Blood Composition of Ruminants in Relation to Time and Level of Feeding

by

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ABSTRACT

Interest has recently been generated as to the possibility of being able to monitor the nutritional status of ruminants from their blood composition. Such a technique would be useful to both veterinarian and agriculturalist in their function of advising farmers on how to maximize farm profits in animal-orientated enterprises.

A number of blood constituents have been proposed as suitable parameters of the nutritional status of ruminants, but it is not known whether such indices will be satisfactory under the nutritional conditions which prevail in UK agriculture. Consequently lactating beef cows were subjected to dietary deficiencies of energy, and pregnant and lactating ewes to deficiencies of energy, protein and the major minerals. Blood samples were taken from the animals at different times of day and at different stages of pregnancy and lactation.

Level of nutrition was not a significant source of variation in the plasma concentrations of the proposed indicators of energy status (glucose, free fatty acids and ketone bodies), of long-term protein status (albumin and globulin) and of mineral status (calcium and phosphorus). The concentrations of urea in plasma increased with the amount of nitrogen absorbed in excess of requirements.

Pronounced diurnal variations were observed in the plasma concentrations of glucose, free fatty acids, ketone bodies and urea. The concentrations of free fatty acids increased before and decreased after a feed but the patterns were not constant for the other constituents, varying considerably both between [illegible]

experiments, and within experiments with changing physiological state of the animals.

It is concluded that apart from urea, the chosen parameters would be of little value in predicting the adequacy of a diet for a particular class of animal, and the search must continue for more suitable blood indices of nutritional status.

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CHAPTER 1

INTRODUCTION

The economic implications of sub-standard performance in animal production and the role played by undernutrition in such cases are well known. It is, therefore, necessary to assess the adequacy of rations in supplying essential nutrients so that undernutrition will not occur. This to some extent will also help to reduce the incidence of certain deficiency and metabolic diseases such as hypocalcaemia, hypomagnesaemia, ovine pregnancy toxaemia and bovine ketosis. The assessment of nutritional status is becoming increasingly important as farmers attempt to maintain profitabilities by either reducing feed costs and maintaining production (as in many beef herds), by maintaining feed costs and increasing overall production (e.g. in dairy herds reducing the time periods between lactations), or by combinations of these procedures.

For animals consuming fixed amounts of rations of known compositions, nutritional adequacy can be calculated by comparing the intake of individual nutrients with the estimated requirements for those nutrients. However, the level and composition of the food intake of individual animals (particularly grazing animals) is often unknown, so that intake/requirement ratios cannot be directly determined. Here, indirect measurements of nutritional adequacy must be used.

Many of the nutritional criteria for ruminants which have been used in the past (e.g. weight changes, reproductive performance, level of

milk or wool production) have limitations in relation to specific nutritional disorders: they do not give estimates of the production-limiting nutrient, nor of the extent of the imbalance. There is a requirement for criteria that are closely associated with metabolic processes, that can be easily obtained from animals and that can be measured quickly and cheaply.

Studies on naturally occurring nutritional deficiencies and excesses have shown that such imbalances are almost invariably associated with abnormal levels of specific constituents in body tissues and fluids. Thus, by measuring the levels (or changes in levels) of certain body constituents, it may be possible to detect changes in the type or rate of biochemical processes related to growth or to productivity, and hence to assess nutritional status.

The choice of tissue or fluid for analysis varies with the nutrient in question. Blood, milk, faeces, urine and wool have obvious advantages, because of their accessibility without slaughter of the animal. Body tissue sampling may present difficulties, although kidney, liver and tailbone biopsy techniques are available. Whole blood, or more commonly, blood serum or plasma is more widely used for biochemical investigations than any other tissue or fluid of the body.

Although quantitative measurements of particular blood components may yield information of considerable value, the composition of the blood is but a limited indicator of the metabolic status of tissues at the cellular level. Many cellular products do not appear to a detectable

degree in the circulation, and there are few instances of simple linear relationships between tissue and plasma concentrations of a given constituent (White, Handler & Smith, 1968).

The metabolic profile test

The metabolic profile test (Payne, et al, 1970) was designed to assess the nutritional status of dairy herds, using as indices the concentrations of those blood constituents considered to be of importance in the normal functioning, production and reproduction of the dairy cow. Blood constituents used include glucose (energy status), total protein, albumin, globulin, urea, haemoglobin (protein status), sodium, potassium (electrolyte status), calcium, inorganic phosphate, magnesium, copper (mineral status) and haematocrit.

The test is a between-herd comparison of blood chemistry. Blood samples are taken from 21 cows within a herd (7 high-yielding, 7 medium-yielding and 7 dry) and the mean concentrations of the constituents in each group compared with "normal" values. The latter set of figures are the mean constituent concentrations ($\pm$2S.D.) in blood samples obtained from all cows in 13 dairy herds of known high nutritional and management standards (Rowlands & Pocock, 1971). Mean group values lying outside these 95% confidence limits are classed as abnormal, and are thought to reflect imbalances in the intake/requirements ratio of one or more nutrients.

In metabolic profile testing both between- and within-herd variations in blood chemistry are attributed entirely to nutrition, despite the fact

that in the UK there is only limited evidence that nutrient deficiencies have measurable effects on blood composition. The only other source of variation in blood composition which has been incorporated into the test is lactation, although several other sources (e.g. age, season) have been identified (Payne, et al, 1974; Rowlands, et al, 1974; Kithchenham, Rowlands & Shorbagi, 1975). For meaningful between-farm comparisons of blood chemistry the non-nutritional variables which affect blood composition on a within-farm basis should be standardized: the omission of such standardization contitutes a serious criticism of the metabolic profile concept.

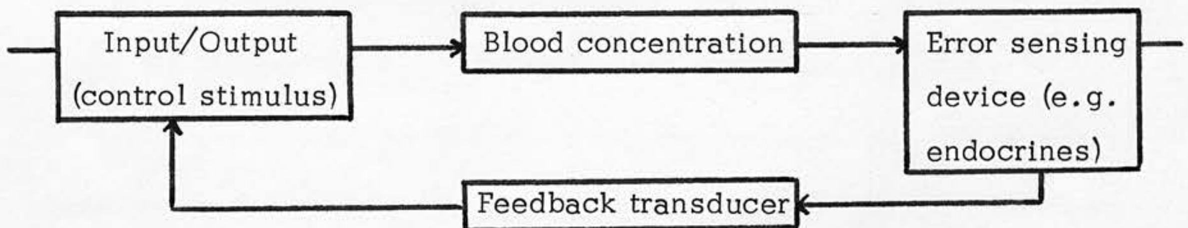
In human medicine, where the equivalent of metabolic profile testing has been used clinically for several years, it is now generally accepted (Robinson, 1971) that reputable normal values, reference points and valid conclusions cannot be established nor drawn from a small number of supposedly healthy individuals. Due regard must be taken of a number of factors, some more controllable than others, that influence biological variation. These factors include genotype, age, sex, stage of breeding cycle, time of day, season, sampling technique, storage of sample, method of analysis and standard of analytical performance (Roil, Suckling & Mattingley, 1974).

A discussion follows of some of the homeostatic mechanisms involved in the regulation of blood composition. This is in turn followed by an examination of some of the nutritional, non-nutritional and technical sources of variation in ruminant blood composition.

Homeostasis of blood constituent concentrations

Homeostasis, the maintenance of a prescribed internal environment in the body of an organism in spite of wide fluctuations in its activity and external environment, is maintained in most biological systems by negative feedback systems. In such mechanisms, a servo-system is actuated by an error signal (the error is the difference between the desired and actual output) and this causes an action which will reduce the error.

This can be represented schematically as a closed cycle system:



The efficiency of the homeostatic mechanisms involved in maintaining blood constituent concentrations varies from highly sophisticated (e.g. calcium) to extremely poor (e.g. urea).

(a) Energy parameters

It is important here to distinguish between caloric homeostasis and the homeostasis of blood glucose (GLUC) concentrations. The ruminant has a general requirement for energy and a specific requirement for GLUC, the requirement for GLUC increasing during pregnancy (Reid, 1968) and lactation (Linzell, 1968).

Plasma GLUC concentrations are maintained by a negative feedback system where the supply of precursors for hepatic gluconeogenesis

(primarily propionate and amino acids) is under close hormonal control. The most important hormones involved are insulin and glucagon (both secreted in the pancreas) and the catecholamines (produced in the adrenal medulla). Insulin promotes storage of fat, and stimulates GLUC uptake by the fatty tissues as well as glycogenesis. Glucagon is antagonistic to insulin in that it promotes the hepatic release of GLUC in hypoglycaemic states, resulting in a rise in blood GLUC concentrations. The catecholamines stimulate glycogenolysis in the liver, resulting in short-lived rises in the blood GLUC level. Catecholamines also have a lipolytic action in adipose tissue.

The above hormonal adjustments are rapid; if a GLUC shortage persists, then more long-term adjustments are necessary. Such actions appear to be brought about by the pituitary, which causes an increased glucocorticoid secretion by the adrenal cortex. Glucocorticoids inhibit the utilization of GLUC, so that the body is able to conserve GLUC and rely on other energy sources (Bergman, 1973).

Caloric homeostasis is controlled by the rate of mobilization/ deposition of fat reserves (the hormonal control of which is described above). The transport form of depot fat is as free (or non-esterified) fatty acids (FFA), which are oxidized by the liver and by the tissues to produce acetyl-CoA. The latter is used in the energy-producing tricarboxylic acid cycle (Reid, 1968), although some is diverted to the synthesis of ketone bodies (acetone, acetoacetate and β-hydroxybutyrate). Normally ketone bodies (KB) act as alternative energy sources (Krebs, 1961), but in severe energy undernutrition the oxidation of FFA

is incomplete and both FFA and KB accumulate in the blood (along with reduced GLUC concentrations).

Hypoglycaemic ketosis may develop without necessarily involving a quantitative caloric deficiency (Adler, 1970), being associated with an increased ratio of ketogenic to glucogenic metabolites available for body maintenance after the GLUC requirements for pregnancy and/or lactation have been satisfied. In such a specific GLUC shortage, despite an adequate caloric intake, the plasma FFA levels would remain normal (with elevated KB and reduced GLUC levels). Should an energy deficiency ensue, the FFA levels would increase as a result of fat mobilization in attempts to maintain caloric homeostasis.

(b) Protein parameters

It is not the aim here to examine the homeostatic mechanisms of all the plasma proteins, but rather to make a brief examination of those mechanisms as related to the metabolism of a single protein - albumin (ALB).

The amount of circulating ALB is the complex end result of synthesis, degradation and distribution: of these the regulation of synthesis, the sites of degradation and the regulation of distribution are unknown (Rothschild, Oratz & Schreiber, 1970).

Nitrogen (N) intake is the most important aspect in the regulation of ALB synthesis, short-term fasts rapidly reducing the amount of ALB synthesized. A regulatory system based on the osmotic pressure of the plasma has also been postulated (a lowering of serum ALB is seen in disease states, characterized by elevations in the γ-globulin fraction, where the colloid content of the body fluids are altered).

Excess thyroid hormones and cortisone stimulate ALB synthesis, and increase ALB degradation rates. There may then be hormonal control of protein homeostasis (Rothschild et al, 1970).

The absolute degradation rates of ALB appear to increase through increased fractional degradation rates. While synthetic rates are acutely affected by a decrease in food intake, the degradation rate only falls after the ALB pool has decreased. Overproduction of ALB is readily compensated by increased degradation (Hoffenberg, 1970). In situations where gastrointestinal or renal losses of ALB are excessive (as in intestinal parasitism and nephrosis), the absolute endogenous rate of degradation is frequently depressed, perhaps in an attempt to prevent further losses of ALB.

The existence of a large extravascular pool of ALB may, in states of plasma hypoalbuminaemia, provide the potential for a transfer of ALB into the intravascular pool. The existence of such a transfer mechanism has yet to be demonstrated, although changes in ALB distribution in humans have been observed in burns, nephrosis and cirrhosis (Rothschild et al, 1970).

Plasma urea (UN) concentrations are determined simply by the difference between the rates of synthesis and degradation: there are no homeostatic mechanisms as such. A model of ovine N metabolism showing the movement between the various N pools was described by Nolan & Leng (1972).

Urea is synthesized in the liver: the synthetic rate is determined

by the availability of ammonia. This ammonia may be derived from the deamination of amino acids (of tissue or intestinal origin), from the breakdown of other tissue compounds, or from rumenal and intestinal absorption. Gut-absorbed ammonia is derived from the fermentation of dietary components and recycled amino acids, and also from the hydrolysis of urea transferred to the gut from the blood (Harrop & Phillipson, 1974). Urea is degraded only in the digestive tract, probably both in the rumen and in the lower intestines (Thornton et al, 1970).

The recycling of UN from the blood to the digestive tract is enhanced during pregnancy (Nolan & Leng, 1970) and is affected by the concentration of the dietary energy intake (Guada, Robinson & Fraser, 1975). The recycling may prevent the accurate prediction of ruminant N status from plasma UN concentrations, particularly in pregnant animals, and in energy and protein deficiencies. Nevertheless, N intake and plasma UN concentrations have been positively correlated, both in growing lambs (Preston, Schnakenberg & Pfander, 1965) and in lactating cows (Prewitt et al, 1971).

(c) Mineral parameters

There are 2 substances responsible for the control of calcium (Ca) and inorganic phosphate (P) levels in extracellular fluid (ECF): parathyroid hormone (PTH) and calcitonin (Simesen, 1970). In the presence of Vitamin D, PTH has a direct action on bone, causing breakdown of the matrix resulting in increased blood Ca and P concentrations. The other site of action is the renal tubules, where

PTH promotes P excretion and stimulates reabsorption of Ca. There are also indications that PTH maintains the blood Ca level by stimulating intestinal Ca absorption (Simesen, 1970).

Calcitonin, the secretory product of the ultimobranchial glands (Copp, 1969), is thought to block bone Ca resorption (or possibly to stimulate bone mineralization) induced by PTH, and thus to lower the blood Ca concentration. Calcitonin has also been reported to resemble PTH, in promoting renal excretion of P.

There are thus two negative feedback systems operating in the control of blood Ca: the effect of blood Ca level on hormone secretion (either PTH or calcitonin), and the subsequent action of the hormones on the net release of Ca from bone. Copp (1969) has described a model of Ca metabolism embracing hormonal and non-hormonal homeostatic mechanisms.

The homeostasis of blood P is more complicated than that of blood Ca, since not only is blood P in equilibrium with bone P (and thus markedly affected by Ca metabolism), but it is also in equilibrium with a large number of organic compounds produced as the result of cellular activity. Anderson et al (1973) gave a speculative model of P metabolism in animals with normal Ca metabolism.

Little is known regarding the factors involved in the regulation of serum magnesium (Mg) concentrations. Blaxter & McGill (1956) suggested that Mg metabolism was regulated by a dynamic equilibrium, in which the skeleton acted as a labile source of Mg. In immature animals, the entire skeleton may act as a Mg reservoir, whereas in the

adult a very large part of the skeleton is thought to be inert. Magnesium homeostasis in the adult may then be the result of a balance between intake from the intestines and renal excretion. It has been demonstrated that in hypomagnesaemic states, Mg homeostasis is critically dependent on the daily Mg intake (Simesen, 1970).

This theory has been refuted by recent work (Gardner, 1973) which has shown that in the pregnant ewe there is an osseous reserve of Mg, available when required for mobilization into ECF. Gardner (1973) showed that no exhaustion of this reservoir occurred with the removal (using dialysis of blood and of the peritoneum) of 5-10% of the whole body Mg.

Nutritional sources of variation in ruminant blood composition

(i) Level of food intake

(a) Energy parameters

Relationships between levels of energy intake and blood concentrations of the energy parameters (GLUC, FFA, KB) have been examined by many workers using both sheep (Annison, 1960; Reid & Hinks, 1962a; Russel, Doney & Reid, 1967; Russel & Doney, 1969; Sykes & Field, 1972b) and cattle (Radloff, Schultz & Hoekstra, 1966; Holmes & Lambourne, 1970; Fisher, Erfle & Sauer, 1971; Hove & Blom, 1973). These studies have shown conclusively that, in animals with high GLUC requirements, reducing the level of food intake causes increased plasma concentrations of FFA and KB, with decreased GLUC levels.

(b) Protein parameters

Despite the difficulties in interpreting plasma ALB concentrations, Sykes & Field (1973) concluded that the concentrations of this particular blood constituent may be a useful index of protein status. There is evidence in both cattle (Payne et al, 1970) and sheep (Sykes & Field, 1973) to suggest that globulins (GLOB) are unaffected by level of nutrition, although Sykes & Field (1973) reported that concentrations of transferrin, the β -globulin associated with iron transport (McFarlane et al, 1969), were clearly differentiated according to the protein status of pregnant sheep.

As mentioned above, significant correlations have been calculated between N intake and blood UN concentrations (Preston et al, 1965; Prewitt et al, 1971).

(c) Mineral parameters

The physiological responses to simple dietary deficiencies of Ca and P have been well known for many years: a fall in the plasma concentrations of the deficient element, an increase in the plasma concentrations of the other, and a withdrawal of both elements from skeletal reserves (Theiler & Green, 1932; Fraser, Godden & Thomson, 1933). These findings were confirmed in recent work (Chicco et al, 1973; Field, Suttle & Nisbet, 1975). The rate of decline in the plasma concentrations of the minerals depends on the degree of dietary deficiency encountered, the mineral requirements of the animal and the relative concentrations of the minerals in the diet (Underwood, 1966).

The plasma Mg concentration is a sensitive measure of Mg status (Blaxter & Rook, 1954), responding rapidly to Mg deficiencies (Swan & Jamieson, 1956). However, once low, the plasma Mg concentrations give no further information on the state of the Mg reserves, the extent of which was considered by Gardner (1973) to be unknown.

(d) Haematocrit

There are conflicting views on the effect of level of food intake on blood haematocrit (PCV). Holmes & Lambourne (1970) reported PCV to vary inversely with the level of energy intake of Hereford heifers, but in pregnant beef cattle Lister *et al* (1973) found that depressing the level of intake to 50% of the *ad libitum* intake also depressed the PCV level.

(ii) Composition of diet

(a) Energy parameters

Annison (1960) found the starch content of the diet offered to sheep influenced the response of the fasting FFA levels to feeding, concluding that the nature of the response was related to the more rapid fermentation of high-starch diets in the rumen. In dairy cows Varman & Schultz (1968) observed that when the ratio of grain to alfalfa hay was increased sufficiently to depress the fat content of the milk, the plasma FFA concentrations decreased. This finding

was confirmed by Holmes & Lambourne (1970), who showed that the plasma FFA concentrations of beef cattle were markedly affected by the amount of grain reaching the small intestine.

The increased production of KB in cattle consuming silage (noted by Knodt, Shaw & White, 1942) is thought to be due to the ingestion of dietary volatile fatty acids (particularly butyric acid) and conversion of ingested butyrate to β-hydroxybutyrate in the ruminal epithelium (Kronfeld, 1972).

(b) Protein parameters

Abou Akkada & el Sayed Osman (1967) observed different ruminal ammonia and blood UN concentrations in animals fed similar N intakes from two different rations (leguminous and non-leguminous), and the discrepancies were attributed to substantially different ruminal fermentation patterns. The authors suggested that N intake may not be a major factor in the control of ruminal ammonia and blood UN levels (a suggestion also made by Lewis, in 1957).

(iii) Time of feeding

In animals fed once per day there is an uneven distribution throughout the day in the supply of nutrients to the tissues, and hence a diurnal variation in blood composition. The buffering effect of the rumen may modify the extent of the diurnal variation, this being less for those constituents (or their precursors) absorbed lower down the gastrointestinal tract (e.g. P, absorbed in the small intestine) than for those absorbed in

the rumen (e.g. propionic acid). The extent of the variations probably diminish with the frequency of feeding: they are probably less in grazing animals, or in animals on ad libitum intakes, than in animals fed once per day.

(a) Energy parameters

There is some disagreement as to the nature of the postprandial response in plasma GLUC levels. Reid (1950) found no evidence of prandial variation in ovine blood sugar concentrations, but in later work (Reid & Hinks, 1962b) it was shown that blood GLUC levels may increase by 10-20 mg/100ml after feeding, particularly in sheep consuming their ration quickly. In sheep consuming a single daily feed, Bassett (1974b) described an initial decrease in plasma GLUC concentrations after feeding, followed by steady rises to maximum concentrations 6-8 h later. Essentially the same results were described by Ambo, Takahashi & Tsuda (1973).

