363	161	157	3.8***
Digestibility	51.3	69.8	70.0	0.7***
Organic matter (g/d)				
Intake	703	501	490	-
Faecal loss	334	138	134	3.3***
Digestibility	52.5	72.4	72.7	0.6***
Nitrogen (g/d)				
Intake	6.7	10.8	10.9	-
Faecal loss	5.1	3.2	3.0	0.4*
Digestibility	24.5	70.7	72.6	4.4***
DOMD (whole ration) (%)	49.6	67.9	67.9	
DOMD (concentrate) (%)	-	80.0	80.1	

Table 5.14 Feed consumption (g DM/d) and nutrient intake (g/d) by sheep receiving a 'control' diet of hay and dairy concentrate or a 'polyol' diet of hay and dairy concentrate containing polyol mixture in Experiment 7.

	Diet	
	Control	Polyol
Chopped hay	356.7	356.7
Dairy concentrate	530.5	-
Dairy concentrate with polyol	-	534.8
Total DM	887.2	891.5
Organic matter	825.9	826.0
Crude protein	128.8	116.7
ADF	178.9	184.9

Table 5.15 The effect of dietary inclusion of polyol on the concentrations (mmol/100ml) of individual VFA in the rumen fluid of 4 sheep in Experiment 7.

	Diet					
	Control	Polyol			Control	
Duration of dietary treatment (days)	14	7	14	21	14	SED
Total VFA	9.28^{d}	9.18^{e}	7.83^{def}	8.48	9.24^{f}	0.40*
Acetic acid	5.84^{a}	5.88^{b}	5.13^{abc}	5.55	5.89^{c}	0.20**
Propionic acid	1.87^{ab}	1.72^{d}	1.30^{ade}	1.44^{b}	1.66^{e}	0.14**
Iso-butyric acid	0.09^{d}	0.09^{e}	0.10^{abd}	0.09^{b}	0.08^{ae}	0.00**
Butyric acid	1.21	1.18	1.07	1.15	1.33	0.09
Iso-valeric acid	0.14	0.16	0.16	0.14	0.14	0.01
Valeric acid	0.13^{de}	0.13^{f}	0.09^{dfg}	0.11^{e}	0.13^{g}	0.01*
$[C_2+C_4]:[C_3]$	3.85^{abd}	4.13^{ce}	4.84^{acf}	4.67^{be}	4.40^{df}	0.20**

Values in the same row with the same superscript differ significantly (a, b and c, $P < 0.01$; others, $P < 0.05$).

decrease in total VFA concentration. Valeric acid also tended to be present in reduced amounts during polyol inclusion. The relative reductions in the concentrations of individual VFA were such that the ratio of the combined concentrations of acetic and butyric acids to propionic acid was significantly increased ($P < 0.01$) 14 and 21 days after polyol introduction. This ratio, although reduced, remained significantly elevated during the final control period.

The relative changes in the concentrations of individual VFA which occurred when polyol diet was offered are shown in Table 5.16 in terms of the effects on the molar proportions of each acid. Notably, the molar proportion of propionic acid present during dietary inclusion of polyol was significantly lower than during the initial control period. Although there was some evidence of a recovery in the proportion of propionic acid in the VFA mixture produced during the final control period, this proportion was still significantly lower than the initial level. The proportions of all but valeric of the remaining VFA were increased during the period of polyol inclusion. These increases were significant for iso-butyric ($P < 0.01$) and iso-valeric ($P < 0.05$) acid.

Changes in rumen fluid pH tended to mirror those in VFA content of the rumen fluid. During the initial and final control periods pH was 6.52 and 5.94 respectively and during successive weeks of polyol inclusion was 6.62, 6.70 and 6.52. Thus pH was significantly higher ($P < 0.05$) than during the initial control period on day 14 of the period for which the animals received polyol, but had returned to the initial control level by day 21. Rumen pH during the final control period was rather low and differed significantly from the remaining values ($P < 0.01$).

Table 5.16 The effect of dietary inclusion of polyol on the molar proportions of individual VFA in the rumen fluid of 4 sheep in Experiment 7.

	Diet					
	Control	Polyol			Control	
Duration of dietary treatment (days)	14	7	14	21	14	SED
Acetic acid	63.70	64.49	65.90	65.72	64.18	0.97
Propionic acid	19.69^{abd}	18.49^{e}	16.33^{ae}	16.92^{b}	17.76^{d}	0.74**
Iso-butyric acid	0.98^{a}	1.03^{d}	1.24^{abde}	1.03^{e}	0.90^{b}	0.07**
Butyric acid	12.69	12.80	13.53	13.49	14.23	0.64
Iso-valeric acid	1.58^{de}	1.80^{f}	1.86^{degh}	1.61^{g}	1.54^{fh}	0.10*
Valeric acid	1.38	1.39	1.15	1.23	1.39	0.11

Values in the same row with the same superscript differ significantly (a, b and c, $P < 0.01$; others, $P < 0.05$).

Discussion

Under the feeding regime adopted in Experiment 5 the animals receiving the control diet were, as intended, in negative energy balance. A situation therefore existed in which the potential of supplementary polyol mixture to contribute to energy metabolism could be assessed. Although the results of the digestibility trial in sheep in Experiment 6 revealed that the hay included in the diets was of poor quality, the performance of the cows receiving the control diet in Experiment 5 was satisfactory. In contrast, 60% of the animals receiving a virtually identical diet which supplied 410g DM/d polyol mixture became clinically ketotic. Evidently, in this instance dietary inclusion of polyol mixture produced changes in metabolism and production indicative of severe energy insufficiency.

The marked hyperketonaemia observed in animals receiving the polyol-supplemented diet was consistent with the high incidence of clinical ketosis in this group and suggested that other animals receiving the polyols may well have been subclinically ketotic. Several characteristics which the responses in these animals had in common with those of animals receiving the control diet suggest that the relatively high rate of ketogenesis in the animals receiving polyol was not solely attributable to an elevated rate of hepatic ketogenesis from FFA mobilised from adipose tissue. FFA concentration tended to be only slightly higher in the plasma of cows receiving the polyol diet, liveweight losses were small in both groups of animals and fatty acid composition of the milk fat did not suggest there to have been a larger contribution to milk fat derived

from mobilised adipose tissue in the animals receiving polyol mixture.

Thus the responses to polyol mixture in Experiment 5 resembled those observed in response to a diet containing xylitol in Experiment 3 in that they were indicative of an effect at the level of rumen metabolism. The changes in VFA concentrations in the rumen associated with dietary inclusion of polyol mixture in Experiment 7 indicate that, even at a level of dietary inclusion as low as 2.7% of DM, these substrates may have been associated with a reduction in total VFA production. Furthermore, the proportionately greater reduction in propionic acid than in butyric and acetic acids indicates that the availability of glucogenic substrate was reduced, both in absolute terms and relative to the amount of ketogenic substrate supplied. Other workers have also noted changes in rumen VFA patterns in animals receiving polyols, although those animals were not in a situation where energy supply was likely to have been as critical as in Experiment 5 and the changes were not associated with significant effects on metabolism or production. Tuori and Poutiainen (1977) found the proportion of butyric acid to be increased, though not significantly, while that of propionic acid was unchanged in the rumen fluid of lactating cows when given a polyol mixture. Slee (1980) found the proportion of acetic acid to be reduced and the proportions of butyric and valeric acids to be increased in the rumen liquor of sheep when a polyol mixture was included in the diet.

SECTION VI

GENERAL DISCUSSION

GENERAL DISCUSSION

Introduction. The ME system introduced by the Agricultural Research Council (1965) provided an objective scientific basis for the assessment of the energy value of ruminant feedstuffs and for the rationing of ruminant farm livestock. Since its adoption in modified form by the advisory services (Ministry of Agriculture, Fisheries and Food, 1975) it has become the agricultural industry's standard in the UK and has done much to improve the precision of ration formulation and animal feeding. The scientific principles underlying the ME system are unquestionably sound but the system has certain well-recognised limitations. Foremost amongst these is the very use of ME to describe energy supply and utilisation, for it has become increasingly apparent that the pattern and efficiency of utilisation of energy can vary with the chemical nature of the nutrient mixture contributing to the dietary ME supply. For example, in the dairy cow the partial efficiency of utilisation of ME for lactation varies over a narrow range of 0.60 to 0.67 (Van Es, 1976) and is similar to the corresponding efficiency for tissue synthesis. There is therefore a simple linear relationship between the ME intake above maintenance and the sum of the energy secreted in milk and deposited in body tissues. However, this linearity obscures the fact that both milk secretion and tissue synthesis have curvilinear dependence on ME supply and the partition of ME use between these two processes is influenced by the chemical composition of the diet. Furthermore, there is evidence that, independent of ME supply, the composition of

the diet may influence the composition of milk and the yields of fat, protein and lactose (Broster et al., 1979; Sutton et al., 1980).

With conventional diets the main sources of energy are carbohydrates (see Thomas & Rook, 1983), the characteristics of which influence the pattern of production of VFA in the rumen and caecum and the absorption of glucose in the small intestine. Thus the amount, composition and physical form of the dietary carbohydrate components can have an important influence on animal performance. This is particularly the case in the lactating dairy cow where the process of milk secretion is highly dependent on a continued and adequate supply of milk precursors and may be sensitive to diet-induced endocrine changes.

The potential impact of changes in the amounts of individual products of carbohydrate digestion on milk yield and composition has been demonstrated in cows in mid-lactation through the responses to intragastric or intravenous infusions of individual VFA or glucose (see Thomas & Chamberlain, 1984). Corresponding effects on the yields of milk constituents have also been observed in studies with diets supplying similar amounts of ME but varying between extremes in carbohydrate composition (Broster et al., 1979; Sutton et al., 1980). Some mechanisms through which these responses may occur have been proposed and are outlined in Fig. 6.01. However, the quantitative basis of the relationships between the products of digestion and the secretion of milk constituents remain poorly understood, as does the regulation of the secretory processes themselves. It is evident from a variety of dietary studies that in some cases the responses observed cannot be explained on the basis of current knowledge. For example, it is not clearly understood why

Table 6.01 Mean effects on the yield of milk fat, protein and lactose of an increased supply of individual end-products of digestion and an outline of the suggested modes of action of the products (From Thomas & Chamberlain, 1984)

End-product of digestion	Milk constituent	Effect on yield†	Suggested mode of action	
Acetate	Fat	+15.6(9)	↑ precursor supply for fatty acid synthesis in mammary gland	↑ substrate supply for ATP production in mammary gland
	Protein	+ 5.5(9)		
	Lactose	+10.3(6)		
Propionate	Fat	-10.5(9)	↑ adipose tissue deposition →↓ availability of milk fat precursors	↑ secretion of insulin
	Protein	+ 4.0(9)	Metabolism in liver →↑ supply of amino acids to mammary gland	
	Lactose	- 0.8(6)		
Butyrate	Fat	+10.6(3)	↑ precursor supply for fatty acid synthesis in mammary gland	
	Protein	- 2.9(3)	Unknown	
	Lactose	- 7.7(2)	Unknown	
Glucose	Fat	- 5.2(7)	↑ adipose tissue deposition →↓ availability of milk fat precursors	↑ secretion of insulin
	Protein	+ 4.4(7)	Metabolism in liver →↑ supply of amino acids to mammary gland	
	Lactose	+ 9.9(2)	Unknown, but probably not due to ↑ precursor supply	

†Values are the mean responses, expressed as a percentage of the control value. Figures in parentheses refer to the number of experiments summarised in the mean. See Thomas & Chamberlain (1984) for the sources of the original data.

isocaloric diets containing a high proportion of barley give substantially higher yields of milk and milk protein than corresponding diets based on maize (Sutton et al., 1980).