Postprandial increases in bovine plasma GLUC concentrations have been reported by several workers (Allcroft, 1933; Holmes & Lambourne, 1970; Hagemeister & Unshelm, 1970; Bowden, 1973), all reporting maximum concentrations at different times after feeding. In direct contrast, Preston & Ndumbe (1961) using calves, and Radloff et al (1966) and Hove & Blom (1973) using high-yielding dairy cattle, described marked postprandial decreases in plasma GLUC concentrations. Halse (cited by Hove, 1974) stated that the downward trend in blood sugar levels after feeding was a

common finding in lactating cows.

There is good agreement on the nature of the postprandial response in the concentrations of FFA and KB: a decrease and an increase respectively (Annison, 1960; Reid & Hinks, 1962b; Hartmann & Lascelles, 1965; Kronfeld, 1965; Slee & Halliday, 1968). Even in cattle fed 6 times per day, there is a fall in plasma FFA concentration following each feed (Holmes & Lambourne, 1970).

(b) Protein parameters

Most of the very small (circa 2%) diurnal variation in plasma total protein (TP) concentrations has been shown (Unshelm, 1969) to be due to postprandial increases in plasma ALB concentrations; GLOB levels remaining constant during the day.

In contrast the plasma UN concentration is known to exhibit considerable diurnal variation, resulting from changes in the ruminal ammonia concentration (Lewis, 1957). There is some uncertainty as to the exact nature of the postprandial response. In Hereford steers, fed on a basal ration supplemented by varying amounts of urea, Thornton (1970a) reported marked postprandial elevations in plasma UN concentrations. The pattern of the changes, the time of maximum concentration and the pre-feeding concentrations were all affected by the level of supplementation. Packett & Groves (1965) reported declining plasma UN levels in the post-feeding period, while Thornton (1970b) noted marked depressions in ovine

plasma UN concentrations after the intravenous infusion of urea solutions of varying strengths.

(c) Mineral parameters

Plasma mineral concentrations in the bovine show little prandial variation (Ehrentraut, Seidel & Bär, 1970); Ca and Mg increasing and P decreasing, following feeding (Unshelm & Rappen, 1968). Ternmouth (1968) described small increases in ovine plasma P levels following feeding.

(d) Haematocrit

Both Christopherson & Webster (1972) and Dooley & Williams (1975a) reported marked postprandial rises in ovine PCV, changes attributed by Dooley & Williams (1975a) to splenal contractions. Unshelm (1968) found small postprandial decreases in bovine PCV, while Bowden (1973) reported that the PCV of beef heifers increased between 1 and 24h after feeding.

Non-nutritional sources of variation in ruminant blood composition

(i) Pregnancy and lactation

Both these physiological states influence blood composition, although studies on the extent of the influences *per se* are rare. The marked changes in plasma volume that occur complicate such studies, as does the fact that cattle may be both pregnant and lactating at the same time.

(a) Energy parameters

The maintenance of caloric homeostasis in pregnant or lactating animals is complicated by the high GLUC requirements of the foetus and mammary gland. Reid & Hinks (1962a) considered that ovine blood GLUC and FFA levels were directly related to the foetal load and to the level of milk production.

There are several reports of pregnancy causing lowered levels of plasma GLUC, and higher levels of FFA and KB (Reid & Hinks, 1962a; Ross & Kitts, 1969; Karihaloo, Webster & Combs, 1970; More, Munshi & Chattopadhyay, 1973). Adler & Lotan (1967) reported high serum FFA levels, and low blood GLUC levels, in ewes with high daily levels of milk production. Rowlands et al (1975) stated that bovine blood GLUC concentrations were lowest during early lactation, while Holmes & Lambourne (1970) reported significantly higher FFA levels in lactating cows than in non-lactating heifers.

(b) Protein parameters

The decreases in ovine ALB and GLOB levels during pregnancy are well documented (Dunlap & Dickson, 1955; Hjelle, 1967; Sykes & Field, 1973). As ALB serves as the major amino acid pool, it is possible that this pool is depleted through an increased requirement for protein precursors during pregnancy (Dimopoullos, 1970).

Sykes & Field (1973) partially attributed the above changes to increases in plasma volume (Barcroft, Kennedy & Mason, 1939). A. R. Sykes (unpublished) has demonstrated that ovine plasma volume

increases at a linear rate from the 15th week of pregnancy, so that unless further protein can be added to the plasma pool, a reduction in plasma protein concentrations of up to 30% must occur as the result of a simple dilution effect (Sykes & Field, 1973).

Additional falls in the plasma GLOB concentrations may occur in late pregnancy as the result of the transfer of β - and γ - globulins to the mammary gland for colostral antibody formation (Pierce & Feinstein, 1965).

In cattle there is little change in the plasma concentrations of ALB or α-globulin throughout pregnancy (Dimopoullos, 1970), while γ-globulin levels decrease markedly in the later stages of pregnancy (Hamana & Usui, 1972; McLennan & Willoughby, 1973).

Although Fell et al (1968) stated that ovine serum ALB concentrations were unaffected by lactation, Mackie & Fell (1971) described significantly shorter ALB half-lives in lactating sheep than in non-lactating controls, a finding associated with post-partum increases in plasma volume. Fell et al (1968) reported that the plasma γ-globulin concentration in sheep increased from the end of pregnancy into the post-weaning period, a rise thought to be due to a compensation (and eventual over-compensation) for the hypogammaglobulinaemia in late pregnancy (Mackie, 1972).

McLennan & Willoughby (1973) reported a tendency of dairy cattle to maintain reduced serum ALB concentrations shortly after parturition, a finding confirmed by Little (1974) and Rowlands et al (1975). Little (1974) reported a significant, positive relationship between bovine serum ALB concentration and the stage of lactation during

the first 120 days post-partum.

In ewes on adequate energy intakes Robinson, Scott & Fraser (1973) described a decrease in plasma UN concentrations associated with pregnancy. These workers concluded that this improved efficiency of protein utilization was the direct result of an increased N requirement for fetal growth.

(c) Mineral parameters

There is disagreement as to the effect of pregnancy on bovine P concentrations. Blood P levels have been shown to increase (Lane, Campbell & Krause, 1968), and serum levels to decrease (Seidel & Schröter, 1970; Hewett, 1974), with advancing pregnancy.

Payne & Leech (1964) reported a marked effect of lactation on plasma P levels in dairy cows; while Lane et al (1968) found blood levels of Ca, Mg and P to be directly affected by the stage of lactation. Concentrations of Mg in lactating dairy cows are often higher than levels in equivalent, non-lactating animals (Payne et al, 1973, 1974; Rowlands et al, 1975).

Ovine plasma copper (Cu) levels fall during pregnancy and rise at parturition (Howell, Edington & Ewbank, 1968).

(d) Haematocrit

Lane & Campbell (1969) reported significant effects of pregnancy and lactation on bovine PCV, the highest values being observed in the latter half of pregnancy.

(ii) Season

Seasonal variation in ruminant blood composition is probably caused by a combination of several factors (Rowlands et al, 1975). These include nutrition (usually poorer in autumn and winter), climate (increased maintenance requirements under adverse weather conditions) and pregnancy and lactation.

(a) Energy parameters

Payne et al (1973, 1974) reported seasonal variations in the blood GLUC concentrations of dairy cows, levels being higher in winter than in summer. Leat (1974) found ovine plasma FFA levels to be lowest during winter and spring, and highest during summer and autumn.

(b) Protein parameters

Sykes & Field (1974) described seasonal changes in the plasma ALB, GLOB and UN concentrations of free-grazing hill sheep. Lowest levels of all 3 constituents were observed in mid- and late winter. Highest plasma protein concentrations were seen in late summer, somewhat later than the equivalent maximum UN concentrations. The changes were attributed by Sykes & Field (1974) to seasonal variations in protein intake.

In dairy cows, Payne et al (1973, 1974) and Rowlands et al (1975) reported that serum ALB and UN concentrations were higher in summer than in winter, changes attributed by the authors to fluctuations in protein intake.

(c) Mineral parameters

Sykes & Field (1974) described seasonal variations in the plasma mineral concentrations of hill sheep: Ca decreased, and P increased, over the period November-April. Wiener & Field (1974) described a steady decline in ovine plasma Cu levels from November until the following June.

There is little seasonal variation in bovine blood mineral concentration (Payne et al, 1973, 1974; Rowlands et al, 1974). The largest such variation is probably that of Mg, levels of which are highest in June and lowest in September (Rowlands et al, 1974).

(d) Haematocrit

Rowlands et al (1974) reported an effect of season on the bovine PCV, higher values being observed in summer than in winter.

(iii) Stress

The stress involved in the restraint, venipuncture and unfamiliar environment of experimental animals is known to influence blood composition (Gartner, Ryley & Beattie, 1965; McNatty, Cashmore & Young, 1972; Pearson & Mellor; 1976).

(a) Energy parameters

The elevation of ruminant plasma GLUC concentrations under stressed conditions is well known. Reid & Mills (1962) reported pronounced, but variable, increases in the blood GLUC levels of animals exposed to various forms of stress. May, Marschang &

Aciocirlanoaie (1974) attributed elevations in bovine blood sugar levels at venipuncture to transient stresses caused by previous venipunctures. These authors concluded that this effect of stress was of weak intensity and short duration.

Excitement causes elevated plasma FFA levels (Kronfeld, 1965; Slee & Halliday, 1968; Holmes & Lambourne, 1970), the extent of such elevations diminishing with repeated handling and blood sampling. Patterson (1963) observed that when taking 2 consecutive blood samples from the same animal, the stress involved in taking the first caused elevated FFA levels in the second.

(b) Protein parameters

Using indwelling catheters, Gartner et al (1965) reported that the resting levels of the circulating proteins were significantly lower than levels obtained at venipuncture. Ovine TP and GLOB concentrations are unaffected by the frequency of animal handling (Gartner et al, 1970), and are highly repeatable (Healy & Falk, 1974).

(c) Mineral parameters

In beef cattle, Gartner et al (1965) reported that both blood and plasma P concentrations were elevated during stress, and that there appeared to be a direct relationship between the degree of stress and the plasma P level. Gartner et al (1969) stated that there was no consistent effect of stress on bovine plasma P concentration, although Gartner et al (1970) observed a positive relationship between stress and ovine plasma P concentration.

The well-established link between stress and ovine hypocalcaemia was closely examined by Moseley & Axford (1973), who reported reduced plasma Ca levels when animals were stressed. These workers attributed the hypocalcaemias to the movement of Ca from the plasma into adipose tissue, concurrent with a stress-induced fat mobilization.

Stress related increases (up to 30%) in ovine plasma Cu levels following minor surgical operations were noted by Suttle, MacRae & Mitchell (unpublished).

(d) Haematocrit

Gartner et al (1965) demonstrated an effect of stress on bovine PCV levels: when stressed, the only animals which showed no significant changes in PCV levels were those which had been splenectomized and tranquillized. Increases in PCV levels were thus partially attributed to splenal contractions, a subject studied more closely by Dooley, Hecker & Webster (1972) and by Dooley & Williams (1975b).

(iv) Age

(a) Energy parameters

Using the results of several metabolic profile tests in dairy cattle of known ages, Kitchenham et al (1975) demonstrated positive effects of age on blood GLUC concentrations.

(b) Protein parameters

The increase in bovine serum TP concentrations with age is well documented (Larson & Touchberry, 1959; Mylrea & Healy, 1968). Tumbleson, Burks & Wingfield (1973) described linear increases with age in bovine TP concentrations, stating that the γ-globulin fraction was responsible for the change. Kitchenham et al (1975) described significant age effects for ALB (-ve), GLOB (+ve) and UN (-ve).

(c) Mineral parameters

Payne & Leech (1964) stated that in dairy cows the plasma Ca and P concentrations decline with advancing age. Similar Ca (Vrzgula, 1963; Tumbleson et al, 1973) and P (Gartner et al, 1966; Mylrea & Bayfield, 1968; Lane et al, 1968) relationships have been noted elsewhere. Both positive (Kitchenham et al, 1975) and negative (Gartner et al, 1966) relationships have been reported between age and bovine serum Mg concentrations.

Hayter, Wiener & Field (1973) reported higher levels of plasma Cu in young lambs than in adult sheep, a finding also reported by Suttle (1975).

(d) Haematocrit

Lane & Campbell (1969) and Kitchenham et al (1975) demonstrated significant, positive effects between age and bovine PCV.

(v) Breed

(a) Energy parameters

Significant breed differences in ovine plasma FFA concentrations have been reported by several workers (Halliday et al, 1969; Russel & Doney, 1969). The latter set of workers attributed the effects to differences between the breeds in their maintenance requirements.

Bowden (1973) reported an effect of breed on bovine GLUC levels; the latter have also been considered (Heyns, 1971; Olbrich et al, 1971) to be affected by species (Bos taurus v. Bos indicus).

(b) Protein parameters

Perk & Lobl (1959) reported higher levels of γ-globulin, and lower ALB levels, in Holstein-Friesian cattle than in equivalent Damascene cattle. Olbrich et al (1971) reported that Scottish Highland cattle maintained higher levels of TP, and lower UN levels, than equivalent Zebu cattle.

(c) Mineral parameters

Using a single sampling of 2 flocks of sheep, Wiener & Field (1971) showed significant breed variations for blood and plasma Ca, P, Mg and Cu concentrations. The contribution of breed (or cross breed) to the total variation was less than 10% for plasma Ca and blood P concentrations. Wiener, Field & Wood (1969) reported that breeds differ significantly in their blood Cu concentrations, with the Scottish Blackface having consistently the lowest and the Welsh

Mountain the highest values.

Heyns (1971) reported higher concentrations of Cu, and lower P concentrations, in Afrikaaner cattle than in Friesland cattle.

(d) Haematocrit

Both Olbrich et al (1971) and Bowden (1973) described effects of breed on bovine PCV, the former finding higher levels in Zebus than in Highland cattle, and the latter reporting higher levels in Angus cattle than in Herefords.

(vi) Climate

(a) Energy parameters

Reid (1962) found ovine blood GLUC concentrations to increase with climatic stress, and Patterson (1963) reported increases in ovine and bovine plasma FFA levels that were apparently due to adverse weather conditions. Slee & Halliday (1968) observed large increases in the plasma FFA concentrations of sheep exposed to severe cold, although mild exposure (3-8°C below the critical temperature, for periods of several days) produced little change.

Halliday et al (1969) found ovine serum protein levels to fall during acute cold exposure; changes attributed by the authors to an increased protein catabolism.

(c) Mineral parameters

Inclement weather has been implicated in certain mineral

deficiency states (Allcroft, 1947; Inglis, Weipers & Pearce, 1959), although in these studies the effect of climate on mineral metabolism was not examined per se. Sykes, Field & Slee (1969) found exposure to +8°C to cause a 12% reduction in plasma Mg levels from levels at +30°C, while acute cold exposure caused marked reductions in plasma Ca and Mg concentrations, with increased P levels.

(d) Haematocrit

Slee (cited by Sykes et al, 1969) found acute cold exposure to cause a 13% increase in ovine PCV; exposure to +8°C having no effect.

(vii) Disease

(a) Protein parameters

In most infections there is a shift in the plasma protein profile. The most striking change is a decrease in the ALB fraction, this being the result of either an inhibition in synthesis or a more rapid catabolism. The decrease may be a response to increased GLOB concentrations - in most infections there is an increase in the α-globulin level, β-globulins increase in hepatic disorders, while γ-globulins increase as the response of the reticulo-endothelial system to antigens (Dimopoullos, 1970).

The changes occurring in the plasma proteins of animals infected with a variety of gastrointestinal parasites do not appear to differ from the changes occurring in other infections. Thus Coop, Sykes & Angus (1976) showed significantly lower ALB levels, and higher GLOB levels, in lambs infected with Trichostrongylus colubriformis (2500

larvae/day for 14 weeks) when compared with controls or pair fed animals. In investigations into the turnover rates of ALB and GLOB in sheep with chronic fascioliasis, Holmes et al (1968) reported a decreased ALB half-life, presumed to be due to the loss of ALB into the gastrointestinal tract.

Using sheep infected with Ostertagia circumcincta, Holmes & MacLean (1971) and Mulligan (1972) showed an increased output of urinary N, and an elevated blood UN concentration, during the acute phase of infection. These findings were similar to those in animals infected with T. colubriformis (Roseby & Leng, 1974). The rise in blood UN concentrations may be due to an increased protein catabolism.

(b) Mineral parameters

The only effect of infection with T. colubriformis on ovine mineral metabolism noted by Coop et al (1976) was a lowering of plasma P levels from the 3rd week of infection. Infections cause increased plasma Cu levels (Beisel, Pekarek & Wannemacher, 1974).

(viii) Miscellaneous

Siemon & Moodie (1972) described significantly lower P levels in bovine venous blood than in arterial blood. Parker & Blowey (1974) studied the composition of bovine blood taken from 3 different sources (jugular vein, coccygeal vein, coccygeal artery) and showed that there were small differences between the levels of some blood constituents at these sources.

This list of sources of variation in ruminant blood composition may be summarized as follows:

	Energy intake	Nitrogen intake	Mineral intake	Dietary composition	Prandial variation	Pregnancy	Lactation	Season	Stress	Age	Breed	Climate	Disease	Miscellaneous
GLUC	+				+	+	+	+	+	+	+	+		
FFA	+			+	+	+	+	+	+		+	+		
KB	+			+	+	+	+					+		
TP						+	+		+	+		+	+	
ALB		+				+	+	+	+	+	+	+	+	
GLOB		+				+	+		+	+	+	+	+	
UN		+		+	+	+		+		+	+		+	
Ca			+				+	+	+	+	+	+		
Mg			+				+			+	+	+		
P			+			+	+	+	+	+	+	+	+	+
Cu						+		+	+	+	+		+	
PCV	+				+	+	+	+	+	+	+	+		

(where + denotes a significant effect)

There follows an examination of some of the technical sources of variation in ruminant blood composition.

Technical problems associated with the determination of blood composition

(i) Analytical error

Analytical errors may be systematic, random or intrinsic, the latter being the most important source of error in automated assays (Robinson, 1971).

Systematic errors occur both between laboratories and between batches, within the same laboratory (Straumfjord & Copeland, 1965). They are brought about by such factors as inaccurate standards and reagents and, in manual assays, by personal bias.

Random errors affect all analyses to different degrees, may be positive or negative, and are generally attributable to the analyst himself. The use of automated equipment has largely removed the possibility of random errors of human origin.

Intrinsic errors, by definition, are those which are inherent to different analytical methods. They include variations in specificity, which for blood and plasma GLUC assays (Pennock et al, 1973) varies from low (e.g. ferricyanide and neocuproin methods) to high (e.g. glucose oxidase methods). Pennock et al (1973) showed that the low specificity of some of the GLUC assays was due to the interference of hexoses.

Further examples of non-specific assays include the colorimetric assays of Ca (Baak, Heck & van der Slik, 1975), ALB by the dye method of Ness, Dickerson & Pastewka (1966), and FFA, where the use of different materials in the extraction mixture results in widely

different estimates of plasma concentration (A. C. Field, unpublished).

Other sources of intrinsic error include poor instrument performance and, in those methods which require it, the precipitation of protein prior to direct determinations. There are many instances of plasma constituents being absorbed on to the protein precipitate.

Intrinsic errors are additive. Thus the extent of this source of error in an assay is a function of the number of procedures in the assay, and is particularly important in assays that use a "by difference" technique (e.g. GLOB) and those assays which require the subtraction of appreciable blanks (e.g. ALB).

(ii) Storage conditions

Considerable changes occur in the composition of blood, serum and plasma on standing at room temperature (Bach & Hibbitt, 1958; Wilson, Guillan & Hocker, 1972). In attempts to stop these changes, specialized storage conditions and preservatives are used: the stability of blood samples in transit is vital in those situations where the analytical laboratories are situated some considerable distance from the farm.

Probably the best known change that occurs in blood composition upon standing is glycolysis. In cattle and sheep the rate of glycolysis is a function of the leucocyte concentration (Anderson, 1969), so that glycolysis may be effectively prevented by the separation, using centrifugation, of the leucocytes from plasma. As there is no GLUC in the erythrocytes of sheep and cattle, plasma GLUC is in any case

preferable to blood GLUC when attempting to determine the concentration of GLUC in the ECF of these animals (Anderson, 1969).

Inorganic fluorides can stop or greatly reduce glycolysis, and to some extent coagulation (Kaneko, 1970). Until recently the most common preservative/anticoagulant mixture was sodium fluoride and potassium oxalate, but the finding that this mixture alters the osmotic equilibrium in ruminant blood, leading to crenation of the erythrocytes, lowered PCV levels and reduced plasma constituent concentrations, has limited its use.

An alternative mixture (containing potassium oxalate, ammonium oxalate and ammonium fluoride) was proposed by Anderson (1969) and was shown by Manston, Whitlock & Young (1974) to be preferable to the sodium fluoride/potassium oxalate mixture.

In the present work glycolysis was prevented by separating the plasma from the leucocytes as soon as possible after sampling, using heparin as the anticoagulant. Heparin is without effect on glycolysis or on the osmotic equilibrium in whole blood (Anderson, 1969).

Whole blood haemolyses on thawing, and is therefore never stored frozen. On the other hand, plasma and serum samples are usually stored frozen, and there is some evidence that the storage temperature and the duration of the storage period may affect serum composition. Thus at temperatures of -20°C for periods of up to 2 years, concentrations of the β-globulins increase (Kuttler & Marble, 1959), while decreases in the γ-globulin levels have also been noted (Senft, Manteuffel & Meyer, 1975).

Outline of Research Programme

In the single-suckling beef herd, the main cost is the nutrition of the dam, and many farm systems have been developed which exploit the capacity of the cow to deplete and replete body reserves according to the availability of cheap food. It would be useful to be able to monitor the nutritional status of such animals in order to avoid those metabolic disorders such as ketosis which are induced by extreme levels of undernutrition, thus allowing the maximum economic benefit to be obtained from strategically reducing the maternal plane of nutrition.

It is generally recognized that energy is the most common nutritional deficiency of suckling beef cattle, although protein deficiency is now thought to occur in outwintered beef animals. In recent times it has been claimed that the energy status of dairy cattle can be assessed from the GLUC concentrations of blood or plasma, but Adler (1970) has pointed out that GLUC is not an index of energy deficiency but of the adequacy of gluconeogenesis to meet the GLUC requirements of milk production and/or pregnancy. Thus the relationship between GLUC concentration and energy status is an indirect one, and it is not known if this relationship will hold for lactating beef cattle with their low milk yields relative to those of dairy cows.

The "true" index of energy status is the plasma concentrations of FFA, which are the transport form of fat in the blood and which are elevated whenever the animal requires to mobilize fat from the fat depots to meet the energy deficiency. Although FFA are an ideal marker of

energy status, doubts have been expressed as to their value in practice. For instance, it is known that their concentrations in plasma are sensitive to the stress caused by the act of bleeding itself.

Another suggested blood parameter for monitoring energy status is KB, but it is generally accepted that KB are similar to GLUC in that they reflect GLUC status rather than energy status.

Cattle experiments

The main aim here was to investigate the value of the above parameters in monitoring the energy status of lactating beef cattle. To achieve this object it is necessary to measure the concentration of the blood constituents in groups of animals on different energy intakes, ranging from frank deficiency to adequacy, and to proportion the variation in these concentrations to those factors which are known or thought to modify blood composition. Apart from energy status the main factors investigated were stage of lactation, which is closely related to level of daily milk production, and the time of feeding. It is also important that the beef cattle should be consuming diets typical of those used in practice.

Fortunately Dr. Lowman and his colleagues at the Edinburgh School of Agriculture were already conducting an experiment, suitable for the present purposes, in which the energy requirements of lactating beef cows were being assessed by feeding three levels of a silage/rolled barley diet. Although it was thought that energy was the sole deficiency, opportunity was taken to measure the effect of lactation and time of feeding on the blood concentrations of a selection of blood parameters

which have been used in the past to monitor the status of the animal to a variety of essential nutrients.

Sheep experiments

Sheep are also able to withstand undernutrition by the mobilization of body reserves and the extent of the undernutrition may be considerable, particularly in outwintered pregnant or lactating ewes on the hill (Reid, 1968). Although the blood composition of such animals has been used on occasions as an index of their status to particular nutrients, little attention has been given to the general effects on blood composition of pregnancy, lactation and time after feeding and their interactions with level of nutrition.

The objects of these experiments were two fold:

(i) to investigate the relationship between blood composition and level of feeding for the most essential macro-nutrients and to see whether these relationships are modified by gestation and lactation. The degree of deficiency of energy, protein, calcium, phosphorus and magnesium imposed was comparable with that found in the field situation.

(ii) to investigate the within-day variation in blood composition and to see how this variation is related to level of nutrition, frequency of feeding and stage of pregnancy and lactation.

For ease of presentation the sheep experiments are described under

two headings: the first deals with the effect of level of feeding on the blood composition of ewes in early and late gestation and in early lactation. The second is concerned with the interrelationships between frequency of feeding and the within-day variation in the blood composition of ewes at the above three stages in the reproductive cycle.

CHAPTER 2

MATERIALS AND METHODS

This chapter describes the materials and methods common to all experiments: blood sampling, chemical analyses, statistical analyses and notes on Figures and Tables.

Blood sampling

A standard method of blood sampling was used in all groups of animals: every attempt was made to minimize the inherent stress factors imposed on the animals by the methods used in restraint and in venipuncture.

Cattle were restrained with a halter and neck-yoke in a holding crate: blood vessels were raised using a choke rope. Sheep were restrained by an assistant during blood sampling, which took place either in individual pens, an exercise yard or a holding pen. The necks of the sheep were clipped to facilitate location of blood vessels.

Blood samples were obtained from the left jugular vein using heparinized, copper-free Vacutainers (Becton-Dickinson & Co. Ltd., New Jersey). The heparin consisted of 0.2 ml, subsequently freeze dried, of a 0.5% aqueous solution of PULARIN (Evans Medical Ltd., Speke, Liverpool) per 20 ml Vacutainer. Plasma was separated by centrifugation: at 1300g for 12 minutes in Experiments C1 and C2, and at 1540g for 10 minutes in all other Experiments. To avoid leucocytic glycolysis, plasma separation took place immediately after sample collection (within 15 minutes for Experiments C2 and S2, and within 45 minutes for all other Experiments). Aliquots of whole blood were taken prior to plasma separation and were used for PCV estimations. Plasma was kept at $-20^{o}C$ in polystyrene tubes prior to chemical analysis.

Chemical analysis

The blood constituents examined in the present work are not the same as those proposed by Payne et al (1970). Plasma FFA and KB concentrations, considered by Bowden (1971) to be better parameters of energy status than are blood (or plasma) GLUC concentrations, were assayed in most of the experiments described here. Concentrations of sodium (Na), potassium (K) and haemoglobin were not determined. Plasma Na concentrations do not reflect Na status (Morris & Gartner, 1971), and the detection of K deficiencies (considered by the A.R.C. in 1965 to be unlikely in the U.K.) using plasma K levels is impractical (Pradhan & Hemken, 1968).

In most of the experiments described here the plasma concentrations of GLUC (mg/l), FFA (μ equiv/l), TP (g/l), ALB (g/l), GLOB (g/l), UN (mg/l), Ca (mg/l), Mg (mg/l) and P (mg/l) were determined: in some experiments, plasma Cu (mg/l) and blood PCV (%) levels were also determined.

Apart from Mg (Ca in Experiment C3) and PCV, all assays were made using colorimetric methods (i.e. those involving a change in absorption within the visible light range, 400 - 700nm). With the exception of FFA and KB, all colorimetric assays were carried out using automated flow-lines.

Flow-lines consist of a number of modules (such as mixing coils, dialysers, heating coils, incubation coils and colorimeters) linked together by a network of glass tubing through which reagents are pumped continuously by a peristaltic pump. A sampler module presents samples in turn to a sampling probe which measures the required amount of material to be injected into the system. Samples follow each other successively along the tubing, and carry-over between samples is reduced by introducing a regular pattern of air bubbles into the liquid

stream. The bubbles prevent the samples from diffusing into each other, and also scour the walls of the tubing. There is an interval after each sample has been introduced into the system during which a saline wash flows through the sampling probe: the wash period can be varied to reduce the amount of carry-over to acceptable limits.

Flow-lines used were capable of performing 40-50 estimations per hour (using sampler timings of 24 seconds sample : 45 seconds wash). Absorbances of standards and unknowns were recorded as peaks on a moving chart recorder attached to the colorimeter. Peak heights were measured using a pencil follower (Cetac Systems Ltd., Glasgow) which determined the X and Y co-ordinates of the peaks and displayed them on a digital display module. The digital output of the pencil follower was recorded on punch tape, and the tape fed directly into a PDP-8f computer.

There follows a summary of each of the analytical methods used.

(i) Automated Assays

Glucose

Assays of GLUC were made using a glucose oxidase method devised by Trinder (1969). The basis of this method is the oxidation of glucose by glucose oxidase, giving hydrogen peroxide as one of the products. On treatment with peroxidase, hydrogen peroxide yields oxygen which oxidizes a suitable colourless substrate (in this case phenol, in the presence of 4-aminophenazone) to a coloured compound. The two solutions required are a diluent (containing 4-aminophenazone) and a colour reagent (containing glucose oxidase, peroxidase and phenol).

Azide was added to both solutions as a preservative. Although the use of a wetting agent would have been beneficial, no suitable agent was available.

In Experiments C1 and C2 the sample was introduced as the supernatant after plasma protein precipitation (using 4% trichloroacetic acid, TCA). In subsequent experiments dialysis replaced precipitation as the method used to remove plasma proteins (see flow diagrams 1 and 2). Standards used (200,400,600,800,1000 mg/l) consisted of aqueous solutions of inorganic glucose (BDH product No. 22056).

Total protein

Plasma TP concentrations were estimated using the biuret method described by Weichselbaum (1946). In the presence of a tartrate diluent, copper in alkaline solution reacts with the peptide bonds of proteins to give a deep purple complex. Only one reagent (containing tartrate, copper sulphate, potassium iodide and sodium hydroxide) was used in the assay; wetting agents were not required (see flow diagram 3).

Aqueous solutions (1, 2, 4, 6, 8, g/l) of bovine albumin fraction V (BDH product No. 44155) were used as standards. In Experiments C3, S1 and S2 the protein content of the stock albumin solution was estimated using the Kjeldahl method, assuming protein = N x 6.25.

Albumin

Plasma ALB concentrations were determined using the method described by Ness et al (1965). This is based on the quantitative binding of the anionic dye, 2-(4-hydroxyazobenzene)-benzoic acid

(HABA), with ALB. Results from Experiments S1 and S2 were corrected for the lower binding capacity of ovine ALB with HABA (A.R. Sykes, unpublished). Only one reagent (HABA) was used, and this was occasionally found to require filtering before use. No wetting agents were required.

In Experiments C3, S1 and S2, albumin blanks (caused by such interferences as bilirubin, haemolysis or turbidity-Robinson, 1971) were determined and subtracted from the estimated ALB concentrations. Albumin blanks were determined in a flow-line identical to that used for ALB determinations (flow diagram 4), except that HABA was replaced by phosphate buffer. The same standards as those used in TP estimations were used in both ALB and ALB blank determinations.

Globulin

Plasma GLOB concentrations were taken as the arithmetical difference between concentrations of TP and ALB. This method is considered less precise than direct GLOB assays (Robinson, 1971), although the latter are unreproducible.

Urea nitrogen

Concentrations of UN in plasma (Experiments C3, S1 and S2) and deproteinized (using 4% TCA) plasma samples were determined using the method described by Marsh, Fingerhut & Miller (1965). When urea is heated with substances containing 2 adjacent acetyl groups (such as diacetyl), coloured compounds are formed. In the present work diacetyl

monoxime was used under acid conditions, in the presence of thiosemicarbazide (the latter compound intensifies the colour of the reaction product, enabling the determination to be run without using concentrated acids).

Two reagents were used: a colour reagent (containing diacetyl monoxime and thiosemicarbazide) and an acid reagent (see flow diagrams 5 and 6). Both reagents required the addition (Iml/l) of a wetting agent (Brij-35: polyoxyethylene lauryl ether, BDH product No. 56003). Determinations were made using aqueous solutions (50,100,150,200, 250 mg UN/1) of inorganic standards (BDH product No. 22018).

Calcium

The Ca content of plasma (Experiments S1 and S2) and deproteinized plasma (using 4% TCA) plasma samples was determined using the method of Gitelman (1967). The sample is treated with o-cresolphthalein complexone solution (containing 8-hydroxyquinoline to eliminate interference from Mg) and colour developed by adding a diethylamine/potassium cyanide base. For Ca estimations in TCA supernatants, ethylene diamine tetra acetic acid (EDTA) was added to the colour reagent to complex a pre-determined amount of Ca. In the other experiments, the sample was diluted with, and dialysed into, a stream of 0.25N hydrochloric acid.

Three reagents (acid, dye and base) were used, all requiring the addition (1 ml/l) of wetting agents (Brij-35). Flow diagrams 7 and 8

show the flow-lines used. In Experiment C3, concentrations of Ca in a 1/25 dilution of plasma with an aqueous solution of lanthanum chloride (6.5 ml/l of a stock 10% aqueous solution of $LaCl_3.7H_2O$; BDH product No. 14041) were determined using the spectrophotometric method of Trudeau & Freier (1967).

Standards used in all experiments consisted of aqueous solutions (80, 90, 100, 110, 120 mg/l) of inorganic Ca (BDH product No. 22028). In Experiments S1 and S2 , protein (4g/l of bovine albumin fraction V) was added to all standards because of the increased Ca dialysis rate in the presence of protein (Lott & Herman, 1971).

Inorganic phosphate

In Experiments C1 and C2, the TCA supernatant was treated with an acid molybdate reagent, the latter reacting with P to form phospho-molybdic acid (Fiske & Subbarow, 1925). The hexavalent molybdenum of the phospho-molybdic acid may be reduced by means of 1-amino, 2-naphthal, 4-sulphonic acid (ANSA) to give a blue compound which can be read colorimetrically at 682nm. Two reagents (acid molybdate and ANSA) were used; wetting agents were not required (see flow diagram 9).

In subsequent experiments, plasma P concentrations were determined using the method of Robinson, Roughan & Wagstaff (1971). Here plasma is diluted with, and dialysed into, an aqueous 1% sulphuric acid solution. The P in the dialysate is coupled with a molybdivanadate reagent, and the intensity of the resulting yellow complex read at 403nm. Two

reagents are required: a diluent of 1% sulphuric acid (requiring the addition (1 ml/l) of octan-2-ol as a wetting agent) and a colour reagent (a mixture of solutions of ammonium meta vanadate and ammonium molybdate in 50% nitric acid) - see flow diagram 10.

Aqueous solutions (20, 40, 60, 80, 100 mg/l) of potassium ortho phosphate (BDH product No. 10203) were used as standards.

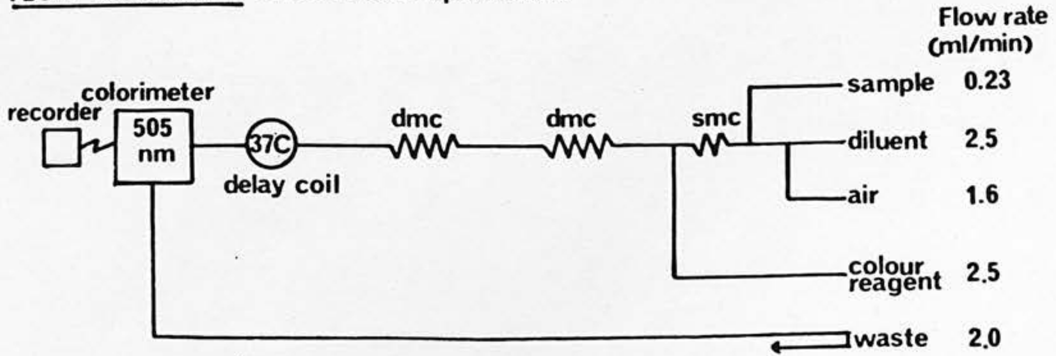
Magnesium

Concentrations of Mg in plasma (Experiments C3, S1 and S2) and deproteinized plasma samples were determined using AAS (Stewart, Hutchinson & Fleming, 1963). Plasma was diluted 1/25, and TCA supernatant 1/5, with an aqueous $LaCl_3$ solution (see Ca assay). Inorganic standards (5, 10, 15, 20, 25 mg/l) were used.

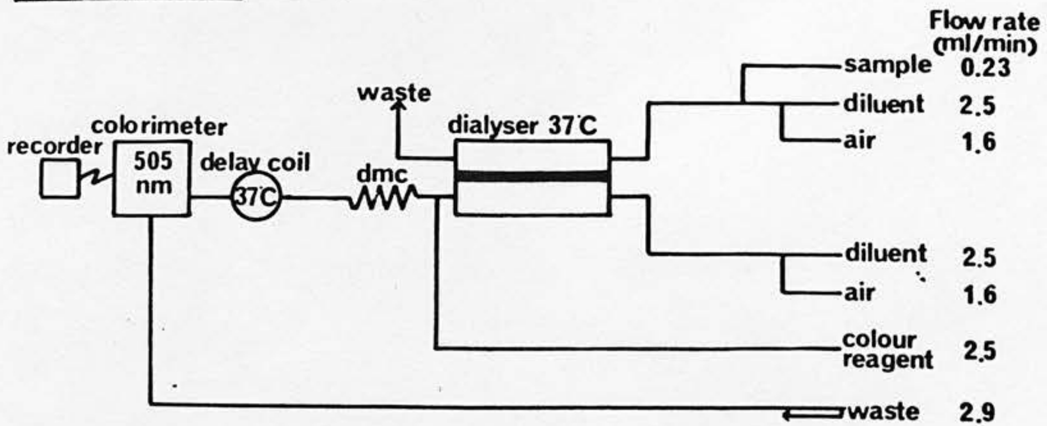
Copper

Plasma Cu concentrations were estimated using the method of Summers (1960). Plasma is treated with 1 N hydrochloric acid to liberate protein-bound Cu, before dialysing into a stream containing isotonic saline. Colour development takes place upon the addition of an oxalyldihydrazide/acetaldehyde colour reagent in ammonia: the resulting colour is read at 552 nm. Three reagents were used: acid, saline and colour reagent. The colour reagent was unstable, and was made freshly each day. Wetting agents were not required (see flow diagram 11). Inorganic standards in the approximate range 0.3 - 1.5 mg/l (BDH product No. 14034) were used.