The relationships between diet and milk production are modified by the physiological state of the animal. During early lactation the mammary gland is afforded priority over other tissues for absorbed nutrients and body tissue is mobilised to augment substrate supply for milk synthesis. As discussed in Section I, the homoerhetic mechanisms involved in this are poorly understood. Reduced sensitivity of the pancreas to insulinotrophic agents (Lomax et al., 1979), diminished inhibition by glucose of adipose tissue lipolysis (Metz & van den Bergh, 1977), increased numbers of β-adrenergic receptors per adipocyte (Jaster & Wegner, 1981) and changes in circulating levels of hormones and in the sensitivities of target tissues appear likely to be contributory factors. As well as directly affecting hormonal responses to nutrient inputs and the sensitivity of metabolic processes to changes in substrate supply and hormone levels, homeorhetic influences on nutrient utilisation also determine the effective nutrient status of the animal under given dietary circumstances. This in turn sets the homeostatic regulation of nutrient utilisation and influences nutrient availability for milk synthesis. Thus, milk production responses to a specific dietary change may vary depending on the cow's stage of lactation. For example, in early lactation when homeorhetic influences promote the utilisation of adipose tissue, the long-chain fatty acids released may serve both as an energy source for milk synthesis and as precursors of milk fat. Under these circumstances, high concentrate-low forage diets depress milk fat content and the yields

of all milk constituents through an effect on milk yield (Flatt, Moe, Munson et al., 1969). In mid-lactation, however, the same diets have a specific effect on milk fat. The diets suppress milk fat content and yield and promote adipose tissue synthesis as in early lactation, but milk yield is not reduced because the mammary gland no longer depends on the oxidation of long-chain fatty acids for ATP production.

The early lactation period can be seen as being characterised by:

(a) a potentially large imbalance in the cow's supply and demand for energy and especially glucose;

(b) the use of mobilised long-chain fatty acids for ATP production in the mammary gland as well as for milk fat synthesis;

(c) special features of homeorhetic and homeostatic regulation of nutrient use.

In view of this, it seems reasonable to expect that milk production in early lactation will be especially influenced by changes in diet composition, and that there will be a sensitivity to dietary manipulations leading to an increased glucose supply or to a change in the secretion of homeostatic hormones. This thesis was in effect the starting point for the experiments in Section IV and Section V which examined the effects on milk yield and composition of the dietary replacement of barley with alternative carbohydrate sources selected to produce a differing pattern of glucose supply and endocrine response. The alternative carbohydrate sources were: maize and xylitol (see Section III) (Experiment 3); formaldehyde-treated barley and a xylitol-enriched polyol mixture (Experiment 4) and a polyol mixture (Experiment 5). In each case the dietary treatments

were intended to produce physiologically significant changes in glucose supply without a large modification in diet formulation. This was designed to avoid extremes of diet composition (see Sutton et al., 1980) and to highlight the responses which may be produced by moderate changes in diet corresponding to those typically found in the dairy industry in diets for early-lactation cows.

The effects of diet on glucose supply. In Experiment 3 it is likely that approximately 100g/d less starch was digested in the rumen from the diet supplemented with maize rather than barley. According to the values for starch content, digestion in the rumen and disappearance from the small intestine assumed for these cereals by Armstrong and Smithard (1979) the concurrent increase in starch digestion in the small intestine would have increased intestinal absorption of glucose by almost 200g/d. Preservative treatment of barley appears on the basis of Dacron bag incubations to reduce the ruminal degradation of its starch by approximately 50% (M. Kassem, personal communication). Such a reduction in ruminal starch digestion when treated barley replaced untreated barley in Experiment 4 may have increased intestinal absorption of glucose by as much as 1400g/d. Starch digested in the small intestine is likely to have been absorbed efficiently and without extensive metabolism (Janes et al., 1985a). Thus, the replacement of barley with maize (Experiment 3) or treated barley (Experiment 4) would have substantially increased glucose supply. The impact of this on glucose availability to the tissues was, however, almost certainly partly offset by a reduction in endogenous glucose production. Ruminal propionic acid production would be concurrently reduced and the availability of this

substrate appears to be the major determinant of its contribution to gluconeogenesis (Brockman & Greer, 1980). Added to this, gluconeogenesis is suppressed when increased amounts of glucose are absorbed (Bartley & Black, 1966; Clark et al., 1977; Baird et al., 1980; Janes et al., 1985a). Enteroglucagon released in response to glucose in the duodenum may have a role in this suppression (Berzins & Manns, 1979) but it has also been suggested that an increased concentration of glucose in blood perfusing the liver may itself be involved as a mediating factor (Janes et al., 1985b).

The potential for xylitol and other polyols given in Experiments 3, 4 and 5 to contribute to glucose supply can be assessed from the results of Experiments 1 and 2 (see Section III) and from information in the literature. The rate of disappearance of xylitol dosed into the rumen of sheep in Experiment 1 suggested that more than 95% of xylitol entering the rumen was likely to escape fermentation. Also in this experiment, and in agreement with the observations of Lister and Smithard (1984), recoveries at the duodenum indicated that this xylitol was efficiently absorbed prior to the duodenum (Table 3.02). Furthermore, as anticipated from work on xylitol metabolism in non-ruminant tissues (Woods & Krebs, 1973; Froesch, 1976), the results of Experiment 2 confirmed that absorbed xylitol contributes to glucose availability. The indications were that glucose was produced from xylitol with an efficiency of 40-60% under the conditions of Experiment 2. These findings suggest that the xylitol given in the xylitol/barley supplement (Experiment 3), the xylitol-enriched polyol mixture (Experiment 4) and in the polyol mixture (Expriment 5) could potentially supply in the region of 200, 130 and 40g glucose/d respectively. However, the true amounts of

glucose supplied by these dietary inclusions may in fact have been less than this if adaptive changes within the microbial population increased ruminal fermentation of xylitol. Lister and Smithard (1984) found evidence in in vitro studies for such changes in microbial populations exposed to a polyol mixture for 3 weeks, but concluded that the proportion of xylitol fermented during a time-period equivalent to rumen turnover time would not be significantly increased. However, the responses in plasma glucose and ketone body concentrations and in milk production to dietary inclusion of xylitol and polyol mixture in Experiments 3 and 5 respectively were inconsistent with the resistance to ruminal fermentation and the glucogenic properties of xylitol observed in short-term studies (see Section III), and as such indicated that there had been some increase in xylitol fermentation in these circumstances.

The contribution to glucose supply made by the polyols other than xylitol in the mixtures offered in Experiments 4 and 5 must inevitably have been small, given their generally poor resistance to fermentation by rumen microorganisms, especially after adaptation has occurred in the microbial population (Lister & Smithard, 1984) and given the low levels at which these substrates were included in the diets. Having said this, the results of Experiment 7 indicate that dietary inclusion of polyols can indirectly influence glucose supply by altering VFA production. The inclusion of polyol mixture in the same proportion of the diet as in Experiment 5 (2.7% of total DM) was associated with changes in rumen fermentation pattern in sheep (Tables 5.15 and 5.16). Early in vitro work by Poutiainen et al. (1976) indicated that polyols might increase the proportion of

propionic acid in the VFA mixture produced, particularly when incubated with adapted rumen liquor. More recently Lister and Smithard (1984) have found indications that fermentation pattern in in vitro incubations with smaller amounts of polyol was shifted following adaptation towards a higher acetic acid:propionic acid ratio as a result of reciprocal changes in the proportions of both VFA. The extent to which VFA concentrations measured in rumen liquor reflect their production is open to question for diets containing concentrates (Sutton & Morant, 1978). However, the changes in the concentrations of all major VFA when polyol mixture was included in the diet in Experiment 7 indicated that their production, and especially that of acetic and propionic acids, was reduced during polyol inclusion. The ratio of the concentrations of acetic and butyric acids to the concentration of propionic acid was significantly increased during dietary inclusion of polyol mixture. However, were polyols to have caused similar changes in ruminal VFA production in Experiments 4 and 5, the impact of these changes on glucose availability was likely to have been small in comparison with those of the other dietary treatments imposed in Experiments 3 and 4. Although it is not possible to be certain whether similar changes in VFA production occurred during dietary inclusion of xylitol in Experiment 3, the tendency for plasma glucose concentrations to be low and ketone body concentrations to be high in animals receiving the xylitol/barley supplement suggests this to be so.

The effects of diet on energy metabolism. Increased absorption of glucose from the small intestine when maize (Experiment 3) or formaldehyde-treated barley (Experiment 4) replaced barley in the

diet was not accompanied by changes in plasma glucose, β-hydroxybutyrate, acetoacetate or FFA concentrations (Tables 4.07 and 4.18). Likewise, partial replacement of barley with an isoenergetic amount of a xylitol-enriched polyol mixture (Experiment 4) was without significant effect on these parameters. Evidently, despite the substantial demands of the mammary gland for glucose, plasma glucose concentration was not modified by considerable differences in glucose supply. Although the absence of changes in glucose concentration may obscure differences in the availability and utilisation of glucose for milk production (Buckley, Herbein & Young, 1982), the close similarities within each experiment in the effects of the diets on the plasma concentrations of the whole range of metabolites measured and the similarities in the yields of milk constituents suggest that the differences in glucose availability associated with these diets were accommodated, such that energy metabolism and the availability and utilisation of substrates for milk synthesis were virtually unaffected.