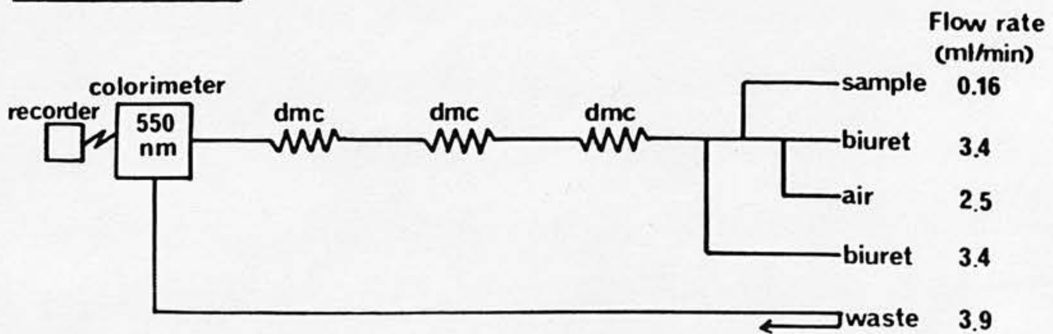
FLOW DIAGRAM 1: GLUC(TCA supernatant)



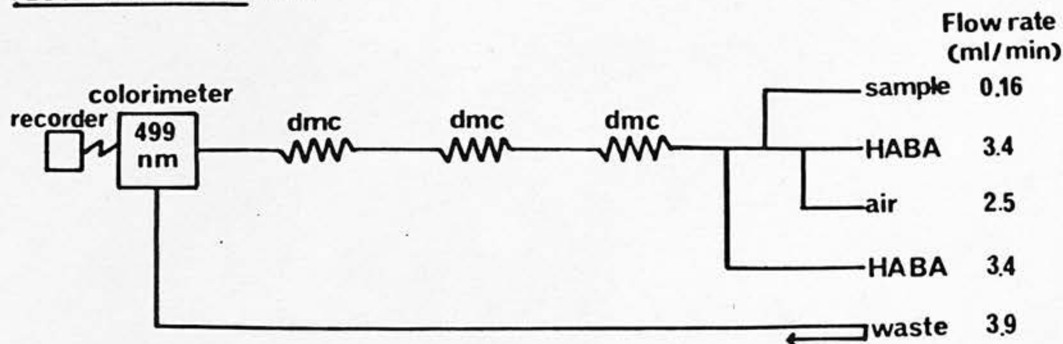
FLOW DIAGRAM 2: GLUC(plasma)



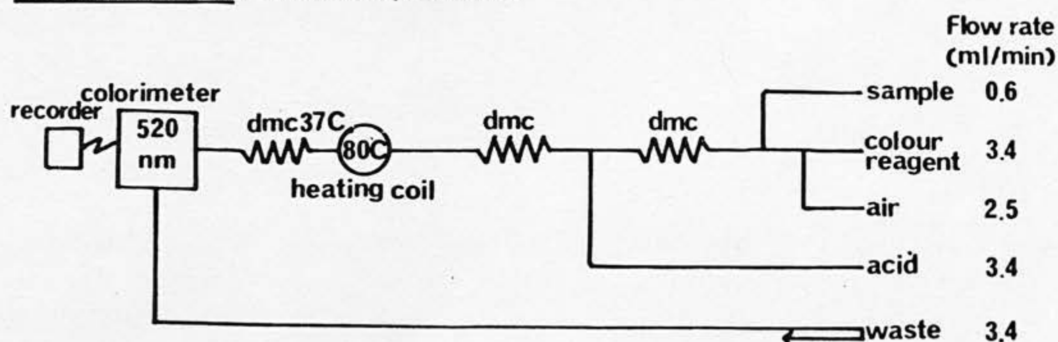
FLOW DIAGRAM 3: TP



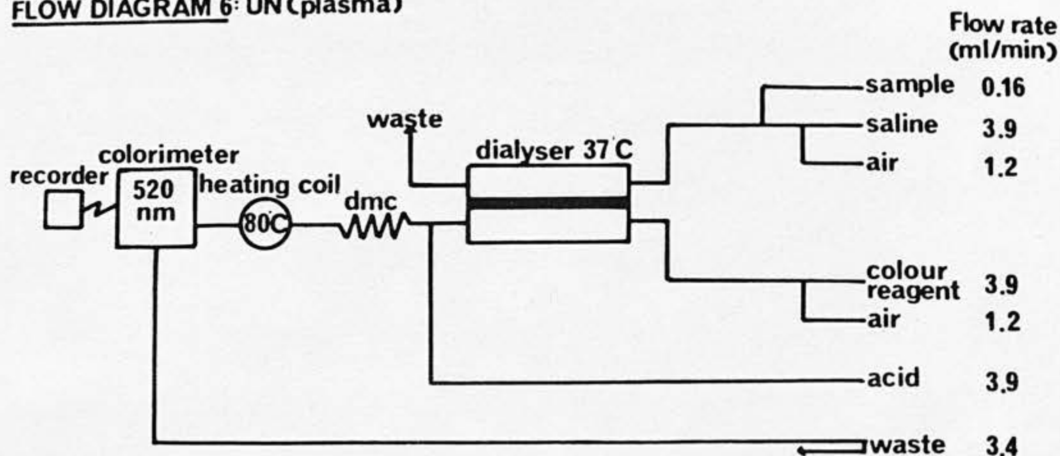
FLOW DIAGRAM 4: ALB

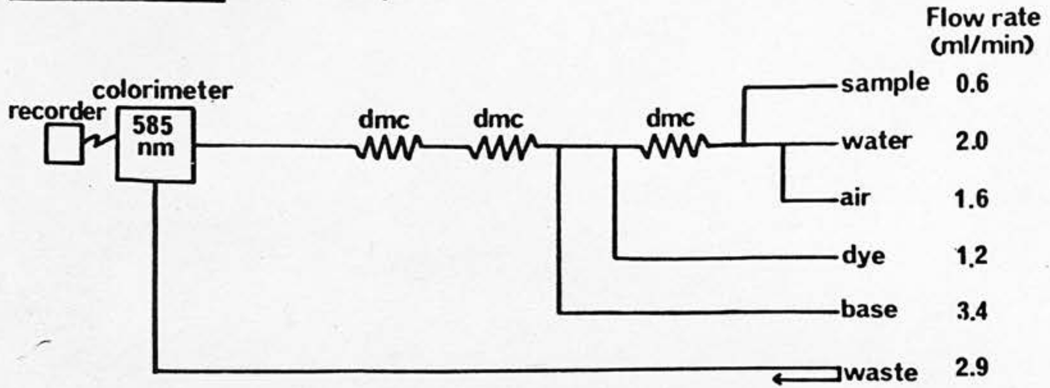


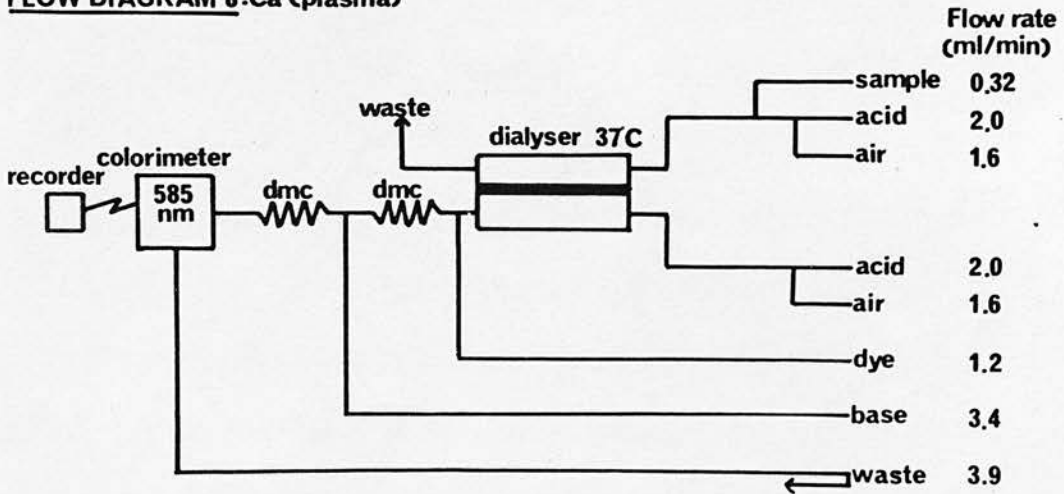
FLOW DIAGRAM 5: UN (TCA supernatant)



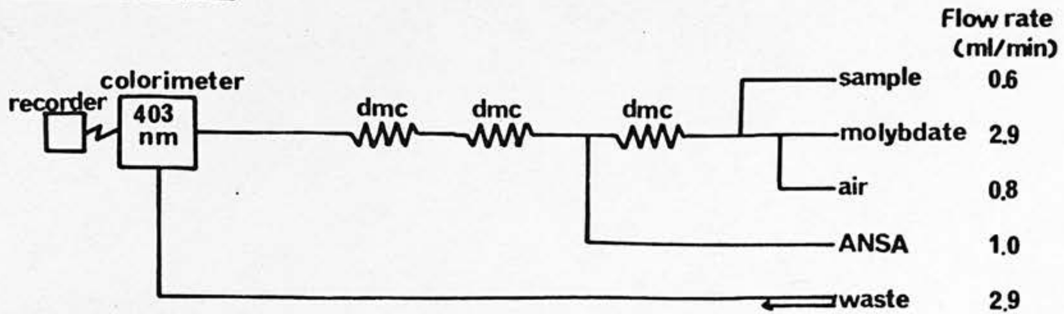
FLOW DIAGRAM 6: UN (plasma)



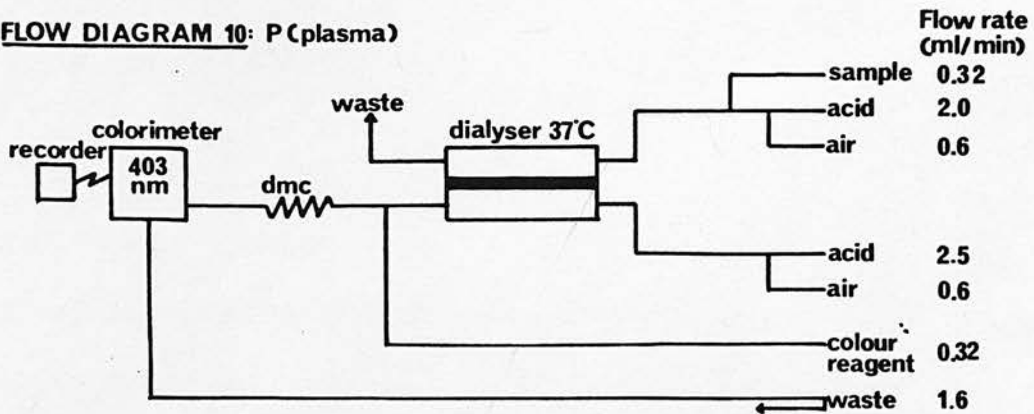

FLOW DIAGRAM 7: Ca (TCA supernatant)
Flow rate (ml/min)
recorder
colorimeter
585 nm
dmc
dmc
dmc
sample 0.6
water 2.0
air 1.6
dye 1.2
base 3.4
waste 2.9


FLOW DIAGRAM 8: Ca (plasma)
Flow rate (ml/min)
recorder
colorimeter
585 nm
dmc
dmc
waste
dialyser 37C
sample 0.32
acid 2.0
air 1.6
acid 2.0
air 1.6
dye 1.2
base 3.4
waste 3.9

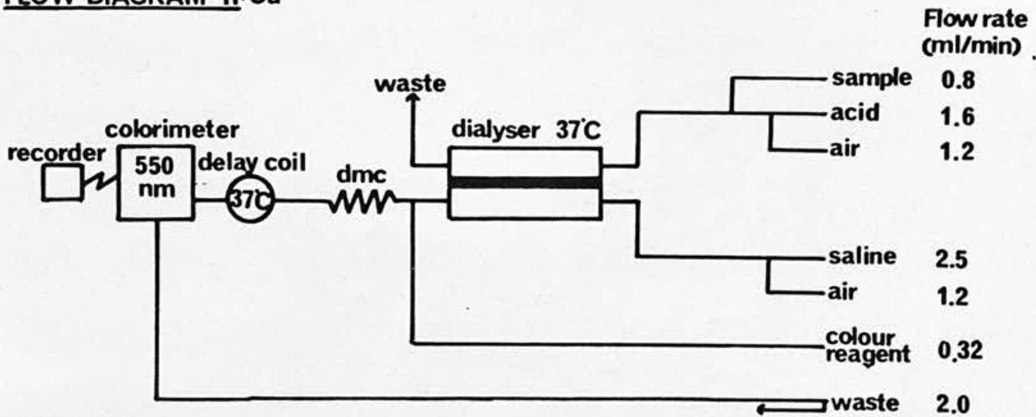
FLOW DIAGRAM 9: P (TCA supernatant)



FLOW DIAGRAM 10: P (plasma)



FLOW DIAGRAM 11: Cu



(ii) Manual Assays

Haematocrit

Blood PCV levels were determined using a Hawksley micro-haematocrit centrifuge (Dooley et al, 1974).

Free fatty acids

Three reagents were used in FFA determinations:

1. Extraction mixture: iso-propanol : n-heptane : N hydrochloric acid in the ratio (by volume) 40 : 10 : 1

2. Copper reagent: (per litre of water): 70 ml triethanolamine, 3 ml glacial acetic acid, 32.5g Cu $(NO_3)_2.3H_2O$, 62.5g K_2SO_4, 170g Na_2SO_4.

3. Colour reagent: 2.03g sodium diethyldithiocarbamate/1 butan-2-ol.

The extraction of FFA from plasma prior to colorimetric determination was achieved using the method of Dole (1956). Plasma or standard (0.5 ml, standards blown dry to correct for dilution effect) was added to the extraction mixture (5 ml) and the solutions mixed (using a vortex shaker) for 10 seconds. After standing for 5 minutes, heptane (3 ml) and water (2 ml) were added, the solutions mixed for 30 seconds and allowed to stand for a further 30 minutes.

The colorimetric FFA determination used was a combination of the methods of Duncombe (1963) and Lauwerys (1969). When shaken with copper nitrate solution in chloroform, FFA form soluble Cu salts. Copper determinations on the chloroform layer (using diethyldithiocarbamate as the colour reagent) give estimates of the FFA concentrations in the plasma.

The top layer (circa 3.5 ml) of the end-product of the Dole extraction was removed (lower layers discarded) and to it added circa 2.5 ml 0.01 N sulphuric acid. The resulting mixture was shaken for 30 seconds, allowed to settle, and 2 ml of the top layer removed (lower layers discarded). To this was added 2 ml chloroform and 2 ml of the copper reagent. This mixture was shaken for 30 seconds and centrifuged (at 2500g) for 10 minutes. The top 2 ml of the resulting complex was removed (lower layers discarded), and to it added 0.2 ml of the colour reagent. This solution was shaken for 10 seconds and read at 428 nm.

Standards used consisted of solutions (500, 1000, 1500, 2000, 2500 μ equiv/1) of palmitic acid in n-heptane. Skilled personnel were able to make approximately 50 FFA determinations per day.

Ketone bodies

The distillation method of Reid (1960) was used to determine the KB concentrations in deproteinized (using sodium hydroxide and acid zinc sulphate) plasma samples. This method is based on the conversion of all KB to acetone, the distillation of the acetone so formed, and the colorimetric determination of acetone by reading the intensity of the colour of the reaction product of acetone with ethanolic salicylic aldehyde in alkaline solution.

Standards used consisted of aqueous solutions of acetone (1g/1) and of sodium β-hydroxybutyrate (2.2112 g/1).

Analytical quality control

Quality control in automated assays consisted of checks performed by the computer on various criteria: an example of the computer print-out is given in Appendix I. The criteria used included the regression coefficient and residual variance of the best line through the standards, and also the mean difference between duplicates. Samples were analysed in batches (usually of 64 samples); if the difference between individual duplicates, within batches, exceeded 3 times the median duplicate difference (= S.D.) then the results for that sample were rejected.

Inorganic standard curves were corrected against Versatols (pooled human serum, standardized for several constituents: William R. Warner, Eastleigh, Hants.), and the factor by which the estimated standards were multiplied as a result of the Versatol correction was printed out. Versatols were not used in Cu or protein assays; in the latter the N content of the bovine albumin used as the standard was used as a correction factor.

This use of correction factors was considered to provide adequate quality control between batches. Within batches, quality control was monitored by assaying pooled ovine plasma samples, one after every 10 samples. Machine drift within runs was thus monitored by the changes in pool plasma concentrations, and also by the changes in initial and final base line and Versatol levels. The order of samples within a

typical run was 5 standards, blank, Versatol, 4 unknowns, pool plasma, 10 unknowns, pool plasma, 10 unknowns, pool plasma, 10 unknowns, pool plasma, 10 unknowns, pool plasma, 10 unknowns, pool plasma, 10 unknowns, blank, Versatol.

Quality control in FFA and KB assays was also computerized: typical computer print-outs are given in Appendix II. In FFA assays the computer determined the best line through the 5 standards; also calculated were the regression coefficient and the residual variance. Pooled plasma samples were used to correct the inorganic standards in the same manner as Versatols were used in automated assays.

The recovery rates of acetone and butyrate in KB assays were calculated by the computer, and were combined to give a constant multiplication factor. In the calculation of this factor it was assumed that the ratio (acetone + acetoacetate) : (butyrate) was 1 : 3 (Reid, 1960).

Statistical analysis

Analysis of experimental results

A Least-Squares, Maximum Likelihood General Purpose programme (LSMLGP: Harvey, 1968) was used in the statistical analysis of Experiments C1, C2, S1 and S2. In each experiment the programme was used to determine the variation in blood constituent concentrations attributable to group, time and the interaction. For each constituent the programme determined the overall means, standard deviations, least-

squares means and standard errors. Also calculated was an analysis of variance table, comprising degrees of freedom, sums of squares, mean squares and variance ratios. For examples of LSMLGP output, see Appendices III and IV.

LSMLGP was also used to determine the simple and residual correlation coefficients between blood constituent concentrations. In many cases the coefficients may be due to random effects, while others may be due to metabolic and physiological inter-relationships. For this reason, it is difficult to explain each correlation. The residual correlation coefficients in each of the experiments (except Experiment C3) are shown in Appendices V - X.

The least-squares, maximum likelihood technique involves the principle of attributing equal weight to each class of a given effect, thus making it possible to study the effect of a certain factor without it being biased by the uneven distribution of other effects in the material. Since each class of each effect was allowed to exert an equal effect, irrespective of the number of observations in the class, the least-squares means often differed from the overall means.

LSMLGP used two statistical models:

i) $y_{ij} = \mu + g_i + t_j + E$

ii) $y_{ij} = \mu + (g.t)_{ij} + E$

where y_{ij} = the blood constituent concentration of the 'i'th group at the 'j'th time period.

μ = overall mean

g_i = the effect of the 'i'th group

t_j = the effect of the 'j'th time period

$(g.t)_{ij}$ = the effect of the interaction between group and time period

and E = Error

Experiment C3 was analysed using Student's t-test to compare the mean group values, and to estimate the significance of the prandial variation.

Abbreviations

The following abbreviations have been used in Tables and Figures:

n = number of observations

S.D. = standard deviation

S.E. = standard error

df = degrees of freedom

$F_{j,k}$ = variance ratio with j,k degrees of freedom

The following symbols have been employed to denote the different statistical levels of probability:

NS = $P > 0.05$

* = $P < 0.05$

* * = $P < 0.01$

* * * = $P < 0.001$

Notes on figures

The lactation curves shown in Figure C1.1 were derived from the actual daily milk yields using an equation (Wood, 1969) which reduced the large fluctuations in the daily milk yields of individual cows. Similar techniques were used in the estimation of maximum milk yields, and also in the calculation of the weight changes in Figure C1.2.

In Figures C1.3 - C1.8 the lines of best fit through the least-squares mean time values for each of the 20, ten day time periods were determined by regression techniques using GENSTAT, a statistical programme developed at the Rothamsted Experimental Station, Herts.

Values obtained for samples taken at 09.00h in Figures C2.1 - C2.7 are shown twice on the same Figure, as are the 08.00h values in Figures S2.01 - S2.17.

The mean values for the weight of each animal in each of 14, ten day time periods were used to calculate the mean group weight changes shown in Figure S1.01, using GENSTAT.

Where the clarity of Figures may have been lost by the inclusion of error terms on all of the points, then only a minimum of such terms have been included.

Notes on Tables

Pooled S.D.'s were derived using the formula:

$$\text{Pooled S.D.} = \sqrt{s_1^2(i_1 - 1) + s_2^2(i_2 - 1) + \ldots / (i_1 - 1) + (i_2 - 1) + \ldots}$$

where i_a = number of observations, with estimated variance s_a^2.

In Tables S1.2 - S1.5, the intakes of individual animals in Group A were taken as the mean daily amount consumed by the collectively fed group, so that no error terms can be given with these figures.

CHAPTER 3

CATTLE EXPERIMENTS

The aims of the present studies with cattle were firstly to see what changes in blood composition were induced by changing the energy status of lactating animals, and secondly whether the induced changes could be used as indices of energy status. Not all blood constituents which are sensitive to energy status are suitable for the latter purpose, since the usefulness of an index depends upon a high proportion of the observed variation in its concentration being attributable to energy status. It was clearly impractical to examine all the causes of variation in blood composition that are listed in the Introduction, and the choice was to a great extent dictated by the experimental facilities available.

In lactating cattle the obvious factors with the potential to alter blood composition are milk yield, stage of lactation and prandial effects, as well as the extent of energy undernutrition imposed on the animal. Obviously in the ideal situation each factor would be investigated independently and its importance assessed. Unfortunately in the available experimental material certain of these factors were confounded in the experimental design: feed intake was fixed throughout lactation and consequently the degree of the energy deficit or excess was correlated with milk yield. Thus the effects of stage of lactation and energy deficit were confounded. Despite this and other limitations certain conclusions could be reached as to the significance of the non-nutritional factors within the general context of the problem of monitoring energy status in lactating cattle.

The work to be described in this chapter relates to two beef cattle experiments, although the results are presented in three sections. The experimental designs were basically the same, differing only in the breed and number of cattle used and whether the cows were machine milked or suckled by their calves. The first section deals with the changes in blood composition observed during the first 200 days of lactation, the second with the changes in blood composition observed during a 24h period and the third with the effect of time of feeding on blood composition.

Experiment C1. Variations in bovine blood composition attributable to stage of lactation and to energy status

INTRODUCTION

The drain of nutrients to the mammary gland in milk production is known to influence plasma concentrations of those consituents thought to monitor energy status (Holmes & Lambourne, 1970; Rowlands et al, 1975) protein status (McLennan & Willoughby, 1973; Little, 1974) and mineral status (Payne & Leech, 1964; Lane et al, 1968). However, the changes in plasma composition have only rarely been monitored throughout an entire lactation (Hewett, 1974), and no previous attempts have been made to compare the effects on blood composition of stage of lactation and of level of nutrition.