In line with this, there was no clear evidence in Experiment 4 of diet-induced endocrine changes which could be attributed to differing direct or indirect effects on hormone secretion of the likely differences in the composition of the mixtures of nutrients absorbed (Table 4.18). This is not inconsistent with the patterns of hormonal response observed by Sutton et al. (1980). These were variable and not clearly related to diet, despite there being relatively large differences between diets in the amounts of propionic acid and glucose absorbed. As the animals in Experiment 4 were in the early stages of lactation a diminished responsiveness of the pancreas to insulinotrophic agents (Jenny et al., 1974; Lomax et

al., 1979, Sartin, Cummins, Kemppainen, Marple, Rahe & Williams, 1985) may have also contributed to the similarities between the effects of these diets on insulin secretion. Insulin concentrations did tend to be slightly lower and growth hormone concentrations to be slightly higher in animals receiving treated barley or xylitol-enriched polyol mixture rather than untreated barley. However, the reductions in the insulin:growth hormone ratio seem unlikely to have been large enough to have modified the supply of precursors at the mammary gland. More data on the effects of precisely defined physiological changes in hormone levels are required before it will be possible to assess accurately the magnitude of changes in individual hormones and in their relative concentrations necessary to alter nutrient metabolism. Furthermore, comparisons of hormone levels from different studies may be of limited validity because of the many factors which influence hormone levels in the animal and because of differences which may arise from differing assay techniques. However, changes in hormone concentrations associated with the replacement of untreated barley with treated barley or xylitol-enriched polyol mixture, although they were in directions which might have been expected to repartition nutrients towards milk secretion rather than tissue synthesis (see Section I), were small in comparison with the ranges of concentrations reported by others for lactating cows (Bines et al., 1983; Herbein, Aiello, Eckler, Pearson & Akers, 1985; Sartin et al., 1985) and those observed by Sutton et al. (1980), admittedly for more extreme diets.

In contrast with the apparent insensitivity of the animals' metabolism to the manipulation of glucose supply by altering the

amount of glucose absorbed from the small intestine, metabolic responses were clearly modified by dietary inclusion of xylitol in Experiment 3 and more so by the inclusion of polyol mixture in Experiment 5. The tendency for plasma glucose concentrations to be lower and ketone body concentrations to be higher in animals receiving the diet supplemented with xylitol/barley rather than the other supplements (Table 4.07) suggests that during prolonged exposure adaptive changes in the rumen microbial population increase xylitol fermentation to a greater extent than would be anticipated on the basis of in vitro studies (Lister & Smithard, 1984). Animals receiving polyol mixture rather than barley in Experiment 5 also tended to have higher plasma ketone body concentrations, and in 60% of these animals this was associated with the development of clinical ketosis and a subsequent anorexia. When considered together with the effects of polyol mixture on ruminal VFA concentrations observed in Experiment 7 (Table 5.15), the metabolic effects of dietary polyols appear to derive mainly from their effects on fermentation pattern. In view of the similarities between the metabolic responses to xylitol and those to polyol mixture it seems that the effects of xylitol on VFA production may have resembled those of the polyol mixture. Ruminal concentrations of propionic, acetic and butyric acids are all reduced when polyol mixture is included in the diet (Experiment 7). Therefore it would appear that the ketonaemia in animals receiving xylitol and/or polyol mixture, rather than being a result of increased ketogenesis from ruminal VFA, was caused by a reduction in the availability of glucogenic substrate (propionic acid) leading to a relatively high rate of adipose tissue mobilisation and an increased hepatic ketogenesis from mobilised FFA.

Having said this, plasma FFA concentrations in Experiments 3 and 5 (Tables 4.07 and 4.15) and liveweight changes in Experiment 5 (Fig. 5.02) did not reflect such changes.

How the relatively small changes in the mixture of digestion end-products absorbed which were likely to have been associated with the inclusion of polyol mixture in the diet should have produced such marked effects on metabolism in Experiment 5 is unclear. As hormone concentrations were not measured in these animals the roles of the basal endocrine status of the animals and the specific hormonal responses evoked by the dietary polyol mixture as factors in the aetiology of the cases of clinical ketosis cannot be assessed. However, the fact that the animals received the diet containing polyol mixture prior to calving and during the initial stages of lactation, when homeorhetic 'drives' would be especially strong and when energy imbalance was particularly large (Table 5.11) probably contributed to the marked metabolic and production effects which were ultimately apparent.

The effects of diet on milk production. In line with the responses in plasma metabolite and hormone concentrations to diet, the yields of milk and milk constituents did not differ significantly between isoenergetic diets in Experiment 3 or Experiment 4, although again the responses to the xylitol/barley supplemented diet tended to differ slightly from those to diets supplemented with barley or maize in Experiment 3 (Tables 4.03, 4.04, 4.14 and 4.15). By contrast, in Experiment 5 animal health and lactational performance was clearly influenced by dietary inclusion of polyol mixture.

The relationship between lactose yield and plasma glucose concentration demonstrated using intravenous infusions of insulin in goats (Rook & Hopwood, 1970) and the failure of intraruminally infused propionic acid (see Rook, Balch & Johnson, 1965) and in some cases of intra-abomasally infused glucose (Clark et al., 1977) to increase milk yield have demonstrated that, if plasma glucose concentration is maintained within the normal physiological range, glucose supply per se is unimportant in the regulation of lactose secretion. Consistent with this, in Experiments 3 and 4 isoenergetic diets which were likely to differ in the amounts of glucose which they supplied did not differ significantly in their effects on either plasma glucose concentration or lactose yield. However, in some circumstances propionic acid and intestinally-absorbed glucose have been demonstrated to have differing effects on lactose synthesis. For example, greater lactose yields were reported by Sutton et al. (1980) for high-concentrate diets containing barley rather than maize. The differences in response patterns between Experiments 3 and 4 and those of Sutton et al. (1980) are undoubtedly partly a reflection of the relatively smaller differences between treatments in the availabilities of propionic acid and glucose in these experiments, but may also be a consequence of endocrine responses peculiar to early lactation. Sutton (1981) has proposed that lactose yield may be positively related to the absolute rate at which propionic acid is produced in the rumen. It is tempting to suggest that the similar responses to diets containing carbohydrates which differed in the rate at which they were fermented in the rumen in Experiments 3 and 4 may be a consequence of the elimination of some endocrine-mediated effect deriving from this characteristic of

digestion. However, the tendency for lactose yield to be lower for the xylitol/barley supplemented diet rather than the other supplemented diets suggests that the rate of propionic acid production may, in the absence of a concurrent increase in intestinal absorption of glucose, influence lactose synthesis. According to the indications from VFA infusion studies, however, it is the availability of acetic acid rather than that of propionic or butyric acid which is positively related to milk yield (Rook et al., 1965). If, as indicated by the effects of polyol mixture on rumen VFA concentrations in Experiment 7, the production of butyric and acetic acids as well as propionic acid was reduced in animals receiving the xylitol/barley supplement as compared with the other supplements this may have contributed to the slightly poorer yield of lactose for that supplement. However, this explanation is not entirely satisfactory because VFA production was also likely to have been reduced when maize replaced barley.

The effects of dietary carbohydrate on milk fat secretion are widely judged to be related to the insulin-mediated effects of glucogenic precursors on adipose tissue metabolism (McClymont & Vallance, 1962). In view of the absence of significantly differing effects of the diets on plasma glucose and FFA concentrations or on insulin concentrations, the similarities in the yields of fat and individual fatty acids between the diets in Experiments 3 and 4 (Tables 4.06 and 4.17) are not inconsistent with this proposal. The similarities between these responses to the barley and maize diets and also to the untreated and treated barley diets are consistent with the suggestion of Sutton (1981) that the effects of propionic acid and glucose on fat secretion are similar.

The diet containing xylitol/barley in Experiment 3 tended to reduce the output of milk fat and more specifically of $C_{18:0}$ and $C_{18:1}$ fatty acids in comparison with the other isoenergetic diets. As plasma metabolite concentrations in animals receiving this supplement were generally consistent with poorer glucose status it is difficult to reconcile the combination of depressed long-chain fatty acid secretion in the milk fat and the responses in metabolite levels with the glucogenic theory of McClymont and Vallance (1962). This theory proposes that an increase in glucose availability to the animal leads to an insulin-mediated increase in adipose tissue deposition and a reduction in the availability of milk fat precursors at the mammary gland and thereby reduces the output of long-chain fatty acids in milk.

Milk protein secretion is increased in response to intraruminal infusions of propionic acid (Rook & Balch, 1961) and, independently of energy intake, in animals receiving increasing amounts of readily-fermentable carbohydrate (Sutton et al., 1980). Contrary to the implications of this, diets containing carbohydrate sources which were likely to differ in their effects on propionic acid production had similar effects on milk protein yield in Experiment 3 and Experiment 4. This may be attributable in Experiment 3 to the differences in propionic acid production between the diets being too small to effect a change in protein synthesis. The reduction in propionic acid availability when treated barley rather than untreated barley was given (Experiment 4) was likely to have been comparatively large. The absence of an effect on milk protein synthesis in this instance may have arisen as the result of a concurrent increase in the passage of dietary protein to the small

intestine; preservative-treatment of barley reduces the rate of ruminal degradation of its protein (M. Kassem, personal communication).

In contrast to the results of Experiments 3 and 4 those of Experiment 5 demonstrate that in some circumstances, as anticipated from the precarious glucose status of the cow during early lactation, energy metabolism and milk production are responsive to differences in the amount of glucose and glucogenic substrate supplied via the diet at a given energy intake. The hypoglycaemia and hyperketonaemia exhibited by 60% of the animals receiving polyol at a level of 2.7% of the total dietary DM was accompanied by effects on milk production which were characteristic of clinical ketosis. Prior to the manifestation of clinical signs, yields of milk and milk constituents for animals receiving the polyol mixture generally did not differ significantly on a weekly basis from those for animals receiving barley (Table 5.07 and Fig. 5.03). This is consistent with the proposal of Baird (1982) that it is the inability of the animal to accommodate inadequacies in substrate availability by reducing milk secretion which predisposes it to ketosis. It is, however, unclear why the relatively small differences in nutrient supply which were likely to exist between the diets given in Experiment 5 should have been so effective in altering animal performance.

The apparent inconsistencies between Experiment 5 and the previous experiments emphasise the complexities of the relationships between diet, nutrient availability and nutrient utilisation during early lactation, and the need for a quantitative understanding of the interactions between the processes involved. Interest is currently increasing in the use of computer modelling to identify and quantify

the nutritional, physiological and other factors which control nutrient utilisation in the lactating cow. Current knowledge has been assimilated into models to describe the processes influencing digestion in the rumen (France, Thornley & Beever, 1982), the metabolism of absorbed nutrients by growing sheep (Gill, Thornley, Black, Oldham & Beever, 1984) and mammary gland function (assuming constant substrate supply) (Neal & Thornley, 1983). At present this approach is useful in the evaluation of current concepts in dairy cow nutrition and in the identification of specific areas where research is required. As they become more refined models should increase the accuracy with which the dietary inputs necessary for the production of a desired combination of milk constituents can be predicted.