ANIMALS AND MANAGEMENT

Twenty-five White Shorthorn x Galloway (Blue-grey) cows, aged 2-15 years with a mean of 9.2 $\pm$ 3.6 years, were transferred at parturition from pasture to individual stalls in a conventional byre and randomly allocated

into Groups L (8 animals), M (9 animals) and H (8 animals). Animals in these groups received feed allowances of silage and rolled barley (Table C1.1) providing respectively a calculated 90, 125 and 175% of their estimated maintenance requirements for metabolizable energy (ME). The requirements of the animals were calculated from their liveweights 12h after calving, using the A.R.C. (1965) figure of 0.489 MJ/kg metabolic body weight $(W^{0.75})$, with no allowances being made for milk production.

Core samples taken from the silage pit showed that the silage offered to the animals contained 21% dry matter (D.M.), with 9.2 MJ ME and 12.4 g N/kg D.M. Corresponding figures for the barley were 75%, 13 MJ and 15 g (R. A. Edwards, personal communication). Each animal was offered 5 kg silage at 07.00h and the rest of the daily ration at 13.30h. All animals had free access to tap water. Silage refusals, removed and weighed each morning before fresh silage was offered, were in general confined to Group H animals and were around 2.8 kg/day. The refusals were excluded from the total rations offered in all calculations of ME status.

Animals were machine milked twice daily, weighed weekly and blood sampled fortnightly. Milking commenced each day at 07.10 and 15.00h, and blood sampling and weighing at 09.00h. The sequence in which the cows were sampled was chosen at random on each occasion.

RESULTS

Milk yield

As lactation advanced the milk yields in all groups fell from around 8.3 kg/day in the second ten day time period to around 3.5 kg/day in the twentieth period. The mean milk yields of each group were similar at all

times, and the lactation curves of the groups are shown in Fig. C1.1.

Liveweight change

All animals lost weight for some time after calving, and Fig. C1.2 shows the rates of change of mean weight of each group over the first 200 days of lactation. Maximum rates of weight loss of 0.92 kg/day in Group L, 0.95 kg/day in Group M and 0.20 kg/day in Group H were seen 30-40 days after calving. Animals in Groups L, M and H had minimum weights at around 125,90 and 65 days after calving, when their mean milk yields were around 4.2, 5.1 and 6.4 kg/day respectively.

Changes in plasma composition

Table C1.2 shows the significances of the main effects, group and time, and their interaction, as sources of variation in plasma composition. Stage of lactation was a significant source of variation in plasma concentrations of GLUC, KB, TP, GLOB, UN and P, but was not for ALB, Ca and Mg. No significant effects on plasma composition of level of feeding or of the interaction between level of feeding and stage of lactation were found, and Table C1.3 shows for each group the adjusted mean concentrations of each constituent.

Energy parameters

Plasma GLUC levels (Fig. C1.3) declined linearly from around 910 mg/l in period 1 to around 700 mg/l in period 20, a fall of approximately 1 mg/l/day. This time relationship accounted for 73.4% of the total variation in levels of this constituent. No precise comparison can be made between the plasma GLUC concentrations in the present work and

the results of Payne et al (1970) because the latter referred to blood reducing sugars. However, it is possible to convert the "normal" range given by Payne et al (1970) to plasma GLUC, using the mean PCV level given by these workers. The GLUC concentrations obtained within 75 days of calving in the present experiment were then above the 95% confidence limit suggested by Payne et al (1970) for concentrations of this metabolite.

Plasma KB levels (Fig. C1.4) also declined markedly over the experiment, from a maximum of around 26 mg/l in period 1 to a minimum of around 11 mg/l in period 17. The time relationship accounted for 88.7% of the total variation. These KB concentrations are within the normal range ($<$ 70 mg/l: Kronfeld, 1957) and are below the levels observed by other workers using high-yielding dairy cows (Radloff et al, 1966; Fisher et al, 1975). The low values confirm that the silage was of adequate quality, since poor quality silages usually have a high butyric acid content and butyric acid is known to be strongly ketogenic (Pennington, 1952).

Protein parameters

Plasma TP, GLOB and UN levels (Figs. C1.5, C1.6 and C1.7) varied in non-linear fashions throughout the experiment and the time relationships accounted for 71.0, 77.4 and 46.3% respectively of the total variation. The minimum TP and GLOB levels, 68.0 and 39.8 g/l, were observed in period 15 and the maxima, 75.0 and 47.4 g/l, some 120 days earlier. Plasma ALB levels remained relatively constant at 25.0 - 27.6 g/l. Plasma UN concentrations were at a minimum, 53.8 mg/l, 60 days after calving and at a maximum, 82.7 mg/l, some 90 days later.

Levels of TP, GLOB and ALB were within, and those of UN below, the range suggested for these constituents by Payne et al (1970).

Mineral parameters

There was a slow decline in plasma P concentrations throughout the experiment, from 52 mg/l in period 1 to 46 mg/l in period 13 (Fig. C1.8). The time relationship accounted for 39.8% of the total variation. Plasma Ca and Mg concentrations remained relatively constant throughout the experiment at 94.0 - 106 and 19.5 - 23.3 mg/l respectively. Concentrations of all the mineral parameters were effectively within the range suggested by Payne et al (1970).

DISCUSSION

The variation in the degree of energy deficiency of the animals, as evidenced by the rates of weight change, was a complicating factor in the assessment of the relation between plasma composition and energy deficiency. Unfortunately, weight changes themselves cannot be used as a measure of energy deficiency because of the difference in the chemical composition of such changes as first predominantly fat and then protein is catabolized in attempts to maintain caloric homeostasis. Furthermore, changes in water retention in undernourished animals can mask changes in tissue mass (Sykes, 1974).

The estimations of the ME intakes of the groups (Table C1.1), critical to interpretations of the blood composition studies, were based primarily on the predicted ME content of the silage. The latter predictions were derived from a relationship obtained with wether sheep fed adequate energy

intakes (Alderman et al, 1969). Since it is known that rations are used with greater efficiency at low levels of D.M. intake and at sub-maintenance energy intakes (Blaxter, 1962), it was considered important in the present work to obtain an independent estimate of the energy intake of the animals.

Such an estimate may be obtained if it is assumed that at zero weight change, the total ME requirements of the animals are equal to the ME supplied by the diet (Table C1.4). The actual ME deficiencies of the individual animals during the periods of weight loss were derived by subtracting from the constant ME intake determined in Table C1.4 the ME requirements for maintenance and lactation. These calculations resulted in the changes in ME deficiencies shown in Fig. C1.9, and their main effect was to reduce the extent of the estimated ME deficiencies from those estimated in Table C1.1.

To investigate the efficiencies with which the ME in silage was utilized by the 3 groups, it is necessary to assume that the contribution of barley to the overall ME intake was constant, although it is appreciated that barley as well as silage may have been utilized with different efficiencies by the different groups. It is thought that the error introduced by this procedure is small, since barley only provided around 30% of the total ME intake. Table C1.4 shows that at zero weight change the silage consumed by Groups L, M and H had energy concentrations of 14.2, 9.87 and 8.48 MJ/kg D.M. respectively, compared with the estimated concentration of 9.2 MJ/kg D.M. This suggests that the silage consumed by the animals in Group L was used more efficiently than was the silage consumed by animals in Groups M and H.

Unfortunately, the only potential indices of ME status available in this experiment were GLUC and KB (Bowden, 1971). Lack of experimental facilities made it impossible to measure the plasma concentrations of FFA, the "true" measure of energy deficiency (Adler, 1970).

In lactating animals GLUC is required in large quantities for the synthesis of lactose (Linzell, 1968), and these requirements as well as the energy deficit would be greatest at maximum milk yield when plasma GLUC levels would be expected to be at a minimum. As lactation advances and milk yields fall, then plasma GLUC levels would be expected to rise, and such negative relationships between milk yield and plasma GLUC concentration have been demonstrated (Hewett, 1974). In the present work, however, there was a positive relationship between plasma GLUC and milk yield, while other work (Bertoni, 1976; Head et al, 1976) has shown constant GLUC levels in lactating dairy cows from 10-150 days after calving. Further work on the changes in plasma GLUC concentrations in animals with declining milk yields is indicated.

If it is accepted that low GLUC levels in lactating cows are indicative of energy deficiencies (Payne et al, 1970), then the finding in the present work of higher GLUC levels in the period of energy deficiency than in the period of energy excess suggests that it is not energy deficiency but some other factor which is responsible for the pattern of change in plasma GLUC levels seen throughout lactation.

The most probable source of the variations is lactation itself, and relationships between plasma hormone concentrations and the intensity of lactation have been previously demonstrated (Hart et al, 1975; Head et al,

1976). Other possible but less likely sources of the variations are the hormones involved in the reproductive cycle before conception and in pregnancy after conception. There is no information in the literature relating concentrations of these hormones to changes in plasma GLUC concentrations.

The changes in plasma UN concentrations are against a background of a constant N intake and absorption of amino acids (AA) from ruminal bacteria and food protein. In addition there will be some ammonia absorbed from the rumen (Lewis, 1957) and caecum (Thornton et al, 1970). Thus the changes in plasma UN values are probably related to variations in the AA requirements for milk production and to the anabolism/catabolism of body protein. If the AA requirements for milk production were the main determinant of plasma UN levels then one would expect peak milk yield to coincide with minimum plasma UN concentration. This was not the case: milk yield was declining before minimum UN levels were reached.

The lack of significant group differences in the concentrations of the potential indices of nutritional status, despite the large differences in ME status between the groups, would seem to indicate that blood composition may be of little use in the estimation of energy status under field conditions. However, it is necessary to eliminate the possibility that the time of sampling may have been responsible for these negative findings. It is known that the energy products of fermentation are produced rapidly after feeding, and all blood samples were taken 2.5 - 4h after animals had all consumed the same amount of silage. It is therefore conceivable that the

animals were in a transiently uniform nutritional status at the time of blood sampling.

The investigation of within-day variation in the blood composition of some of the animals used in this experiment forms the subject of the second cattle experiment.

Experiment C2. <u>Variations in bovine blood composition attributable to time of day and to energy status</u>

INTRODUCTION

Diurnal variations in the plasma concentrations of those constituents thought to monitor energy status (Allcroft, 1933; Radloff et al, 1966; Hagemeister & Unshelm, 1970; Hove & Blom, 1970), protein status (Unshelm, 1969; Thornton, 1970a) and mineral status (Unshelm & Rappen, 1968; Ehrentraut et al, 1970) have been previously reported and may have been responsible in the previous experiment for the lack of significant effects of level of feeding on plasma composition.

Few of these previous observations of diurnal variation in plasma composition were made in animals consuming high-roughage diets typical of single-suckling beef enterprises, nor were attempts made to reduce the amount of stress to which the animals were subjected during blood sampling. It is known that bovine blood composition is affected by stress, and in particular by the stress of serial restraints and venipunctures on the same day (Patterson, 1963; Gartner et al, 1965; May et al, 1974).

Information was thus required on the extent of diurnal variations in the plasma composition of animals consuming silage and sampled under conditions of minimum stress.

ANIMALS AND MANAGEMENT

This experiment was conducted with 22 of the 25 lactating Blue-grey cows used in Experiment C1, and took place approximately 50-80 days after calving. There were 8 animals in Group L, and 7 in Groups M and H. Table C2.1 shows the mean composition (R. A. Edwards, personal

communication) of the silage and barley consumed by the animals during this experiment, and these compositions were only slightly different from those given in Table C1.1. for the 200 days of Experiment C1.

Management of the animals was as described in Experiment C1, but the byre in which the animals were housed was illuminated continuously during a "dummy run" and also during the experiment. The dummy run was performed in an attempt to accustom the animals to their modified environment, and was identical to the actual experiment except that no blood samples were taken.

Blood samples were taken from the animals at 5 times during the day: 09, 15, 17, 19 and 22.00h. Each animal was sampled once daily on each of the ten days of the experiment, so that there were 2 independent blood samples per animal at each time of day. No more than 5 animals were sampled at any one time.

RESULTS

Milk yield

The mean 4% fat corrected milk yield of the animals was around 5.5 kg/day in each group (Table C2.2).

Liveweight change

The mean liveweight changes of the animals in Groups L, M and H during this experiment were -0.57, -0.32 and +0.02 kg/day respectively. These changes were derived from curves similar to those shown in Fig. C1.1.

Energy status

The estimated mean ME deficits of animals in Groups L and M were 22

and 4 MJ/day respectively, while animals in Group H were considered to be consuming excess ME over their requirements (Table C2.2). As in Fig. C1.9 of Experiment C1 it was possible to make an independent assessment of the ME deficits of the groups, assuming that the ME requirements of the animals at zero weight change were equal to their ME intakes and ignoring any changes in silage composition. Subtracting from these ME intake figures the mean ME requirements of the animals for maintenance and lactation during this experiment yielded figures of 10 and 7 MJ/day as the mean ME deficits of animals in Groups L and M. Group H animals were again considered to be consuming excess ME over their requirements.

Changes in plasma composition

Table C2.3 shows the significances of group, time of day and their interaction as sources of variation in plasma composition. Time of day was a highly significant source of variation in plasma concentrations of GLUC, FFA, KB, ALB, UN, Ca and Mg but was not for TP, GLOB, P and Cu. With the exception of TP and GLOB, there were no significant effects on blood composition of group. None of the interactions between group and time of day were significant, and Table C2.4 shows the adjusted mean concentrations of each constituent.

Energy parameters

Concentrations of all three energy parameters were affected markedly by feeding. Plasma GLUC (Fig. C2.1) and FFA (Fig. C2.2) concentrations both declined markedly within 3.5h of feeding, from 835 to 705 mg/l for GLUC and from 509 to 267 μ equiv/l for FFA. Plasma KB levels (Fig.

C2.3) increased from 17.2 to 27.9 mg/l over the same time period.

Although no overall effect of group on the concentrations of the energy parameters was noted, the 09.00h GLUC and KB concentrations in Group L were significantly ($P < 0.001$) different from the 09.00h concentrations in Groups M and H. Concentrations of FFA did not show any significant effect of group before or after feeding.

Protein parameters

Plasma TP and GLOB concentrations were relatively constant throughout the day at around 75.0 and 43.1 g/l respectively. There was a significant postprandial rise in plasma ALB concentrations from 31.4 g/l 4.5h pre-feeding to 32.7 g/l 3.5h post-feeding (Fig. C2.4). Plasma TP and GLOB concentrations in Group M animals were significantly higher at 78.2 and 47.3 g/l than were the corresponding levels in Groups L (72.6 and 40.7 g/l) and H (74.4 and 41.2 g/l).

Concentrations of UN in plasma increased from around 60 mg/l 4.5h before feeding to around 80 mg/l 5.5h after feeding (Fig. C2.5). The only effect of level of feeding was that concentrations were higher in Group H than in Group L or M, but this difference of around 14 mg/l was not significant.

Mineral parameters

The magnitudes of the statistically significant effects of time on plasma Ca (Fig. C2.6) and Mg (Fig. C2.7) concentrations were small: Ca concentrations increased from 99.2 mg/l 1.5h after feeding to 103 mg/l some 6h later. The corresponding rise for Mg concentrations was

from 21.8 to 23.8 mg/l. Concentrations of P and Cu were constant throughout the day at 48.7 and 0.95 mg/l respectively.

DISCUSSION

The postprandial changes in plasma GLUC, FFA and KB concentrations, although occurring more rapidly, are qualitatively similar to changes reported in dairy animals yielding up to 40 kg milk/day and consuming adequate amounts of hay and concentrates (Radloff et al, 1966; Hove & Blom, 1973).

The fall in plasma FFA levels upon feeding is well known (Annison, 1960) and is considered to be the result of the rate of lipogenesis in adipose tissue exceeding the rate of lipolysis, resulting in a net uptake of FFA from the blood (Reid, 1968). Even in cattle consuming 6 equal feeds per day there is a fall in FFA levels following each feed (Holmes & Lambourne, 1970).

The increase in plasma KB levels upon feeding is also well known (Knodt et al, 1942) and the rise is presumably due to ketogenesis: the conversion of ingested butyrate to β -hydroxybutyrate during ruminal absorption has been suggested as a likely source of such ketogenesis (Kronfeld, 1972).

In contrast, there is still controversy as to the nature of the postprandial response in plasma GLUC concentrations. Several workers using both sheep (Reid & Hinks, 1962b; Ambo et al, 1973; Bassett, 1974b) and cattle (Allcroft, 1933; Holmes & Lambourne, 1970; Hagemeister & Unshelm, 1970; Bowden, 1973) have demonstrated postprandial increases in the plasma concentrations of this metabolite, but Radloff et al (1966) and Hove & Blom (1973) noted postprandial falls in the plasma GLUC concentrations of dairy cows. Results

similar to those of the latter workers have also been reported from work using young calves (Preston & Ndumbe, 1961). Halse (cited by Hove, 1974) stated that the downward trend in blood sugar levels after feeding was a common phenomenon in lactating cows.

Plasma UN concentrations are known to exhibit considerable diurnal variation, this being the result of changes in ruminal ammonia concentrations (Lewis, 1957). As with GLUC, there is some uncertainty as to the exact nature of the postprandial response. The increases reported here, to maximum levels some 6.5h after feeding, agree with results obtained by Thornton (1970a) who used beef cattle offered a basal low-N ration supplemented by varying amounts of urea. In contrast, Packett & Groves (1965) reported declining plasma UN levels in the post-feeding period. The rate of recycling of urea to the rumen from the blood (Harrop & Phillipson, 1974) may be an important variable affecting the postprandial response in plasma UN levels.

The small postprandial increases in the plasma concentrations of ALB, Ca and Mg are not considered biologically significant; they are similar to earlier observations (Unshelm, 1969; Unshelm & Rappen, 1968).

The absence of group differences in plasma composition until approximately 20h after the main feed was offered may be related to the bulky nature of the diet: the rate of consumption of the silage was probably similar in all groups for several hours after feeding. Thus for some time animals would be in positive balance with respect to those nutrients which are rapidly absorbed from the rumen, notably the energy products of fermentation. Group differences in plasma composition would only develop when the rate of

absorption of a nutrient was less than the animal's requirements for that nutrient. This theory is supported by the finding that FFA concentrations in all samples taken up to 8.5h after feeding were similar to those found in well-nourished sheep and cattle.

On examination the significantly higher mean TP and GLOB levels in Group M than in Groups L and H were due to 2 animals with mean GLOB levels of 51.5 and 51.8 g/l, compared with the overall mean of 43.2 g/l. These 2 animals were 10 and 15 years old respectively, and it is considered that the hyperglobulinaemia may have been age-induced as such effects have been previously reported (Tumbleson et al, 1973).

The results of this experiment show that plasma composition is affected only to a limited extent by nutritional status, but is affected to a much greater extent by within-day factors. Because the pattern of feeding was constant, it is not possible to attribute with certainty the variations in plasma composition to prandial effects. Nevertheless, most of the changes in plasma composition which did occur appeared to be directly related to feeding, and other work has shown the importance of prandial effects when studying plasma composition.

The third cattle experiment was performed to confirm the prandial nature of the within-day variation in the plasma composition of lactating beef cows consuming silage-based rations. In order to make a precise study of the prandial effects, the animals used were only fed once per day and were sampled at pre-determined time intervals before and after feeding. In general, the animals were kept under management conditions similar to those described above.

Experiment C3. Variations in bovine blood composition attributable to time of feeding and to energy status

INTRODUCTION

In the previous experiment it was considered that the large within-day variations in plasma composition shown there may have been prandial in origin. Unfortunately the animals were fed more than once per day, thus reducing the effect on plasma composition of each individual feed and the times at which blood samples were taken did not allow precise studies of prandial fluctuations to be made.

The present study was carried out to determine the extent of the prandial fluctuations in the blood composition of animals similar to those used in the previous experiments, but which were fed only once per day.

ANIMALS AND MANAGEMENT

Fifty-two Hereford x British Friesian cows, aged 4-6 years with a mean of 4.4 $\pm$ 0.6 years, were randomly allocated shortly after parturition into groups L (20 animals), M (12 animals) and H (20 animals). As in Experiment C1, animals in these groups received feed allowances (Table C3.1) providing respectively 90,125 and 175% of their estimated ME requirements for maintenance 12h after calving. No ME allowances were made for lactation, and the compositions of the concentrate mixes offered to the groups were varied in attempts to ensure similar N intakes *in toto* by all animals.

The present experiment took place around 130 days after calving, and Table C3.1 shows the mean compositions (R. A. Edwards, personal communication) of the silage and of the concentrates offered to the animals over the

three days of the experiment. Animals were fed once daily, at 09.00h in Group L, at 11.00h in Group M and at 10.00h in Group H.

The cows were suckled by their Charolais x calves from 3 to 2.5h before feeding and from 5 to 4.5h after feeding: the calves did not have access to their dams' ration so that the milk yields of the cows could be estimated by weighing the calves before and after each suckling. In all other aspects management of the cows was as described in Experiment C1.