Aside from illustrating the need for such advances, the results of the present work also have several practical implications relating to the formulation of diets for early-lactation cows. Evidently, despite the considerable stress on glucose metabolism imposed by the requirements of the mammary gland at this time, moderate dietary changes designed to increase glucose supply by enhancing intestinal absorption of starch are without effect on energy metabolism or milk production. Thus, there appears to be no advantage in terms of milk production to be gained from selecting carbohydrate sources which are relatively slowly fermented in the rumen, such as raw maize, rather than barley for inclusion in diets given during early lactation.

In general, the responses of animals receiving xylitol and/or polyols in the diet failed to demonstrate these substances to be of value in improving the balance between nutrient supply and demand during early lactation. Despite the potentially glucogenic nature of

xylitol and the success with which commercial products containing polyol mixtures together with propylene glycol and sodium propionate have been used in the prophylactic treatment of ketosis, diets containing polyols were in these experiments frequently associated with relatively high rates of ketogenesis. In circumstances where the animals were apparently especially sensitive to nutritional imbalances polyol mixture actually increased the incidence of clinical ketosis. It would appear from this that the beneficial properties of the commercially available products derive from the recognised glucogenic substrates, propylene glycol and sodium propionate, which are present in relatively large proportions. Given the low levels of inclusion of polyol mixture in the diet which produced clearly detrimental effects on animal health and lactational performance in Experiment 5 it seems inadvisable to include these substances in diets for lactating cows in other than very small amounts.

REFERENCES

ABE, M. and KANDATSU, M. 1969. Jap. J. Zootech. Sci. 40: 313-319.

ADLER, J.H., WERTHEIMER, E., BARTANA, U. and FLESH, J. 1963. Vet. Rec. 75: 304-307.

AGRICULTURAL RESEARCH COUNCIL 1965. The Nutrient Requirements of Farm Livestock. No. 2. Ruminants. HMSO, London.

AGRICULTURAL RESEARCH COUNCIL 1980. The Nutrient Requirements of Ruminant Livestock. Commonwealth Agricultural Bureaux, Slough.

ANNISON, E.F. 1956. Biochem. J. 64: 705-714.

ANNISON, E.F., BICKERSTAFFE, R. and LINZELL, J.L. 1974. J. agric. Sci. Camb. 82: 87-95.

ANNISON, E.F., LINDSAY, D.B. and WHITE, R.R. 1963. Biochem. J. 88: 243-248.

ANNISON, E.F. and LINZELL, J.L. 1964. J. Physiol., Lond. 175: 372-385.

ANNISON, E.F., LINZELL, J.L., FAZAKERLEY, S. and NICHOLS, B.W. 1967. Biochem. J. 102: 637-647.

ANNISON, E.F. and McDOWELL, G.H. 1980. Proc. Nutr. Soc. Aust. 5: 96-103.

ANNISON, E.F. and WHITE, R.R. 1961. Biochem. J. 80: 162-169.

ARCUS, A.C., ELLIS, M.J., KIRK, R.D., BEAVAN, D.W., DONALD, R.A., HART, D.S., HOLLAND, G.W. and REDEKOPP, C. 1976. Aust. J. biol. Sci. 29: 223-236.

ARMSTRONG, D.G. and BEEVER, D.E. 1969. Proc. Nutr. Soc. 28: 121-131.

ARMSTRONG, D.G. and SMITHARD, R.R. 1979. Proc. Nutr. Soc. 38: 283-294.

ASANO, T., GREENBERG, B.Z., WITTMERS, R.V. and GOETZ, F.C. 1977. Endocrinology 100: 339-345.

ASH, R. and BAIRD, G.D. 1973. Biochem. J. 136: 311-319.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS 1975. (eds W. Horwitz et al.). AOAC, Washington, DC.

BAIRD, G.D. 1977. Biochem. Soc. Trans. 5: 819-827.

BAIRD, G.D. 1981. Proc. Nutr. Soc. 40: 115-120.

BAIRD, G.D. 1982. J. Dairy Sci. 65: 1-10.

BAIRD, G.D., HEITZMAN, R.J. and HIBBITT, K.G. 1972. Biochem. J. 128: 1311-1318.

BAIRD, G.D., HIBBITT, K.G., HUNTER, G.D., LUND, P., STUBBS, M. and KREBS, H.A. 1968. Biochem. J. 107: 683-689.

BAIRD, G.D., LOMAX, M.A., SYMONDS, H.W. and SHAW, S.R. 1978. Proc. Nutr. Soc. 37: 94A(Abstr.).

BAIRD, G.D., LOMAX, M.A., SYMONDS, H.W. and SHAW, S.R. 1980. Biochem. J. 186: 47-57.

BAIRD, G.D., REID, I.M., LOMAX, M.A., SYMONDS, H.W., ROBERTS, C.J. and MATHER, D. 1977. Proc. Nutr. Soc. 36: 40A(Abstr.).

BAIRD, G.D., SYMONDS, H.W. and ASH, R. 1975. J. agric. Sci., Camb. 85: 281-296.

BAIN, A. 1980. The Fate of the Polyol Xylitol in the Ruminant. BSc Thesis, University of Newcastle.

BALCH, C.C., BROSTER, W.H., ROOK, J.A.F. and TUCK, V.J. 1965. J. Dairy Res. 32: 1-11.

BALDWIN, R.L. and ALLISON, M.J. 1983. J. Anim. Sci. 57: Suppl. 2: 461-477.

BALDWIN, R.L., REICHL, J.R., LOUIS, S., SMITH, N.E., YANG, Y.T. and OSBORNE, E. 1973. J. Dairy Sci. 56: 340-349.

BALDWIN, R.L. and SMITH, N.E. 1971. J. Dairy Sci. 54: 583-595.

BALLARD, F.J., FILSELL, O.H. and JARRET, I.G. 1972. Biochem. J. 226: 193-200.

BALLARD, F.J., HANSON, R.W. and KRONFELD, D.S. 1969. Fed. Proc. 28: 218-231.

BARRY, J.M. 1964. Biol. Rev. 39: 194-213.

BARTLEY, J.C. and BLACK, A.L. 1966. J. Nutr. 89: 317-328.

BASSETT, J.M. 1970. Aust. J. biol. Sci. 23: 903-914.

BASSETT, J.M. 1971. Aust. J. biol. Sci. 24: 311-320.

BASSETT, J.M. 1972. Aust. J. biol. Sci. 25: 1277-1287.

BASSETT, J.M. 1974. Aust. J. biol. Sci. 27: 167-181.

BASSETT, J.M. 1975. In Digestion and Metabolism in the Ruminant (ed. I.W. McDonald and A.C.I. Warner), pp. 383-398. University of New England Publishing Unit, Armidale, Australia.

BASSETT, J.M. 1978. Proc. Nutr. Soc. 37: 273-280.

BASSETT, J.M. 1980. In Hormones and Metabolism in Ruminants, pp. 65-79. Agricultural Research Council, London.

BASSETT, J.M., WESTON, R.H. and HOGAN, J.P. 1971. Aust. J. biol. Sci. 24: 321-330.

BASSLER, K.H. 1969. In Pentoses and Pentitols (ed. B.L. Horecker, K. Lang and Y. Takagi), pp. 190-196. Springer-Verlag, Berlin.

BATH, I.H. and ROOK, J.A.F. 1963. J. agric. Sci., Camb. 61: 341-348.

BAUMAN, D.E. 1976. Fed. Proc. 35: 2308-2313.

BAUMAN, D.E., BROWN, R.E. and DAVIS, C.L. 1970. Archs Biochem. Biophys. 140: 237-244.

BAUMAN, D.E. and CURRIE, W.B. 1980. J. Dairy Sci. 63: 1514-1529.

BAUMAN, D.E. and DAVIS, C.L. 1975. In Digestion and Metabolism in the Ruminant (ed. I.W. McDonald and A.C.I. Warner), pp. 496-509. University of New England Publishing Unit, Armidale, Australia.

BAUMAN, D.E., EISEMANN, J.H. and CURRIE, W.B. 1982. Fed. Proc. 41: 2538-2544.

BAUMAN, D.E. and ELLIOT, J.M. 1983. In Biochemistry of Lactation (ed. T.B. Mepham), pp. 437-468. Elsevier, Amsterdam.

BECK, N.F.G. and TUCKER, H.A. 1978. Proc. Soc. exp. Biol. Med. 159: 394-396.

BEEVER, D.E., THOMSON, D.J. and CAMMELL, S.B. 1976. J. agric. Sci., Camb. 86: 443-452.

BEEVER, D.E., THOMSON, D.J. and HARRISON, D.G. 1971. Proc. Nutr. Soc. 30: 86A(Abstr.).

BEEVER, D.E., THOMSON, D.J., PFEFFER, E. and ARMSTRONG, D.G. 1971. Br. J. Nutr. 26: 123-134.

BELL, A.W. 1980. Prog. Lipid Res. 18: 117-164.

BEN-GHEDALIA, D., TAGARI, H., BONDI, A. and TADMOR, A. 1974. Br. J. Nutr. 31: 125-142.

BENNINK, M.R., MELLENBERGER, R.W., FROBISH, R.A. and BAUMAN, D.E. 1972. J. Dairy Sci. 55: 712(Abstr.).

BENSADOUN, A., PALADINES, O.L. and REID, J.T. 1962. J. Dairy Sci. 45: 1203-1210.

BENSON, J.D., ASKEW, E.W., EMERY, R.S. and THOMAS, J.W. 1972. J. Dairy Sci. 55: 83-92.

BERGEN, W.G., PURSER, D.B. and CLINE, J.H. 1968. J. Anim. Sci. 27: 1497-1501.

BERGMAN, E.N. 1973. Cornell Vet. 63: 341-382.

BERGMAN, E.N. and HEITMANN, R.N. 1978. Fed. Proc. Fed. Am. Socs. exp. Biol. 37: 1228-1232.

BERGMAN, E.N. and HOGUE, D.E. 1967. Am. J. Physiol. 213: 1378-1384.

BERGMAN, E.N., KATZ, M.L. and KAUFMAN, C.F. 1970. Am. J. Physiol. 219: 785-793.

BERGMAN, E.N. and KON, K. 1964. Am. J. Physiol. 206: 449-452.

BERGMAN, E.N., ROE, W.E. and KON, K. 1966. Am. J. Physiol. 211: 793-799.

BERGMAN, E.N., STARR, D.J. and REULEIN, S.S.Jr. 1968. Am. J. Physiol. 215: 874-880.

BERZINS, R. and MANNS, J.G. 1979. J. Dairy Sci. 62: 1739-1745.

BICKERSTAFFE, R., ANNISON, E.F. and LINZELL, J.L. 1974. J. agric. Sci., Camb. 82: 71-85.

BICKERSTAFFE, R., NOAKES, D.E. and ANNISON, E.F. 1972. Biochem. J. 130: 607-617.

BINES, J.A., BRUMBY, P.E., STORRY, J.E., FULFORD, R.J. and BRAITHWAITE, G.D. 1978. J. agric. Sci., Camb. 91: 135-150.