Blood samples were taken from each group of cows from 1h before feeding and from 3.5h after feeding. To minimize the residual effect of previous blood samplings on subsequent blood composition, the pre-feeding blood samples were taken on one day and the post-feeding samples taken on the following day. The sequence in which each group of animals was sampled was chosen at random on each occasion.

RESULTS

Milk yield

The estimated mean milk yields of animals in Groups L, M and H during this experiment were 7.25, 7.04 and 8.71 kg/day respectively. Unfortunately, no corrections could be made for the fat contents of these estimated yields.

Liveweight change

The mean rates of liveweight change of animals in Groups L, M and H during this experiment were +0.05, +0.37 and +0.72 kg/day respectively, cessation of weight loss occurring at around 115, 103 and 64 days after calving. These estimates of liveweight changes were derived from relationships similar to those shown in Fig. C1.2.

Energy status

Two estimates of the energy status of the animals can be made. Table C3.2 shows that for ME, animals in Groups L, M and H respectively were undernourished, adequately nourished and more than adequately nourished. These estimations of ME status differ from those made using rates of live-weight change: all animals were gaining weight, and may therefore be considered to be in positive energy balance. As in the earlier cattle experiments, there may have been a differential utilization of the rations by the groups, resulting in actual ME statuses considerably different from those estimated using the predicted ME content of the diet.

Changes in blood composition

Table C3.3 shows the significances of the effects on blood composition of group, before and after feeding, and of feeding within each group.

Pre-feeding, group was a significant source of variation in plasma GLUC, FFA, KB, ALB, UN, Ca, Mg and P concentrations but was not for plasma TP, GLOB and blood PCV levels. Post-feeding group differences in blood composition were less significant, being restricted to plasma GLUC, ALB, Ca, Mg and P concentrations.

Feeding resulted in significant changes in the plasma concentrations of GLUC, FFA, KB, Mg and P, with smaller changes seen in TP, ALB and Ca concentrations, and no significant effect observed in levels of GLOB, UN or PCV.

Energy parameters

Plasma GLUC levels in all groups declined markedly on feeding from 690 - 750 mg/l to 550 - 700 mg/l. The change in GLUC levels

was greater in Groups L and M than in Group H. Plasma FFA levels in Group L also declined on feeding, from 496 to 265 μ equiv/1, but the concomitant declines in Groups M and H were not significant. Plasma KB levels increased substantially in all groups from 12 - 17 mg/1 before feeding to 38 - 43 mg/1 after feeding.

Pre-feeding, Groups L animals maintained lower plasma GLUC levels (690 mg/1), and higher plasma FFA levels (496 μ equiv/1), than did animals in Groups M (756 mg and 282 μ equiv/1) and H (753 mg and 252 μ equiv/1). Plasma KB levels were higher in Group H (17.4 mg/1) than in Groups L (13.9 mg/1) and M (11.8 mg/1).

The higher mean plasma GLUC level in Group H (690 mg/1) than in Groups L (554 mg/1) and M (603 mg/1) was the only significant post-feeding group difference in plasma concentrations of the energy parameters.

Protein parameters

Plasma TP and GLOB concentrations in each group remained effectively constant at around 60 and 36 g/1 respectively, although the small prandial increase in TP levels in Group L from 60.7 to 62.0 g/1 was significant. Plasma ALB levels in Group L increased from 23.5 g/1 before feeding to 25.5 g/1 after feeding, and for this constituent a smaller rise, from 26.0 to 26.6 g/1, was noted in Group H animals. Levels of ALB in the plasma of Group M animals, and of UN in the plasma of animals in each group, were unaffected by feeding.

Before feeding, ALB levels in Group H (26.0 g/1) were higher than levels in Groups L (23.5 g/1) and M (23.4 g/1). After feeding, this

group difference was less significant, levels in Group H (26.6 g/l) being higher than levels in Group M (24.3 g/l). Plasma UN levels were differentiated according to group both before and after feeding, the mean levels in Groups L, M and H being around 120, 100 and 75.0 mg/l respectively.

Mineral parameters

Plasma Ca concentrations in Group H decreased slightly upon feeding from 96.0 to 93.5 mg/l, but there was no effect of feeding upon concentrations of the constituent in the other groups. Plasma Mg concentrations decreased in all groups from 26.2 - 28.0 mg/l before feeding to 23.9 - 26.2 mg/l after feeding. Animals in Groups L and M showed similar prandial rises in plasma P concentrations, from 68.7 and 63.2 mg/l to 71.8 and 70.5 mg/l. The small prandial rise in plasma P concentrations in Group H was not significant.

Plasma concentrations of all three mineral parameters were differentiated according to group both before and after feeding. Plasma Ca levels in Group H at 96.0 mg/l pre-feeding and 93.5 mg/l post-feeding were 7 - 13 mg/l higher than concentrations in Groups L and M; the corresponding Mg levels being 28.0 and 26.2 mg/l and the difference 1.2 - 2.3 mg/l. By contrast, plasma P concentrations at 57.6 mg/l pre-feeding and 59.2 mg/l post-feeding were 6 - 12 mg/l lower than the corresponding levels in Groups L and M.

Haematocrit

No significant effect of group or of feeding on blood PCV levels were noted, the range in levels being from 26.2 to 27.5%.

DISCUSSION

The only biologically significant prandial changes in plasma composition noted here were in concentrations of GLUC, FFA and KB, although small changes did occur upon feeding in the concentrations of most of the other plasma constituents. The prandial changes in the concentrations of the energy parameters reported here are similar in magnitude and in duration to the changes observed in the main feeding period (which occurred at a different time of day) of Experiment C2, thus discounting the theory that the changes in plasma composition observed in that earlier experiment were unrelated to feeding but were due to an inherent circadian rhythm.

As discussed in the previous experiment, the postprandial changes in plasma FFA and KB concentrations are considered to be due respectively to the rate of lipogenesis in adipose tissue exceeding the rate of lipolysis resulting in an uptake of FFA from the blood, and to ruminal ketogenesis from absorbed butyrate. Also discussed in the earlier work was the fact that the postprandial decline in plasma GLUC levels observed there was not in agreement with the bulk of previous work. The results of the present study confirm the existence in lactating beef cows consuming high-roughage rations of a prolonged decline in plasma GLUC concentrations after feeding.

The differing postprandial responses in plasma GLUC concentrations noted by the different groups of workers may have resulted from the differing compositions of the diets used. In most of the work where postprandial increases were noted (e.g. Holmes & Lambourne, 1970; Bowden, 1973; Jenny & Polan, 1975) the rations contained a high proportion of concentrates, while postprandial decreases have in general only been reported in animals

consuming high-roughage diets (Radloff et al, 1966: Hove & Blom, 1973). Further work is required to elucidate the exact mechanisms involved in these differing responses: it is possible that the relatively high energy cost of consuming the high-roughage diets may prove important in such work.

The small postprandial increases in plasma ALB concentrations noted here were similar to changes noted by Unshelm (1969), and may have been caused by reductions in plasma volume (Christopherson & Webster, 1972). However, under such circumstances concomitant changes in plasma GLOB and blood PCV levels would be expected to occur, changes that were not in fact observed, suggesting that other factors were involved in this prandial change in plasma composition.

The decline in plasma Mg concentrations and the increase in plasma P concentrations upon feeding in each group of animals are in direct opposition to earlier results (Unshelm & Rappen, 1968; Ehrentraut et al, 1970), and this difference may also have been due to the nature of the diet used in the present work. Unshelm & Rappen (1968) noted small postprandial increases in plasma Ca concentrations and a similar (but non-significant) effect was noted here in Groups L and M only, implying an effect in the prandial response of plasma Ca levels of level of dietary intake.

Significant effects of feeding on ruminant plasma UN concentrations have been noted previously, both in Experiment C2 and in other work (Lewis, 1957; Thornton, 1970a). The complete lack in the present work of significant prandial variations in plasma UN concentrations was therefore unexpected, although it is possible that changes in concentrations of this plasma constituent did occur more than 3½h after feeding (see Fig. C2.5). As mentioned above,

no significant effects of feeding on blood PCV levels were noted, a result intermediate between the postprandial declines noted by Unshelm (1968) and the increases noted by Bowden (1973). Little of this previous work was in animals consuming high-roughage rations, so that dietary composition may have an effect on the prandial response in plasma UN and blood PCV levels.

The effectively complete inability of the energy parameters to distinguish between groups of animals consuming considerably different amounts of the same diet agrees well with the results of recent work (Erfle et al, 1974; Fisher et al, 1975) where poor correlations were calculated between estimated ME status and plasma GLUC, FFA and KB concentrations. As in Experiments C1 and C2, there may have been different efficiencies of utilization of the rations by the groups, resulting in mean ME statuses different from those calculated in Table C3.2. Nevertheless, there were undoubtedly nutritional differences between the groups, both in rates of liveweight gain and in time elapsed since cessation of weight loss.

It is well known (A.R.C., 1965) that the law of diminishing returns applies to the responses of energy retention in mature animals to successive increments of feed. Suggested reasons for the curvilinearity include the depressions which are known to occur in D.M. digestibility at high feeding levels (Blaxter, 1962), changes in ruminal fermentation and passage of digesta, and differences between the efficiency of energy utilization in anabolism/catabolism of body tissues.

Most of the remaining group differences in plasma composition are small and are not considered biologically significant. The one exception is in

plasma concentrations of UN, which in the past have been shown to be highly correlated with N intake (Prewitt et al, 1971). From the results of this previous work, the plasma UN concentrations in Groups L and M in the present work would be expected to be similar, and to be lower than the levels in Group H (Table C3.2). In fact, the lowest plasma UN levels were seen in Group H animals, and the highest in Group L, with intermediate levels in Group M (Tables C3.4, C3.5). The higher N concentration in the rations offered to the Group L animals (Table C3.1) may be a possible source of the discrepancy between predicted and actual plasma UN concentrations: it is known that groups of animals fed similar total N intakes from different sources maintain different plasma UN levels (Abou Akkada & el Sayed Osman, 1967).

SUMMARY AND CONCLUSIONS

Summary

In Experiment C1, designed to investigate the changes in bovine blood composition attributable to stage of lactation and to level of energy intake, concentrations of some plasma constituents declined with falling daily milk yield while others showed non-linear variations. Of particular interest was the positive relationship between plasma GLUC concentration and daily milk yield. No significant effects of level of energy intake on plasma composition were noted, and it was considered that a differential efficiency of utilization of the rations by the nutritionally distinct groups may have contributed to this lack of significance.

Experiment C2 investigated the possiblity that within-day factors may

have contributed to the lack of significant effects of group in the previous experiment. Marked within-day variations were observed in the concentrations of several plasma constituents, the most pronounced changes being in plasma GLUC, FFA, KB and UN levels. Plasma GLUC levels declined markedly after feeding, this result being in direct contrast to the results of most previous work. The effects of level of feeding on plasma composition were small, and again it was calculated that there were differential efficiencies of utilization of the rations by the groups.

Experiment C3 was successful in confirming the prandial nature of most of the diurnal variations in plasma composition reported in the previous experiment. The most pronounced prandial fluctuations were in plasma concentrations of the energy parameters, the changes being similar in magnitude and duration to those reported in Experiment C2. Blood composition 1h pre-feeding was influenced markedly by level of feeding, the most significant effects again being in plasma concentrations of the energy parameters. Blood composition 3½h post-feeding was affected by level of feeding to a much lesser extent than was pre-feeding blood composition, confirming that the time of blood sampling is an important source of variation in bovine blood composition.

The three cattle experiments showed conclusively that the blood composition of lactating beef cows is modified considerably by stage of lactation and by within-day factors, particularly by time of feeding, but is modified to a much lesser extent by level of feeding. Specialized blood sampling conditions were required before the nutritionally distinct groups of animals could be distinguished using the blood parameters, and even under these conditions there were poor correlations between nutritional status and blood composition.

Conclusion

The mean nutritional status of groups of beef cows, often at widely different stages of lactation and usually fed more than once per day, cannot be accurately predicted using as parameters those blood constituents used in the present study.

Table C1.1. Rations offered, with estimated ME and N balances at maximum milk yield

	Group			Pooled
	L	M	H	S.D.
Weight post-partum (kg)	551	555	525	52
Maximum milk yield (kg/day)	8.30	8.44	8.33	1.59
Conception date (days post-partum)[i]	70	69	100	36
Rations consumed (kg/day)				
Fresh silage offered	16.8	23.4	31.4	1.7
Silage D.M. intake	3.54	4.79	6.06	0.34
Fresh barley offered	1.68	2.39	3.11	0.16
Barley D.M. intake	1.37	1.95	2.35	0.13
ME balance (MJ/day)				
Intake	50.5	69.6	88.9	4.5
Requirements [ii]	93.0	93.9	91.0	9.6
Intake/requirements (%)	54.4	74.5	98.3	7.7
N balance (g/day)				
Intake	57.1	78.4	99.9	5.1
Requirements [iii]	85.0	86.2	84.0	12.4
Intake/requirements (%)	68.1	93.6	121	17.5

i Subsequent parturition date, minus 283 days.
ii 0.489 MJ/kg $(W)^{0.75}$; 4.51 MJ/kg 4% fat milk. (A.R.C., 1965)
iii 0.039 g/kg W; 7.68 g/kg milk. (A.R.C., 1965)

Table C1.2. Significances of sources of variation in blood composition

	Group	Time period	Interaction
	$F_{2,22}$	$F_{19,215}$	$F_{38,215}$
GLUC	NS	* * *	NS
KB	NS	* * *	NS
TP	NS	* * *	NS
ALB	NS	NS	NS
GLOB	NS	* * *	NS
UN	NS	* * *	NS
Ca	NS	NS	NS
Mg	NS	NS	NS
P	NS	*	NS

Table C1.3. Least-squares mean group concentrations ($\pm$ S.E.) of blood constituents

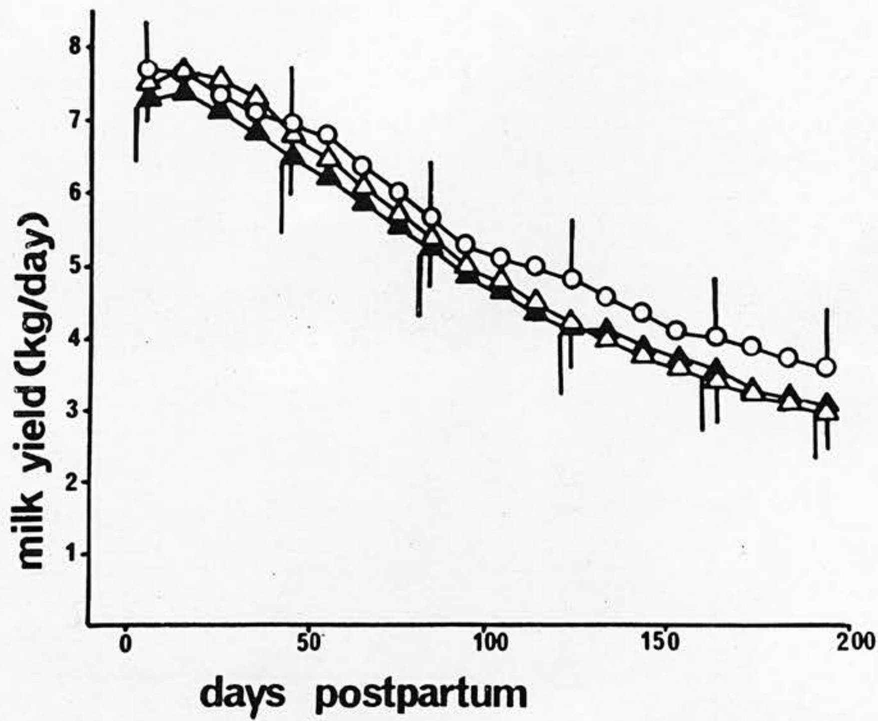
	Group		
	L	M	H
n	100	102	95
GLUC	835 $\pm$ 8.00	842 $\pm$ 8.8	889 $\pm$ 8.9
KB	16.6 $\pm$ 0.89	13.6 $\pm$ 0.98	16.4 $\pm$ 1.00
TP	68.8 $\pm$ 0.51	71.0 $\pm$ 0.56	70.7 $\pm$ 0.57
ALB	26.3 $\pm$ 0.28	26.8 $\pm$ 0.31	27.2 $\pm$ 0.31
GLOB	42.5 $\pm$ 0.48	44.2 $\pm$ 0.52	43.4 $\pm$ 0.53
UN	62.8 $\pm$ 1.99	63.4 $\pm$ 2.18	75.2 $\pm$ 2.20
Ca	97.6 $\pm$ 0.63	96.6 $\pm$ 0.69	99.1 $\pm$ 0.70
Mg	22.0 $\pm$ 0.28	20.6 $\pm$ 0.31	22.1 $\pm$ 0.31
P	48.4 $\pm$ 0.80	47.0 $\pm$ 0.87	46.3 $\pm$ 0.88

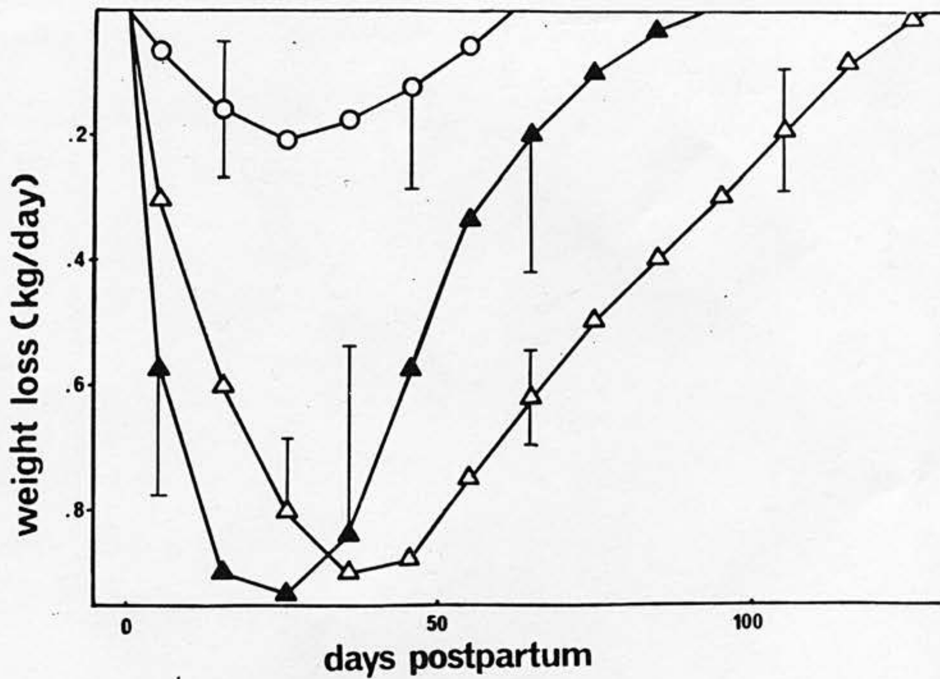
Table C1.4. Calculated ME content of silage, from ME requirements data based on ME requirements at zero weight change.