BINES, J.A. and DAVEY, A.W.F. 1978. Br. J. Nutr. 39: 567-578.

BINES, J.A. and HART, I.C. 1982. J. Dairy Sci. 65: 1375-1389.

BINES, J.A., HART, I.C. and NAPPER, D.J. 1980. Annual Report of the National Institute for Research in Dairying, p. 58.

BINES, J.A., HART, I.C. and MORANT, S.V. 1983. Horm. metab. Res. 15: 330-334.

BINNERTS, W.T., van't KLOOSTER, A.TH. and FRENS, A.M. 1968. Vet. Rec. 82: 470.

BIRD, A.R., CHANDLER, K.D. and BELL, A.W. 1981. Aust. J. biol. Sci. 34: 541-550.

BLAXTER, K.L. and CLAPPERTON, J.L. 1965. Br. J. Nutr. 19: 511-522.

BLIGH, E.G. and DYER, W.J. 1959. Can. J. Biochem. Physiol. 37: 911-917.

BLOOM, S.R., EDWARDS, A.V. and VAUGHAN, N.J.A. 1973. J. Physiol., Lond. 233: 457-466.

BOLTON, S.C., WEEKES, T.E.C., GODDEN, P.M.M. and ARMSTRONG, D.G. 1983. Proc. Nutr. Soc. 42: 48A(Abstr.).

BRENT, B.E. and ADEPOJU, A. 1967. J. Anim. Sci. 26: 1482(Abstr.).

BRITISH STANDARDS INSTITUTION 1963. Methods for the chemical analysis of liquid milk and cream. BS1741.

BRITISH STANDARDS INSTITUTION 1969. Gerber method for the determination of fat in milk and milk products. BS696 Part 2: Methods.

BROCKMAN, R.P. 1976. Can. J. comp. Med. 40: 166-170.

BROCKMAN, R.P. 1978a. Can. Vet. J. 19: 55-62.

BROCKMAN, R.P. 1978b. Fed. Proc. 37: 339(Abstr.).

BROCKMAN, R.P. 1979. Can. Vet. J. 20: 121-126.

BROCKMAN, R.P. 1982. Comp. Biochem. Physiol. 73: 237-238.

BROCKMAN, R.P., BERGMAN, E.N., JOO, P.K. and MANNS, J.G. 1975. Am. J. Physiol. 229: 1344-1350.

BROCKMAN, R.P. and GREER, C. 1980. Aust. J. Biol. Sci. 33: 457-464.

BROCKMAN, R.P. and MANNS, J.G. 1974. Cornell Vet. 64: 217-224.

BROOKS, C.C., GARNER, G.B., GEHRKE, C.W., MUHRER, M.E. and PFANDER, W.H. 1954. J. Anim. Sci. 13: 758-764.

BROSTER, W.H. 1974. Biennial Review of the National Institute for Research in Dairying, pp. 14-34.

BROSTER, W.H., BROSTER, V.J. and SMITH, J. 1969. J. agric. Sci., Camb. 72: 229-245.

BROSTER, W.H., SUTTON, J.D. and BINES, J.A. 1979. In Recent Advances in Animal Nutrition - 1978 (ed. W. Haresign and D. Lewis), pp. 99-126. Butterworths, London.

BRUCKENTAL, I., OLDHAM, J.D. and SUTTON, J.D. 1980. Br. J. Nutr. 44: 33-45.

BRYANT, M.P. and ROBINSON, I.M. 1962. J. Bacteriol. 84: 605-614.

BUCKLEY, B.A., HERBEIN, J.H. and YOUNG, J.W. 1982. J. Dairy Sci. 65: 371-384.

BURRIS, W.R., BRADLEY, N.W. and BOLING, J.A. 1974. J. Anim. Sci. 38: 200-205.

CHALMERS, J.S. 1979. Dietary factors influencing the composition of cows milk with special reference to milk protein. PhD thesis. University of Glasgow.

CHALUPA, W. 1975. J. Dairy Sci. 58: 1198-1218.

CHRISTOPHERSON, S.W. and GLASS, R.L. 1969. J. Dairy Sci. 52: 1289-1290.

CHURCH, D.C. 1976. Digestive Physiology and Nutrition of Ruminants, Vol. 1 (ed. Church, D.C.). O and B Books, Oregon, USA.

CLARKE, E.M.W., ELLINGER, G.M. and PHILLIPSON, A.T. 1966. Proc. R. Soc. Lond. B. 166: 63-79.

CLARK, J.H., SPIRES, H.R., DERRIG, R.G. and BENNINK, M.R. 1977. J. Nutr. 107: 631-644.

COCHRAN, W.G. and COX, G.M. 1950. Experimental Designs. Wiley, New York.

COELHO DA SILVA, J.F., SEELEY, R.C., BEEVER, D.E., PRESCOTT, J.H.D. and ARMSTRONG, D.G. 1972. Br. J. Nutr. 28: 357-371.

COELHO DA SILVA, J.F., SEELEY, R.C., THOMSON, D.J., BEEVER, D.E. and ARMSTRONG, D.G. 1972. Br. J. Nutr. 28: 43-61.

CONNELLY, J.D., HEAD, H.H. and WILLIAMS, W.F. 1964. J. Dairy Sci. 47: 386-390.

CONWAY, E.J. 1957. Microdiffusion Analysis and Volumetric Error, pp. 217-226. Crosby, Lockwood & Sons Ltd, London.

CORNISH-BOWDEN, A. 1981. Basic Mathematics for Biochemists. Chapman and Hall Ltd, London.

COSTA, N.D., McINTOSH, G.H. and SNOSWELL, A.M. 1976. Aust. J. biol. Sci. 29: 33-42.

COTE, J.F., CURTIS, R.A., McSHERRY, B.J., ROBERTSON, J.McD. and KRONFELD, D.S. 1969. Can. Vet. J. 10: 179-187.

COTTYN, B.G. and BOUCQUE, G.N. 1968. J. agric. Fd Chem. 16: 105-107.

COWIE, A.T., FORSYTH, I.A. and HART, I.C. 1980. Hormonal Control of Lactation. Springer-Verlag, Berlin.

CRAMPTON, E.W. and MAYNARD, L.A. 1938. J. Nutr. 15: 383-395.

DAVIS, C.L. and BAUMAN, D.E. 1974. In Lactation: A Comprehensive Treatise, Vol. II (ed. B.L. Larson and V.R. Smith), pp. 3-30. Academic Press, London.

DAWSON, R.M.C. and KEMP, P. 1970. In Physiology of Digestion and Metabolism in the Ruminant (ed. A.T. Phillipson), 504-518. Oriel Press, Newcastle-upon-Tyne.

DE BOER, G., TRENKLE, A. and YOUNG, J.W. 1985. J. Dairy Sci. 68: 326-337.

DENNIS, S.M., ARAMBEL, M.J., BARTLEY, E.E. and DAYTON, A.D. 1983. J. Dairy Sci. 66: 1248-1254.

DEPARTMENT OF AGRICULTURE AND FISHERIES FOR SCOTLAND. 1975. 1st Report of the Feedingstuffs Evaluation Unit. DAFS, Edinburgh.

DEVENDRA, C. and LEWIS, D. 1974. Anim. Prod. 19: 67-76.

DEWAR, W.A. and McDONALD, P. 1961. J. Sci. Fd. Agric. 12: 790-795.

DONEFER, E., LLOYD, L.E. and CRAMPTON, E.W. 1962. J. Anim. Sci. 21: 993(Abstr.).

DRIVER, P.M. and FORBES, J.M. 1978. Proc. Nutr. Soc. 37: 100A(Abstr.).

EL-HAG, G.A. and MILLER, T.B. 1972. J. Sci. Fd Agric. 23: 247-258.

ELLIOT, J.M. 1976. In Proceedings of the Cornell Nutrition Conference. A report of research of the Cornell University Agricultural Experimental Station, p. 59.

ELLIOT, J.M., BARTON, E.P. and WILLIAMS, J.A. 1979. J. Dairy Sci. 62: 642-645.

EL-SHAZLY, K. 1952. Biochem. J. 51: 640-647.

EMERY, R.S. 1979. J. Anim. Sci. 48: 1530-1537.

EMMANUEL, B. 1974. Biochim. biophys. Acta 337: 404-413.

EMMANUEL, B. 1978. Biochim. biophys. Acta 528: 239-246.

ESKELAND, B. and PFANDER, W.H. (1973) J. Dairy Sci. 56: 1340-1343.

EVANS, E., BUCHANAN-SMITH, J.G., MACLEOD, G.K. and STONE, J.B. 1975. J. Dairy Sci. 58: 672-677.

FARHAN, S.M.A. and THOMAS, P.C. 1977. Proc. Nutr. Soc. 36: 57A(Abstr.).

FISHER, L.J. and ELLIOT, J.M. 1966. J. Dairy Sci. 49: 826-829.

FLATT, J.P. 1966. J. Dairy Sci. 49: 230-237.

FLATT, W.P., MOE, P.W., HOOVEN, N.W., LEHMANN, R.P., ORSKOV, E.R. and HEMKEN, R.W. 1969. In Energy Metabolism of Farm Animals (ed. K.L. Blaxter, J. Kielanowski and G. Thorbeck), pp. 221-234. Oriel Press, Newcastle-upon-Tyne.

FLATT, W.P., MOE, P.W., MUNSON, A.W. and COOPER, T. 1969. In Energy Metabolism of Farm Animals (ed. K.L. Blaxter, J. Kielanowski and g. Thorbeck), pp. 235-259. Oriel Press, Newcastle-upon-Tyne.

FORSTER, H. 1974. In Sugars in Nutrition (eds H.L. Sipple and K.W. McNutt), pp. 259-280. Academic Press.

FORSTER, H. 1978. In Xylitol (ed. J.N. Counsell), pp. 43-66. Applied Science Publishers, London.

FRANCE, J., THORNLEY, J.H.M. and BEEVER, D.E. 1982. J. agric. Sci., Camb. 99: 343-353.

FREDERICKSON, D.S. and GORDON, R.S. 1958. Physiol. Rev. 38: 585-630.

FRITZ, I.B. 1961. Physiol. Rev. 41: 52-129.

FROBISH, R.A. and DAVIS, C.L. 1977a. J. Dairy Sci. 60: 204-209.

FROBISH, R.A. and DAVIS, C.L. 1977b. J. Dairy Sci. 60: 268-273.

FROESCH, E.R. 1976. Int. J. Vit. Nutr. Res. 515: 239-241.

FULGHUM, R.S. and MOORE, W.E.C. 1963. J. Bacteriol. 85: 808-815.

GARTON, G.A. 1969. Proc. Nutr. Soc. 23: 131-139.