	Group		
	L	M	H
* ME requirement for maintenance (MJ/day)	47.7	50.3	51.7
ME requirement for lactation (MJ/day)	20.6	22.3	30.2
Total ME requirement (≑ total ME intake) (MJ/day)	68.3	72.6	81.9
ME supplied by barley (MJ/day)	17.9	25.3	30.5
Total ME intake - ME supplied by barley (MJ/day)	51.6	47.3	51.4
Silage D.M. intake (kg/day)	3.54	4.79	6.06
ME content of silage (MJ/kg D.M.)	14.2	9,87	8.48

* The above calculations were based on A.R.C. (1965) data. Using more recent requirements (M.A.F.F., 1975), the ME content (MJ/kg D.M.) of the silages would be:

13.8 9.60 8.23

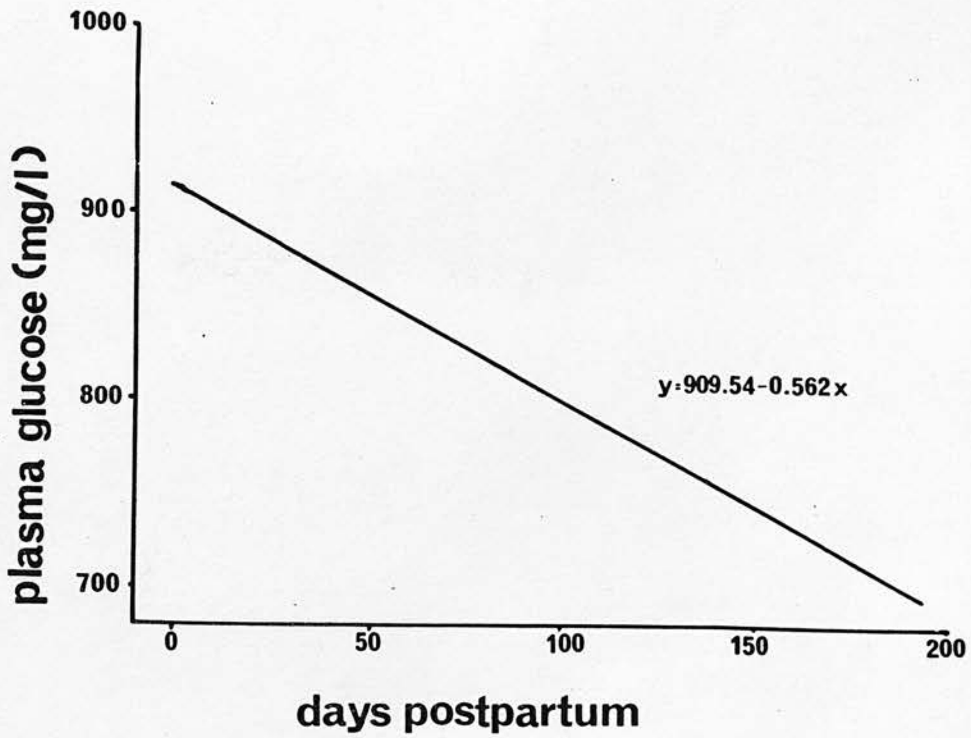


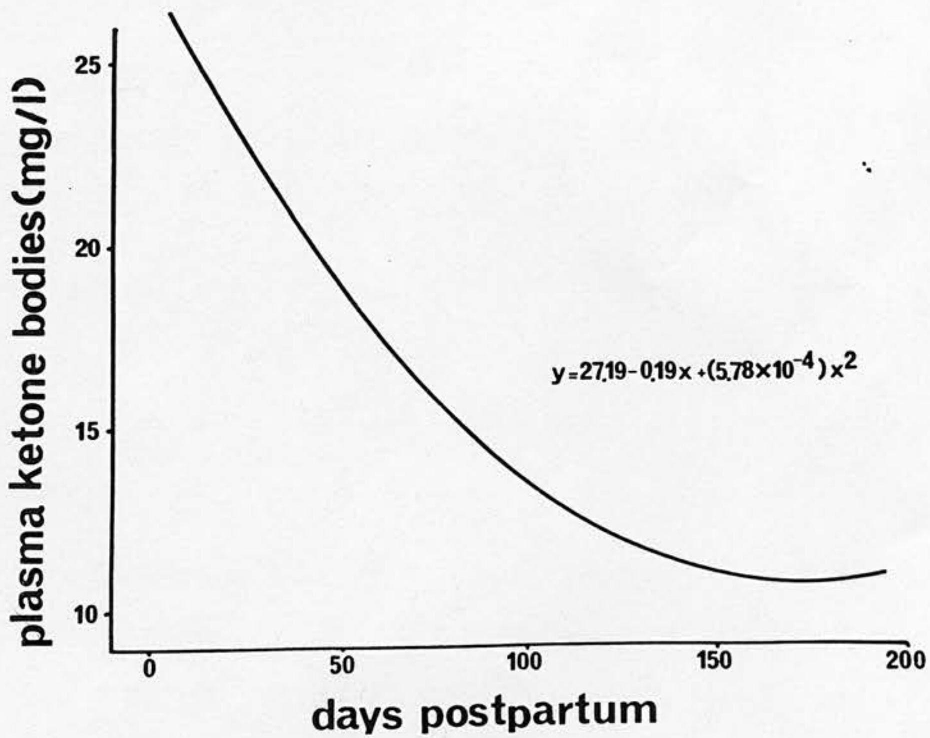


Figures C1.1, C1.2

Lactation curves and post-partum weight changes (± S.E.) in 3 nutritional groups of lactating beef cows.

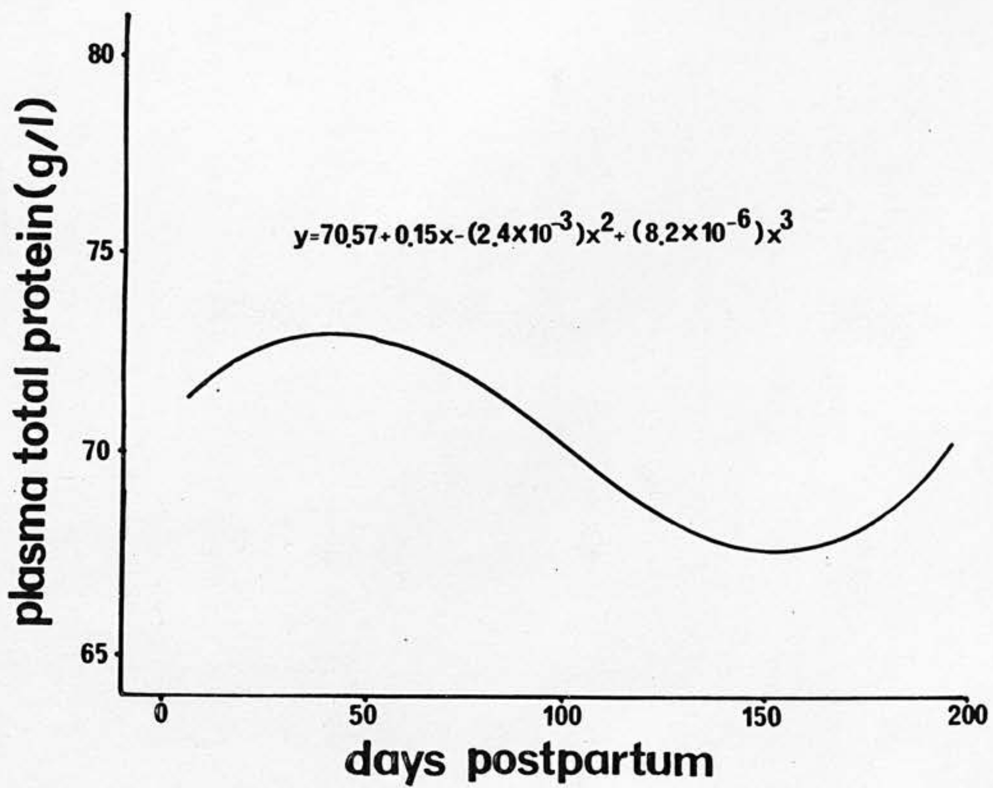
△—△ Group L, ▲—▲ Group M, ○—○ Group H

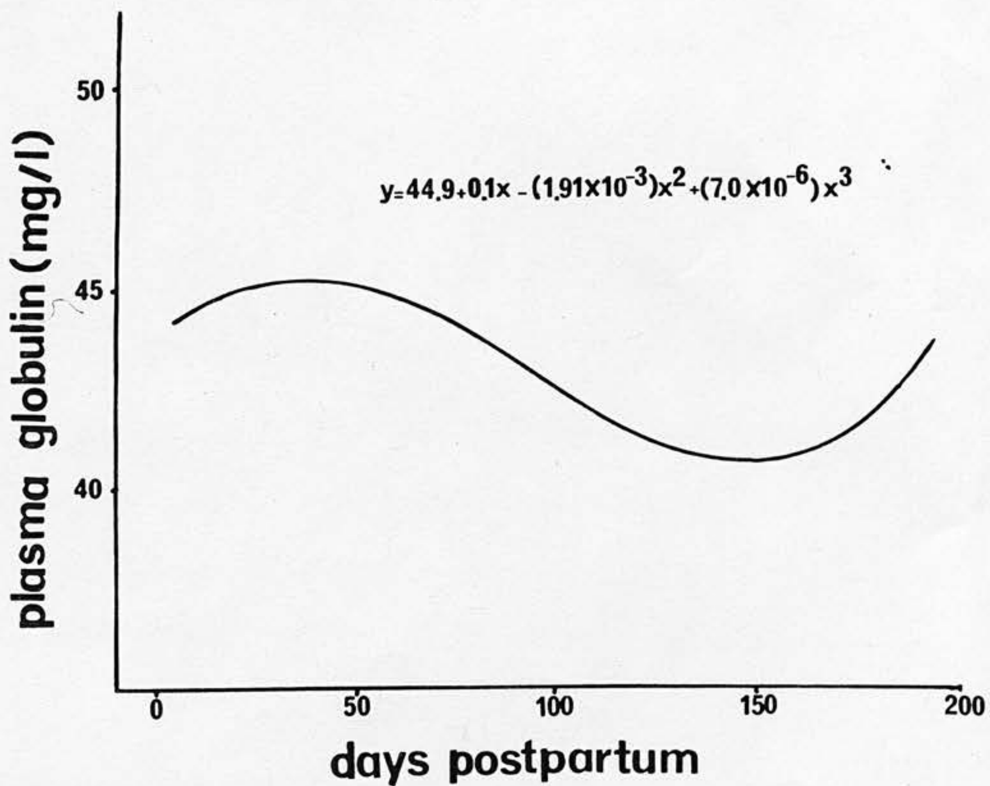




Figures C1.3, C1.4

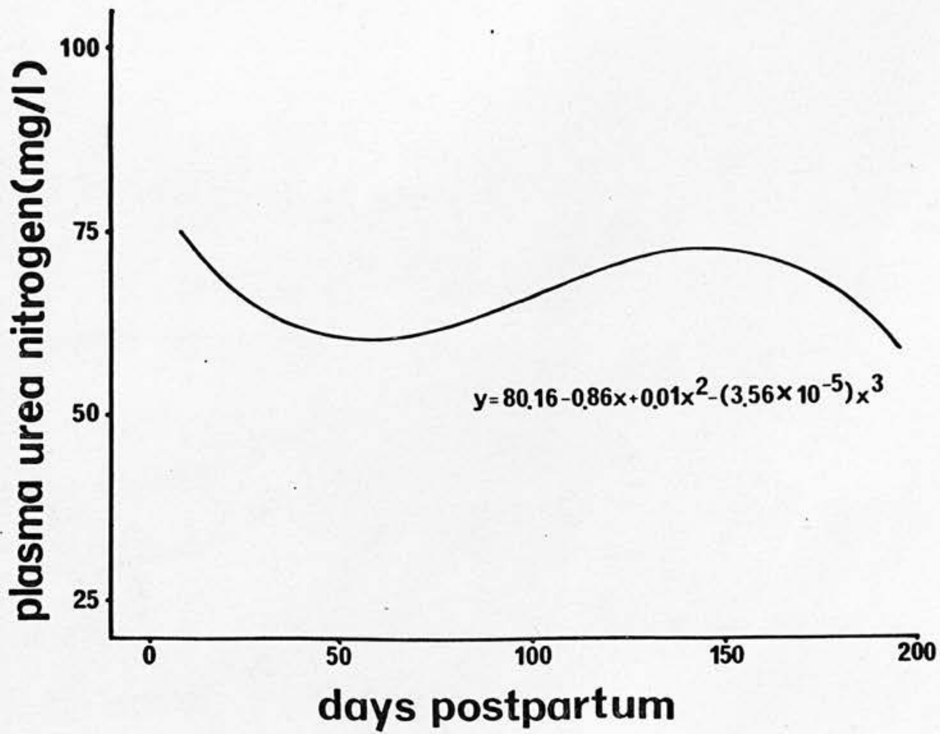
Effect of time post-partum on plasma glucose and ketone body concentrations of lactating beef cows.

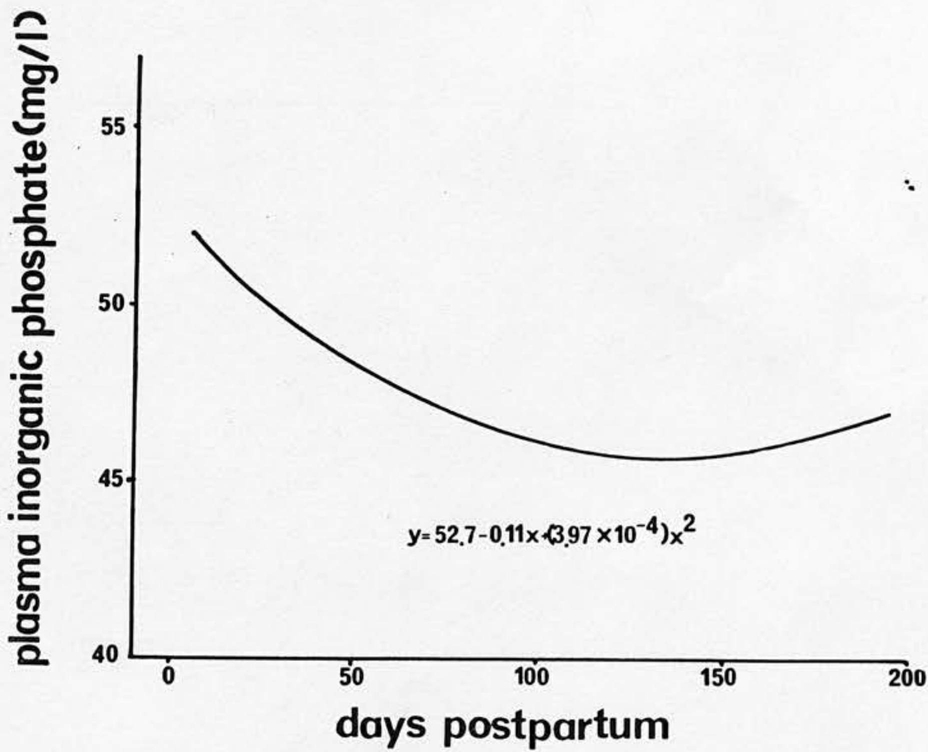




Figures C1.5, C1.6

Effect of time post-partum on plasma total protein and globulin concentrations of lactating beef cows.





Figures C1.7, C1.8

Effect of time post-partum on plasma urea nitrogen and inorganic phosphate concentrations of lactating beef cows.

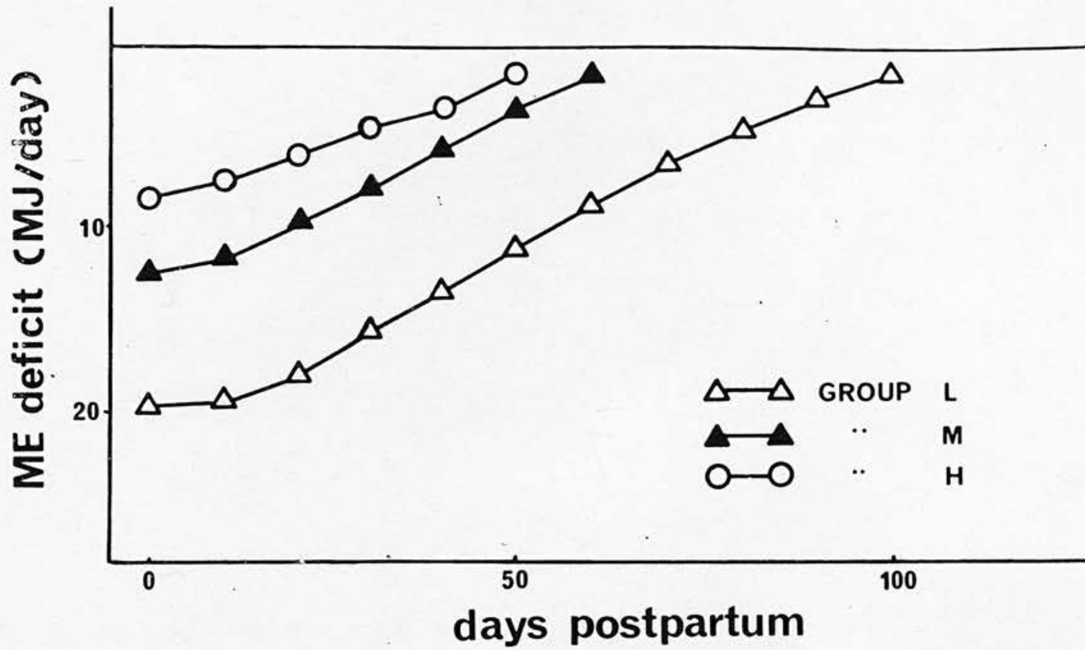


Figure C1.9

Estimated ME deficiencies in 3 nutritional groups of lactating beef cows.

Table C2.1. Composition and rate of feeding of rations

Mean silage composition

D.M.	pH	MAD-F[i]	CP[ii]	DCP[iii]	ME
(%)		(% in D.M.)			(MJ/kg D.M.)
21.6	3.93	38.8	11.3	7.54	9.64

i Modified acid detergent fibre.
ii Crude protein.
iii Digestible crude protein.

Mean barley composition

D.M.	CF[i]	EE[ii]	NFE[iii]	Ash	CP	DCP	ME
(%)			(% in D.M.)				(MJ/kg D.M.)
74.7	3.7	1.2	59.8	1.95	8.1	6.64	12.9

i Crude fibre.
ii Ether extract.
iii Nitrogen free extract.

Rations consumed (kg/day)

	Group			Pooled
	L	M	H	S.D.
Fresh silage offered	16.8	23.6	31.9	1.6
Silage D.M. intake	3.64	4.90	6.26	0.36
Fresh barley offered	1.68	2.43	3.16	0.13
Barley D.M. intake	1.25	1.81	2.36	0.10

Table C2.2. Estimated ME and N balances

	Group			Pooled
	L	M	H	S.D.
Weight (kg)	464	479	489	52
Weight as % of weight at parturition	83.7	85.2	91.2	5.5
Days post-partum	60	51	80	28
Daily milk yield (kg)	5.46	5.51	5.50	2.0
ME balance (MJ/day)				
Intake	51.3	70.3	90.2	4.4
Requirements[i]	73.5	74.3	75.2	9.0
Intake/requirements (%)	70.0	95.0	120	12.8
N balance (g/day)				
Intake	56.9	78.0	99.9	5.1
Requirements[ii]	59.9	60.9	61.2	15.2
Intake/requirements (%)	98.5	140	175	48

i 0.489 MJ/kg $(W)^{0.75}$; 4.51 MJ/kg milk. (A.R.C., 1965)
ii 0.039 g/kg W; 7.68 g/kg milk. (A.R.C., 1965)

Table C2.3. Significances of sources of variation in blood composition

	Group	Time	Interaction
	$F_{2,19}$	$F_{4,183}$	$F_{8,183}$
GLUC	NS	* * *	NS
FFA	NS	* * *	NS
KB	NS	* * *	NS
TP	*	NS	NS
ALB	NS	* * *	NS
GLOB	*	NS	NS
UN	NS	* * *	NS
Ca	NS	* * *	NS
P	NS	NS	NS
Mg	NS	* * *	NS
Cu	NS	NS	NS

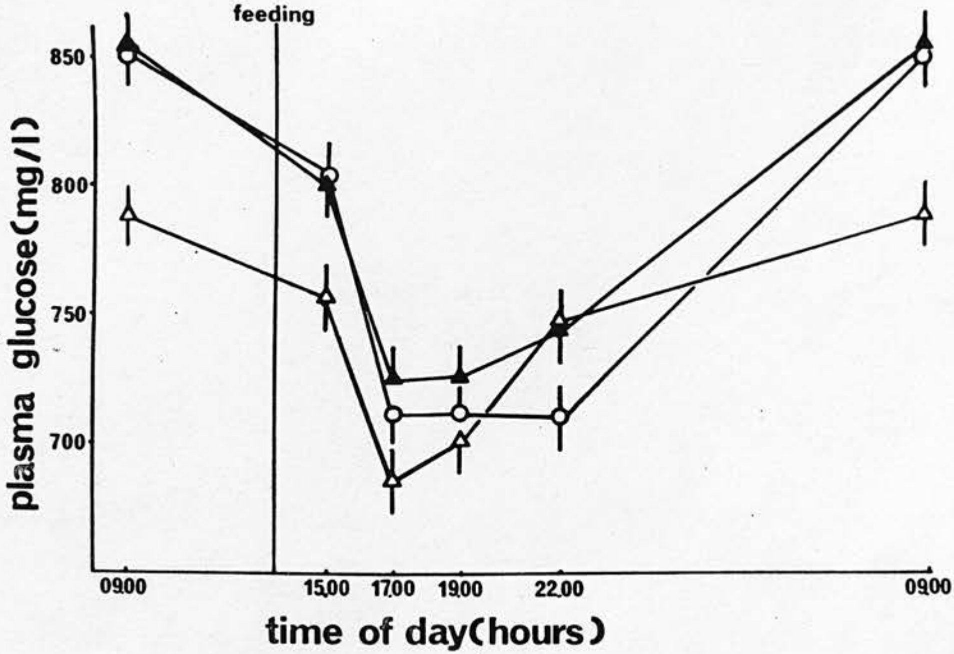
Table C2.4. Least-squares mean group concentrations (± S.E.) of blood constituents