GARTON, G.A., LOUGH, A.K. and VIOQUE, E. 1961. J. Gen. Microbiol. 25: 215-225.

GILL, M., THORNLEY, J.H.M., BLACK, J.L., OLDHAM, J.D. and BEEVER, D.E. 1984. Br. J. Nutr. 52: 621-649.

GIRARD, V. and HAWKE, J.C. 1978. Biochim. biophys. Acta 528: 17-27.

GOERING, H.K. and VAN SOEST, P.J. 1970. Forage Fibre Analysis in Agriculture Handbook No. 379.

GORDON, F.J. 1977. Anim. Prod. 25: 181-191.

GRIMBLEY, F.H. 1956. J. Dairy Res. 23: 229-237.

HAGEMEISTER, H., KAUFMANN, W. and PFEFFER, E. 1976. In Protein Metabolism and Nutrition (ed. D.J.A. Cole, K. Boorman, P.J. Buttery, D. Lewis, R.J. Neale and H. Swan), pp. 425-440. Butterworths, London.

HAMADA, T., ISHII, T. and TAGUCHI, S. 1982. J. Dairy Sci. 65: 1509-1513.

HARFOOT, C.G. 1981. In Lipid Metabolism in Ruminant Animals (ed. W.W. Christie), pp. 21-55. Pergamon Press, Oxford.

HART, I.C. 1980. In Hormones and Metabolism in Ruminants, pp. 47-64. Agricultural Research Council, London.

HART, I.C. 1983. Proc. Nutr. Soc. 42: 181-194.

HART, I.C., BINES, J.A., MORANT, S.V. and RIDLEY, J.L. 1978. J. Endocrinology 77: 333-345.

HART, I.C., BINES, J.A. and MORANT, S.V. 1979. J. Dairy Sci. 62: 270-277.

HAYDON, R.K. 1961. Biochim. biophys. Acta 46: 598-599.

HEALD, P.J. 1951. Br. J. Nutr. 5: 84-93.

HEATH, T.J. and MORRIS, B. 1963. Br. J. Nutr. 17: 465-474.

HEITZMAN, R.J., HIBBITT, K.G. and MATHER, I. 1971. Eur. J. Biochem. 21: 411-415.

HEMBRY, F.G., BELL, M.C. and HALL, R.F. 1967. J. Nutr. 93: 175-181.

HENDERSON, C. 1973. J. agric. Sci., Camb. 81: 107-112.

HENDERICKX, H. and MARTIN, J. 1963. Compt. Rend. Rech. Inst. Rech. Sa. Ind. Agr. Bruxelles 31: 11-66.

HERBEIN, J.H., AIELLO, R.J., ECKLER, L.I., PEARSON, R.E. and AKERS, R.M. 1985. J. Dairy Sci. 68: 320-325.

HERTELENDY, F. and KIPNIS, D.M. 1973. Endocrinology 92: 402-410.

HIBBITT, K.G. 1979. Vet. Rec. 105: 13-15.

HIRATA, Y., FUJISAWA, M. and OGUSHI, T. 1969. In Pentoses and Pentitols (ed. B.L. Horecker, K. Lang and Y. Takagi), pp. 226-229. Springer-Verlag, Berlin.

HOBSON, P.N. and MANN, S.O. 1961. J. gen. Microbiol. 25: 227-240.

HOLDSWORTH, E.S., NEVILLE, E., NADER, C., JARRET, I.G. and FILSELL, O.H. 1964. Biochim. biophys. Acta 86: 240-249.

HOOD, R.L., COOK, L.J., MILLS, S.C. and SCOTT, T.W. 1980. Lipids 15: 644-650.

HOSOYA, N. and IITOYO, N. 1969. In Pentoses and Pentitols (ed. B.L. Horecker, K. Lang and Y. Takagi), pp. 197-199. Springer-Verlag, Berlin.

HOUPT, T.R. and HOUPT, K.A. 1968. Am. J. Physiol. 214: 1296-1303.

HOVE, K. and BLOM, A.K. 1973. Acta Endocrinologica 73: 289-303.

HUME, I.D. and BIRD, P.R. 1970. Aust. J. agric. Res. 21: 315-322.

HUNGATE, R.E. 1966. The Rumen and its Microbes. Academic Press, London.

INGLE, D.L., BAUMAN, D.E. and GARRIGUS, U.S. 1972. J. Nutr. 102: 617-624.

JANES, A.N., WEEKES, T.E.C. and ARMSTRONG, D.G. 1985a. Br. J. Nutr. 54: 449-458.

JANES, A.N., WEEKES, T.E.C. and ARMSTRONG, D.G. 1985b. Br. J. Nutr. 54: 459-471.

JARRET, I.G. 1948. J. Counc. Sci. Industr. Res. Aust. 21: 311.

JARRET, I.G., FILSELL, O.H. and BALLARD, F.J. 1976. Metabolism 25: 523-531.

JASTER, E.H. and WEGNER, T.N. 1981. J. Dairy Sci. 64: 1655-1663.

JAKOB, A., WILLIAMSON, J.R. and ASAKURA, T. 1971. J. biol. Chem. 246: 7623-7631.

JENNY, B.F., POLAN, C.E. and THYE, F.W. 1974. J. Nutr. 104: 379-385.

JONES, G.A., MACLEOD, R.A. and BLACKWOOD, A.C. 1964. Can. J. Microbiol. 10: 371-378.

JOURDAN, M.H., MacDONALD, I. and HENDERSON, J.R. 1972. Nutr. Metab. 14: 92-99.

JUDSON, G.J., ANDERSON, E., LUICK, J.R. and LENG, R.A. 1968. Br J. Nutr. 22: 69-75.

JUDSON, G.J. and LENG, R.A. 1973. Br. J. Nutr. 29: 175-195.

KARR, M.R., LITTLE, C.O. and MITCHELL, G.E. 1966. J. Anim. Sci. 25: 652-654.

KATZ, M.L. and BERGMAN, E.N. 1969. Am. J. Physiol. 216: 953-960.

KAUFMAN, C.F. and BERGMAN, E.N. 1971. Am. J. Physiol. 221: 967-972.

KAUFMAN, C.F. and BERGMAN, E.N. 1974. Am. J. Physiol. 226: 827-832.

KAY, R.N.B. 1969. Proc. Nutr. Soc. 23: 140-151.

KEENEY, M. 1970. In Physiology of Digestion and Metabolism in the Ruminant (ed. A.T. Phillipson), pp. 489-503. Oriel Press, Newcastle-upon-Tyne.

KELLER, V. and FROESCH, E.R. 1971. Diabetologia 7: 349-356.

KENNEDY, P.M., CHRISTOPHERSON, R.J. and MILLIGAN, J.P. 1976. Br. J. Nutr. 36: 231-242.

KNIGHT, R., SUTTON, J.D., STORRY, J.E. and BRUMBY, P.E. 1978. Proc. Nutr. Soc. 38: 4A(Abstr.).

KOSAKA, K. 1969. In Pentoses and Pentitols (ed. B.L. Horecker, K. Lang and Y. Takagi), pp. 212-225. Springer-Verlag, Berlin.

KOUNDAKJIAN, P.P. and SNOSWELL, A.M. 1970. Biochem. J. 119: 49-57.

KRISHNAN, R., JAMES, H.M., BAIS, R., ROFE, A.M., EDWARDS, J.B. and CONYERS, R.A.J. 1980. Aust. J. exp. Biol. Med. Sci. 58: 627-638.

KRISHNAN, R., WILKINSON, I., JOYCE, L., ROFE, A.M., BAIS, R., CONYERS, R.A.J. and EDWARDS, J.B. 1980. Aust. J. exp. Biol. Med. Sci. 58: 639-652.

KRONFELD, D.S. 1965. Vet. Rec. 77: 30-35.

KRONFELD, D.S. 1971. J. Dairy Sci. 54: 949-961.

KRONFELD, D.S., RAGGI, F. and RAMBERG, C.F. 1968. Am. J. Physiol. 215: 218-227.

KRONFELD, D.S. and SIMESEN, M.G. 1961. Am. J. Physiol. 201: 639-644.

KUDO, R.R. 1966. Protozoology. Charles C. Thomas, Springfield, Illinois.

KUZUYA, T. 1969. In Pentoses and Pentitols (ed. B.L. Horecker, K. Lang and Y. Takagi), pp. 230-233. Springer-Verlag, Berlin.

LAARVELD, B., CHRISTENSEN, D.A. and BROCKMAN, R.P. 1981. Endocrinology 108: 2217-2221.

LAMB, J.N., PRESCOTT, J.H.D. and ARMSTRONG, D.G. 1976. In Optimizing the Utilisation of Cereal Energy by Cattle and Pigs, pp. 1-30. Technical Publication of the US Feed Grains Council.

LENG, R.A. 1970. Adv. Vet. Sci. Comp. Med. 14: 209-259.

LENG, R.A. and BRETT, D.J. 1966. Br. J. Nutr. 20: 541-552.

LENNOX, A.M. and GARTON, G.A. 1968. Br. J. Nutr. 22: 247-254.

LINDSAY, D.B. 1971. Proc. Nutr. Soc. 30: 272-277.

LINDSAY, D.B. 1975. Proc. Nutr. Soc. 34: 241-248.

LINDSAY, D.B. 1978. Biochem. Soc. Trans. 6: 1152-1156.

LINDSAY, D.B. 1980. In Hormones and Metabolism in Ruminants (ed. J.M. Forbes and M.A. Lomax), pp. 25-36. Agricultural Research Council, London.

LINZELL, J.L. 1967. J. Physiol., Lond. 190: 333-346.

LINZELL, J.L. and PEAKER, M. 1971. Physiol. Rev. 51: 564-597.

LISTER, C.J. and SMITHARD, R.R. 1984. J. Sci. Fd. Agric. 35: 21-28.

LOMAX, M.A., BAIRD, D., MALLINSON, C.B. and SYMONDS, H. 1979. Biochem. J. 180: 281-289.

McATEE, J.W. and TRENKLE, A. 1971. J. Anim. Sci. 33: 612.-616.

McCLYMONT, G.L. and VALLANCE, S. 1962. Proc. Nutr. Soc. 21: XLI.

McCORMICK, D.B. and TOUSTER, O. 1957. J. biol. Chem. 229: 451-461.

McDONALD, I.M. 1952. Biochem. J. 51: 86-90.

McDOWELL, G.H. 1983. Proc. Nutr. Soc. 42: 149-167.

McGARRY, J.D., ROBLES-VALDES, C. and FOSTER, D.W. 1975. Proc. Natl. Acad. Sci. U.S.A. 72: 4385-4388.

McMENIMAN, N.P., BEN-GHEDALIA, D. and ARMSTRONG, D.G. 1976. In Protein and Metabolism and Nutrition (ed. D.J.A. Cole, K.N. Boorman, P.J. Buttery, D. Lewis, R.J. Neale and H. Swan), pp. 217-229. Butterworths, London.

McNEILL, J.W., POTTER, G.D. and RIGGS, J.K. 1971. J. Anim. Sci. 33: 1371-1374.

MacRAE, J.C. and ARMSTRONG, D.G. 1969. Br. J. Nutr. 23: 377-387.

MADON, R.J., FINLEY, E. and FLINT, D.J. 1984. Lab. Pract. 33: 115-116.

MAKINEN, K.K. and SCHEINEN, A. 1975. Acta Odont. Scand. 33: Suppl. 70: 105-127.

MANNS, J.G. 1972. Can. J. Physiol. Pharmacol. 50: 554-560.

MANNS, J.G. and BODA, J.M. 1967. Am. J. Physiol. 212: 747-755.

MARTIN, R.J., WILSON, L.L., COWAN, R.L. and SINK, J.D. 1973. J. Anim. Sci. 36: 101-106.

MAYES, P.A. and FELTS, J.M. 1967. Nature, Lond. 215: 716-718.

MAYES, R.W. and ORSKOV, E.R. 1974. Br. J. Nutr. 32: 143-153.

MELLANBY, J. and WILLIAMSON, D.H. 1974. In Methods of Enzymatic Analysis 2nd ed. (ed. H.U. Bergmeyer), pp. 1840-1843. Academic Press, New York.

MEPHAM, T.B. 1971. In Lactation (ed. I.R. Falconer), pp. 297-315. Butterworths, London.

METZ, S.H.M. and VAN DEN BERGH, S.G. 1972. FEBS Lett. 21: 203-206.

METZ, S.H.M. and VAN DEN BERGH, S.G. 1977. Neth. J. Agric. Sci. 25: 198-211.

MILLER, E.L. 1973. Proc. Nutr. Soc. 32: 79-84.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD 1975. Technical Bulletin 33: Energy Allowances and Feeding Systems for Ruminants. HMSO, London.

MITCHELL, G.E.JR, LITTLE, C.O., KARR, M.R. and HAYES, B.W. 1967. J. Anim. Sci. 26: 224(Abstr.).

MOE, P.W., FLATT, W.P. and TYRRELL, H.F. 1972. J. Dairy Sci. 55: 945-958.

MOE, P.W., REID, J.T. and TYRRELL, H.F. 1965. J. Dairy Sci. 48: 1053-1061.

MOE, P.W. and TYRRELL, H.F. 1974. J. Dairy Sci. 58: 602-610.

MOE, P.W., TYRRELL, H.F. and FLATT, W.P. 1971. J. Dairy Sci. 54: 548-553.

MOORE, J.H. and CHRISTIE, W.W. 1979. Prog. Lipid Res. 17: 347-395.

MORRISON, I.M. 1972. J. Sci. Fd. Agric. 23: 455-463.

MYERS, L.L., JACKSON, H.D. and PACKETT, L.V. 1967. J. Anim. Sci. 26: 1450-1458.

NEAL, H.D.ST.C. and THORNLEY, J.H.M. 1983. J. agric. Sci., Camb. 101: 389-400.

O'KEEFE, E. and CUATRACASAS, P. 1974. Biochim. biophys. Acta 343: 64-77.

OKORIE, A.U., BUTTERY, P.J. and LEWIS, D. 1977. Proc. Nutr. Soc. 36: 38A(Abstr.).

OLDHAM, J.D. 1978. In Ruminant Digestion and Feed Evaluation (ed. D.F. Osbourn, D.E. Beever and D.J. Thomson), pp. 13.1. Agricultural Research Council, London.

OLDHAM, J.D. 1980. In Feeding Strategies for Dairy Cows (ed. W.H. Broster, C.L. Johnson and J.C. Tayler), pp. 7.1-7.19. Agricultural Research Council, London.

OLDHAM, J.D., HART, I.C. and BINES, J.A. 1978. Proc. Nutr. Soc. 37: 9A(Abstr.).

OLDHAM, J.D., HART, I.C. and BINES, J.A. 1982. Br. J. Nutr. 48: 543-547.

OPSTVEDT, J., BALDWIN, R.L. and RONNING, M. 1967. J. Dairy Sci. 50: 108-109.

ORSKOV, E.R. 1981. In Recent Developments in Ruminant Nutrition (eds W. Haresign and D.J.A. Cole), pp. 258-267. Butterworths, London.

ORSKOV, E.R., FLATT, W.P., MOE, P.W. and MUNSON, A.W., HEMKEN, R.W. and KATZ, I. 1969. Br. J. Nutr. 23: 443-453.

OSBOURN, D.F., BEEVER, D.E. and THOMSON, D.J. 1976. Proc. Nutr. Soc. 35: 191-200.

PALMQUIST, D.L. and CONRAD, H.R. 1971. J. Dairy Sci. 54: 1025-1033.

PALMQUIST, D.L., DAVIS, C.L., BROWN, R.E. and SACHAN, D.S. 1969. J. Dairy Sci. 52: 633-638.

PALMQUIST, D.L. and JENKINS, T.C. 1980. J. Dairy Sci. 63: 1-14.

PAYNE, J.M. 1966. Br. Vet. J. 122: 183-189.

PEARSON, R.M. and SMITH, J.A.B. 1943. Biochem. J. 37: 148-153.

PEEL, C.J., BAUMAN, D.E., GOREWIT, R.C. and SNIFFEN, C.J. 1981. J. Nutr. 111: 1662-1671.

PENNINGTON, R.J. 1952. Biochem. J. 51: 251-258.

PIPEROVA, L.S. and PEARCE, J. 1982. Int. J. Biochem. 14: 351-354.

POTHOVEN, M.A. and BEITZ, D.C. 1975. J. Nutr. 105: 1055-1061.

POTHOVEN, M.A., BEITZ, D.C. and ZIMMERLI, A. 1974. J. Nutr. 104: 430-433.

POUTIAINEN, E., TUORI, M. and SIRVIO, I. 1976. Proc. Nutr. Soc. 35: 140A(Abstr.).

PRIOR, R.L. 1978. J. Nutr. 108: 926-935.

PRIOR, R.L. and CHRISTENSON, R.K. 1978. J. Anim. Sci. 46: 201-210.

PRIOR, R.L. and SMITH, S. 1982. Fed. Proc. 40: 2545-2549.

REYNAERT, R., DE PAEPE, M., MARCUS, S. and PEETERS, G. 1975. J. Endocr. 66: 213-224.

ROOK, J.A.F. 1961. Dairy Sci. Abstr. 23: 251-258.

ROOK, J.A.F. 1976. In Principles of Cattle Production (ed. H. Swan, and W.H. Broster), pp. 221-236. Butterworths, London.

ROOK, J.A.F. and BALCH, C.C. 1961. Br. J. Nutr. 15: 361-369.

ROOK, J.A.F., BALCH, C.C. and JOHNSON, V.W. 1965. Br. J. Nutr. 19: 93-99.

ROOK, J.A.F. and HOPWOOD, J.B. 1970. J. Dairy Res. 37: 193-198.

ROOK, J.A.F., STORRY, J.E. and WHEELOCK, J.V. 1965. J. Dairy Sci. 48: 745-747.

ROOK, J.A.F. and THOMAS, P.C. 1983. In Nutritional Physiology of Farm Animals (ed. J.A.F. Rook and P.C. Thomas), pp. 314-368. Longman, London.

ROSS, J.P. and KITTS, W.D. 1973. J. Nutr. 103: 488-493.

ROTHERA, A.C.H. 1908. J. Physiol., Lond. xxxii, 491.

SALTER, D.N. and SMITH, R.H. 1977. Br. J. Nutr. 38: 207-216.

SARTIN, J.L., CUMMINS, K.A., KEMPPAINEN, R.J., MARPLE, D.N., RAHE, C.H. and WILLIAMS, J.C. 1985. Am. J. Physiol. 248: E108-E114.

SATTER, L.D. and ROFFLER, R.E. 1975. J. Dairy Sci. 58: 1219-1237.

SATTER, L.D. and ROFFLER, R.E. 1977. In Recent Advances in Animal Nutrition (ed. W. Haresign and D. Lewis), pp. 25-49. Butterworths, London.

SATTER, L.D. and SLYTER, L.L. 1974. Br. J. Nutr. 32: 199-208.

SCHAADT, H.Jr., JOHNSON, R.R. and McCLURE, K.E. 1966. J. Anim. Sci. 25: 73-77.

SCHEININ, A. and MAKINEN, K. 1975. Acta Odont-Scand. 33: Suppl. 70: I-XXI.

SCHULTZ, L.H. 1974. In Lactation: A Comprehensive Treatise, Vol. 2 (ed. B.L. Larson and V.R. Smith), pp. 317-353. Academic Press, New York.

SCHWALM, J.W. and SCHULTZ, L.H. 1976. J. Dairy Sci. 59: 255-261.

SCHWARTZ, H.M. and GILCHRIST, F.M.C. 1975. In Digestion and Metabolism in the Ruminant (ed. I.W. McDonald and A.C.I. Warner), pp. 165-179. University of New England Publishing Unit, Armidale, Australia.

SCOTT, T.W., BREADY, P.J., ROYAL, A.J. and COOK, L.J. 1972. Search 3: 170-171.

SCOTT, R.A. and PRIOR, R.L. 1977. Fed. Proc. 36: 1140(Abstr.).

SHIRLEY, J.E., EMERY, R.S., CONVEY, E.M. and OXENDER, W.D. 1973. J. Dairy Sci. 56: 569-574.

SHREEVE, W.W. 1974. Physiological Chemistry of Carbohydrates in Mammals. W.B. Saunders & Co., Philadelphia.

SIDDONS, R.C. 1968. Biochem. J. 108: 839-844.

SIDHU, K.S. and EMERY, R.S. 1972. J. Dairy Sci. 55: 926-930.

SLEE, M.D.R. 1980. Glucose Metabolism in Sheep Fed a Diet Supplemented with Polyhydric Alcohols. MSc Thesis, University of Aberdeen.

SMITH, R.H. 1975. In Digestion and Metabolism in the Ruminant (ed. I.W. McDonald and A.C.I. Warner), pp. 399-415. University of New England Publishing Unit, Armidale, Australia.

SMITH, G.H., CRABTREE, B. and SMITH, R.A. 1983. In Biochemistry of Lactation (ed. T.B. Mepham), pp. 121-140. Elsevier, Amsterdam.

SMITH, R.D., HANSEL, W. and COPPOCK, C.E. 1976. J. Dairy Sci. 59: 248-254.

SMITH, R.H. and MARSTON, H.R. 1971. Br. J. Nutr. 26: 41-53.

SMITH, R.M., OSBORNE-WHITE, W.S. and RUSSELL, G.R. 1969. Biochem. J. 112: 703-707.

SNEDECOR, G.W. and COCHRAN, W.G. 1980. Statistical Methods. 7th ed. Iowa State University Press, Iowa.

SNOSWELL, A.M. and HENDERSON, G.D. 1970. Biochem. J. 119: 59-65.

SOMERS, M. 1961. Aust. J. exp. Biol. Med. Sci. 39: 111, 123, 133, 145.

SOMERS, M. 1969. Aust. J. exp. Biol. Med. Sci. 47: 219-225.

SPITZ, I.M., RUBENSTEIN, A.H., BERSOHN, I. and BASSLER, K.H. 1970. Metabolism 19: 24-34.

STEIN, Y. and SHAPIRO, B. 1959. Am. J. Phys. 196: 1238-1241.

STEVENSON, A.E. and DE LANGEN, H. 1960. N.Z. J. agric. Res. 3: 314-319.

STORRY, J.E. 1981. In Recent Advances in Animal Nutrition - 1981 (ed. W. Haresign), pp. 3-34. Butterworths, London.

STORRY, J.E., BRUMBY, P.E., HALL, A.J. and JOHNSON, V.W. 1974. J. Dairy Res. 41: 165-173.

STORRY, J.E. and ROOK, J.A.F. 1965a. Br. J. Nutr. 19: 101-109.

STORRY, J.E. and ROOK, J.A.F. 1965b. Biochem. J. 96: 210-217.

STORRY, J.E. and ROOK, J.A.F. 1965c. Biochem. J. 97: 879-886.

STORRY, J.E. and ROOK, J.A.F. 1966. Br. J. Nutr. 20: 217-228.

STORRY, J.E. and SUTTON, J.D. 1969. Br. J. Nutr. 23: 511-521.

STRICKLAND, M.J. and BROSTER, W.H. 1981. J. agric. Sci., Camb. 96: 677-690.

SUTTON, J.D. 1976. In Principles of Cattle Production (ed. H. Swan and W.H. Broster), pp. 121-143. Butterworths, London.

SUTTON, J.D. 1981. In Recent Advances in Animal Nutrition - 1981 (ed. W. Haresign), pp. 35-48. Butterworths, London.

SUTTON, J.D. 1984. In Milk Compositional Quality and its Importance in Future Markets (eds M.E. Castle and R.G. Gunn), pp. 43-52. Occ. Publ. 9, British Society of Animal Production.

SUTTON, J.D., BROSTER, W.H., NAPPER, D.J. and SIVITTER, J.N. 1985. Br. J. Nutr. 53: 117-130.

SUTTON, J.D., HART, I.C. and BROSTER, W.H. 1982. In Energy Metabolism of Farm Animals (ed. A. Ekern and F. Sundstol), pp. 26-29. The Agricultural University of Norway.

SUTTON, J.D., OLDHAM, J.D. and HART, I.C. 1980. In Energy Metabolism (ed. L.E. Mount), pp. 303-306. Butterworths, London.

SUTTON, J.D. and MORANT, S.V. 1978. In Ruminant Digestion and Feed Evaluation (ed. D.F. Osbourn, D.E. Beever and D.J. Thomson), pp. 7.1-7.10. Agricultural Research Council, London.

SUTTON, J.D., STORRY, J.E. and NICHOLSON, J.W.G. 1970. J. Dairy Res. 37: 97-105.

SWAN, H. 1976. In Principles of Cattle Production (ed. H. Swan and W.H. Broster), pp. 85-102. Butterworths, London.

SYMONDS, H.W. and BAIRD, G.D. 1975. Br. vet. J. 131: 17-21.

THIVEND, P. 1973. Proc. Nutr. Soc. 33: 7A(Abstr.).

THOMAS, P.C. 1975. World Rev. Anim. Prod. 11: 33-44.

THOMAS, P.C. and CHAMBERLAIN, D.G. 1984. In Recent Advances in Animal Nutrition - 1984 (eds W. Haresign and D.J.A. Cole), pp. 219-243. Butterworths, London.

THOMAS, P.C. and CLAPPERTON, J.L. 1972. Proc. Nutr. Soc. 31: 165-170.

THOMAS, P.C., KELLY, N.C., CHAMBERLAIN, D.G. and CHALMERS, J.S. 1980. In Energy Metabolism (ed. L.E. Mount), pp. 357-362. Butterworths, London.

THOMAS, P.C. and ROOK, J.A.F. 1977. In Recent Advances in Animal Nutrition - 1977 (ed. W. Haresign and D. Lewis), pp. 83-109. Butterworths, London.

THOMAS, P.C. and ROOK, J.A.F. 1983. In Nutritional Physiology of Farm Animals (eds J.A.F. Rook and P.C. Thomas), pp. 558-624. Longman, London.

THOMPSON, G.E., BASSETT, J.M. and BELL, A.W. 1978. Br. J. Nutr. 39: 219-226.

THOMPSON, J.R., WEISER, G., SETO, K. and BLACK, A.L. 1975. J. Dairy Sci. 58: 362-370.

TINDAL, J.S., BLAKE, L.A., SIMMONDS, A.D., HART, I.C. and MIZUNO, H. 1982. Horm. Metab. Res. 14: 425-429.

TINDAL, J.S., KNAGGS, G.S., HART, I.C. and BLAKE, L.A. 1978. J. Endocr. 76: 333-346.

TOPPS, J.H., KAY, R.N.B. and GOODALL, E.D. 1968. Br. J. Nutr. 22: 261-280.

TRENKLE, A. 1971. Proc. Soc. exp. Biol. Med. 136: 51-55.

TRENKLE, A. 1978. J. Dairy Sci. 61: 281-193.

TRIGG, T.E., JURY, K.E., BRYANT, A.M. and PARR, C.R. 1980. In Energy Metabolism (ed. L.E. Mount), pp. 345-349. Butterworths, London.

TUORI, M. and POUTIAINEN, E. 1977. J. Scient. Agric. Soc. Finl. 49: 315-329.

TYRRELL, H.F. and MOE, P.W. 1975. J. Dairy Sci. 58: 1151-1163.

TYRRELL, H.F. and REID, J.T. 1965. J. Dairy Sci. 48: 1215-1223.

ULYATT, M.J., DELLOW, D.W., REID, C.S.W. and BAUCHOP, T. 1975. In Digestion and Metabolism in the Ruminant (ed. I.W. McDonald and A.C.I. Warner), pp. 119-133. University of New England Press, Armidale, Australia.

UNGER, R.H. 1971. Diabetes 20: 834-838.

VAN ES, A.J.H. 1976. In Principles of Cattle Production (ed. H. Swan and W.H. Broster), pp. 237-254. Butterworths, London.

VAN HORN, H.H., ZOMETA, C.A., WILCOX, C.J., MARSHALL, S.P. and HARRIS, B. 1979. J. Dairy Sci. 62: 1086-1093.

VAN ROTH, C. 1939. Vorratspf. Lebensmittelforsch. 2: 22.

VASILATOS, R. and WANGSNESS, P.J. 1979. J. Dairy Sci. 62: Suppl. 1: 230(Abstr.).

VERNON, R.G. 1978. Biochem. Soc. Trans. 6: 988-990.

VERNON, R.G. 1980. Prog. Lipid Res. 19: 23-106.

VERNON, R.G., CLEGG, R.A. and FLINT, D.J. 1981. Biochem. J. 200: 307-314.

WALDO, D.R. 1973. J. Anim. Sci. 37: 1062-1074.

WALLACE, A.L.C. and BASSETT, J.H. 1966. Metabolism 15: 95-97.

WANG, Y.-M. and VAN EYS, J. 1981. Ann. Rev. Nutr. 1: 437-475.

WARD, P.F.V., SCOTT, T.W. and DAWSON, R.M.C. 1964. Biochem. J. 92: 60-68.

WATSON, M.J., SAVAGE, G.P., BROWN, T. and ARMSTRONG, D.G. 1972. Proc. Nutr. Soc. 31: 99A(Abstr.).

WEEKES, T.E.C. and GODDEN, P.M.M. 1980. In Hormones and Metabolism in Ruminants (ed. J.M. Forbes and M.A. Lomax), pp. 99-111. Agricultural Research Council, London.

WEEKES, T.E.C. and WEBSTER, A.J.F. 1974. Proc. Nutr. Soc. 33: 71A(Abstr.).

WEIGAND, E., YOUNG, J.W. and McGILLIARD, A.D. 1972. Biochem. J. 126: 201-209.

WELLER, R.A. 1957. Aust. J. biol. Sci. 10: 384-389.

WELLER, R.A. and PILGRIM, A.F. 1974. Br. J. Nutr. 32: 341-351.

WELLER, R.A., PILGRIM, A.F. and GRAY, F.V. 1971. Br. J. Nutr. 26: 487-497.

WEST, C.E. and PASSEY, R.F. 1967. Biochem. J. 102: 58-64.

WESTON, R.H. and HOGAN, J.P. 1967. Aust. J. agric. Res. 18: 789-801.

WHEELOCK, J.V., ROOK, J.A.F. and DODD, F.H. 1965. J. Dairy Res. 32: 79-88.

WHITEHURST, G.B., BEITZ, D.C., POTHOVEN, M.A., ELLISON, W.R. and CRUMP, M.H. 1978. J. Nutr. 108: 1806-1811.

WILLIAMS, V.J. and CHRISTIAN, K.R. 1956. N.Z. J. Sci. Technol. 38: 403-406.

WILLIAMS, W.F., LEE, S.D., HEAD, H.H. and LYNCH, J.J. 1963. J. Dairy Sci. 46: 1405-1408.

WILLIAMSON, D.H. and MELLANBY, J. 1974. In Methods of Enzymatic Analysis (ed. H.U. Bergmeyer), 2nd ed., pp. 1836-1839. Academic Press, New York.

WILSON, G.F., ADEEB, N.N. and CAMPLING, R.C. 1973. J. agric. Sci., Camb. 80: 259-267.

WILTROUT, D.W. and SATTER, L.D. 1972. J. Dairy Sci. 55: 307-317.

WOLFF, J.E., BERGMAN, E.N. and WILLIAMS, H.H. 1972. Am. J. Physiol. 223: 438-446.

WOODS, H.F. and KREBS, H.A. 1973. Biochem. J. 134: 437-443.

WRETLIND, A. 1975. Nutr. Metab. 18(Suppl. 1): 246-255.

YAMAGATA, S., GOTO, Y., OHNEDA, A., ANZAI, M., KAWASHIMA, S., CHIBA, M., MARUHAMA, Y. and YAMAUCHI, Y. 1965. Lancet, 2: 918-921.

YANG, Y.T. and BALDWIN, R.L. 1973. J. Dairy Sci. 56: 350-365.

ZAMMIT, V.A. 1981. Trends Biochem. Sci. 6: 46-49.