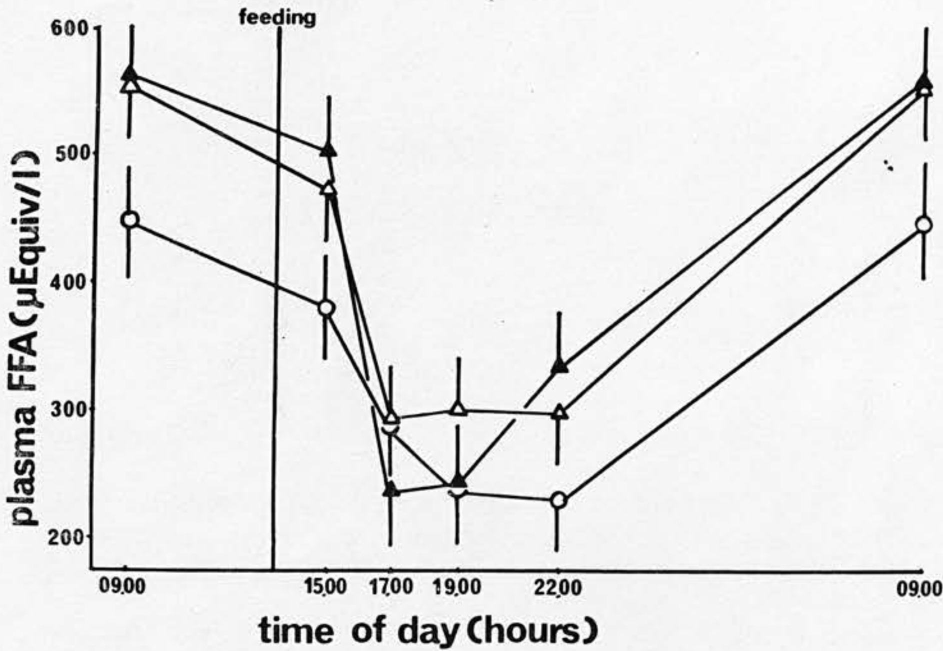
	Group		
	L	M	H
n	80	68	69
GLUC	737 ± 7.0	774 ± 7.6	760.00 ± 7.6
FFA	381 ± 17.6	378 ± 19.1	312 ± 19.0
KB	25.3 ± 0.74	22.7 ± 0.81	25.5 ± 0.80
TP	72.6 ± 0.39	78.2 ± 0.42	74.4 ± 0.42
ALB	31.8 ± 0.16	30.8 ± 0.17	33.2 ± 0.17
GLOB	40.7 ± 0.32	47.3 ± 0.35	41.2 ± 0.35
UN	65.3 ± 1.85	63.9 ± 2.01	77.9 ± 2.00
Ca	100 ± 0.3	100 ± 0.4	100 ± 0.3
P	50.7 ± 0.68	48.1 ± 0.74	47.3 ± 0.73
Mg	23.3 ± 0.19	21.7 ± 0.20	22.9 ± 0.20
Cu	0.87 ± 0.012	1.07 ± 0.014	0.91 ± 0.014

Table C2.5. Least-squares mean time concentrations (± S.E.) of blood constituents

	Sampling Time (h)					
	09.00	15.00	17.00	19.00	22.00	S.E.(i)
n	44	44	44	41	44	
GLUC.	835	790	705	711	735	9.5
FFA	509	451	267	259	287	23.8
KB	19.2	17.4	27.9	29.4	29.2	1.01
TP	74.9	74.7	76.4	74.9	74.5	0.53
ALB	31.3	31.4	32.7	32.3	32.0	0.21
GLOB	43.6	43.3	43.7	42.6	42.5	0.44
UN	59.7	64.2	74.4	79.4	67.2	2.51
Ca	102	99.2	99.8	100	103	0.48
P	49.9	48.1	48.5	48.3	48.8	0.92
Mg	22.3	21.8	22.5	22.8	23.8	0.26
Cu	0.95	0.97	0.98	0.94	0.92	0.017

i S.E.'s for 19.00h sampling marginally higher.





Figures C2.1, C2.2

Least-squares mean values (± S.E.) of plasma glucose and FFA concentrations in 3 groups of lactating beef cows at 5 times during the day.

△—△ Group L, ▲—▲ Group M, ○—○ Group H

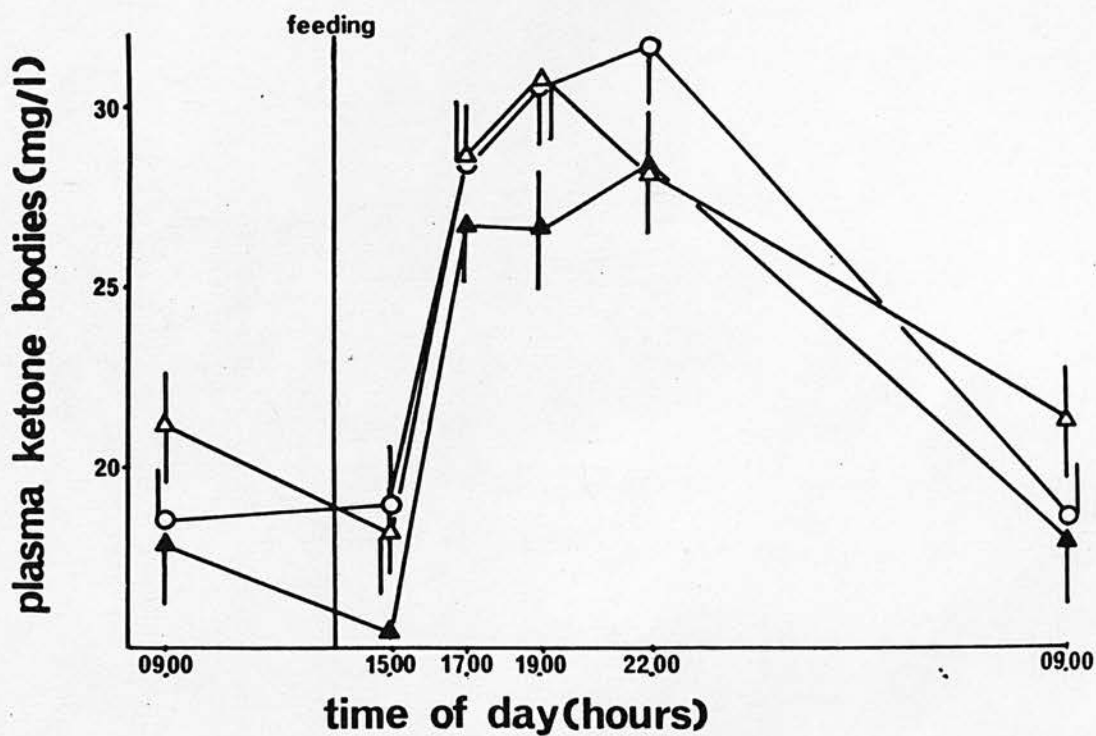
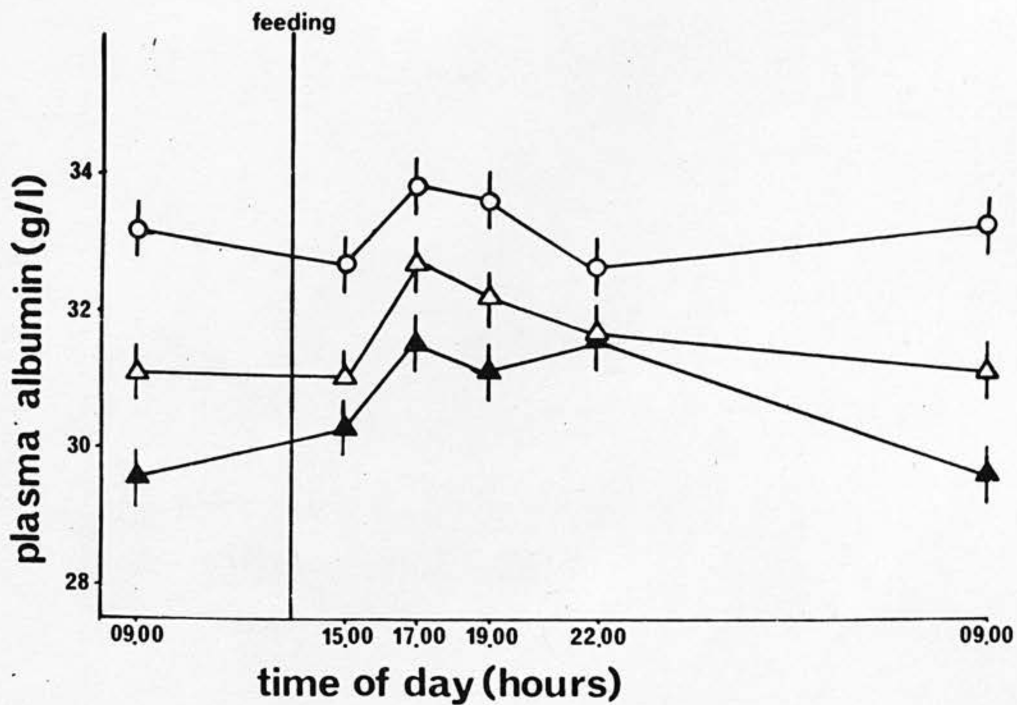
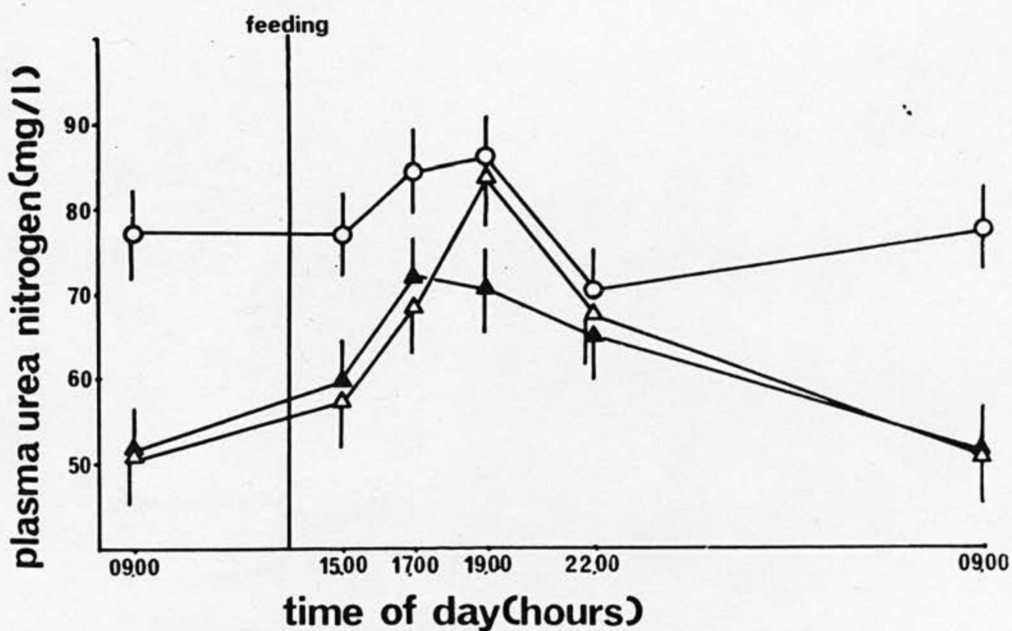


Figure C2.3

Least-squares mean values ($\pm$ S.E.) of plasma ketone body concentrations in 3 groups of lactating beef cows at 5 times during the day.

△—△ Group L, ▲—▲ Group M, ○—○ Group H

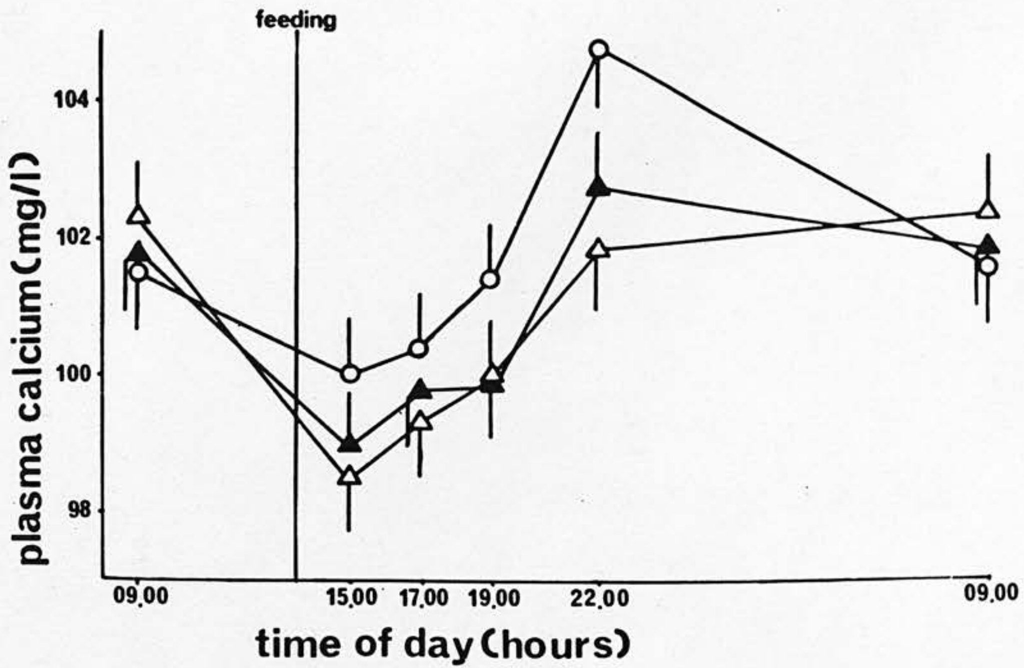


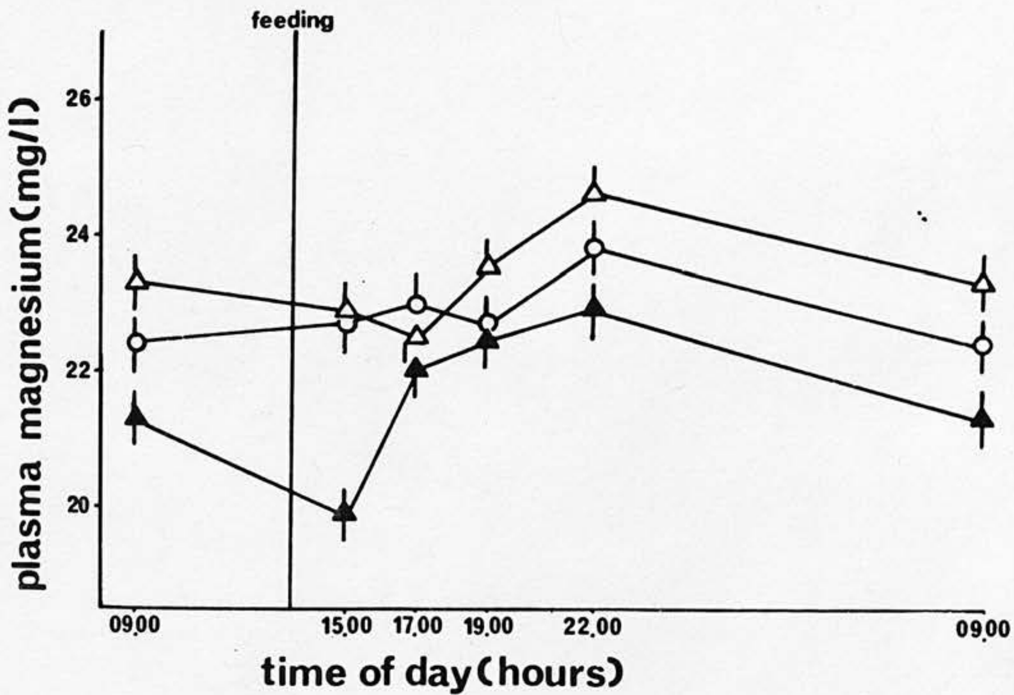


Figures C2.4, C2.5

Least-squares mean values (± S.E.) of plasma albumin and urea nitrogen concentrations in 3 groups of lactating beef cows at 5 times during the day.

△—△ Group L, ▲—▲ Group M, ○—○ Group H





Figures C2.6, C2.7

Least-squares mean values (± S.E.) of plasma calcium and magnesium concentrations in 3 groups of lactating beef cows at 5 times during the day.

△—△ Group L, ▲—▲ Group M, ○—○ Group H

Table C3.1. Composition[i] and rate of feeding of rations

Silage composition

D.M.	pH	MAD-F	CP	DCP	ME
(%)		(% in D.M.)			(MJ/kg D.M.)
28.4	3.76	30.8	12.6	7.81	10.6

Concentrate composition

	D.M.	CF	EE	NFE	Ash	CP	DCP	ME
	(%)			(% in D.M.)				(MJ/kg D.M.)
Group L	85.3	7.2	0.5	33.2	17.9	41.2	37.7	10.5
Group M	84.5	4.6	0.4	62.6	11.7	20.7	15.7	11.5
Group H	85.0	3.7	0.7	78.1	6.4	11.1	8.4	13.1

i For assumptions made in calculation of DCP and ME contents of rations, see Appendix XI.

Rations consumed (kg/day)

	Group			Pooled
	L	M	H	S.D.
Fresh silage offered	18.8	25.0	36.3	2.7
Silage D.M. intake	5.28	7.02	9.65	1.03
Fresh concentrate offered	0.85	1.02	1.38	0.08
Concentrate D.M. intake	0.72	0.87	1.17	0.07

Table C3.2. Estimated ME and N balances

	Group L	Group M	Group H	Pooled S.D.
Weight (kg)	393	409	500	49
Weight as % of weight at parturition	74.1	82.1	95.2	4.9
Estimated milk yield (kg/day)	7.25	7.04	8.71	1.63
Days post-partum	123	138	132	29
ME balance (MJ/day)				
Intake	63.6	84.8	118	11
Requirements[i]	79.3	79.6	95.1	10
Intake/requirements (%)	80.8	107	124	13.6
N balance (g/day)				
Intake	109	110	136	14.7
Requirements[ii]	71	70.1	86.4	13
Intake/requirements (%)	159	161	160	32

i 0.489 MJ/kg $(W)^{0.75}$. (A.R.C., 1965)
5 MJ/kg milk. (R.A. Edwards, unpublished)*

ii 0.039 g/kg W; 7.68 g/kg milk. (A.R.C., 1965)

* for derivation, see M.A.F.F. Bulletin 33 (1975).

Table C3.3. Significances of sources of variation in blood composition

	Group ($F_{2,49}$)		Time (i)		
	Pre-feeding	Post-feeding	Group L	Group M	Group H
GLUC	* * *	* * *	* * *	* * *	* *
FFA	* * *	NS	* * *	NS	NS
KB	* * *	NS	* * *	* * *	* * *
TP	NS	NS	* *	NS	NS
ALB	* * *	*	* * *	NS	*
GLOB	NS	NS	NS	NS	NS
UN	* * *	* * *	NS	NS	NS
Ca	* * *	* * *	NS	NS	*
Mg	* *	* * *	* * *	* *	* * *
P	* *	* * *	*	* *	NS
PCV	NS	NS	NS	NS	NS

(i) Using appropriate 't' test.

Table C3.4. Pre-feeding mean group concentrations of blood constituents

	Group			Pooled	Group Difference[i]		
	L	M	H	S.D.	L v. M	M v. H	L v. H
n	20	12	20				
GLUC	690	756	753	79	* * *	NS	* * *
FFA	496	282	252	145	* * *	NS	* * *
KB	13.9	11.8	17.4	3.7	NS	* * *	* * *
TP	60.7	59.8	61.1	4.3	NS	NS	NS
ALB	23.5	23.4	26.0	2.2	NS	* * *	* * *
GLOB	37.2	36.4	35.1	4.5	NS	NS	NS
UN	122	100	74.0	12.7	* * *	* * *	* * *
Ca	84.6	84.6	96.0	5.7	NS	* * *	* * *
Mg	26.2	26.9	28.0	1.6	NS	NS	* * *
P	68.7	63.2	57.6	9.7	NS	NS	* *
PCV	27.5	27.4	26.2	3.4	NS	NS	NS

(i) Using appropriate 't' test.

Table C3.5. <u>Post-feeding mean group concentrations of blood constituents</u>

	Group			Pooled	Group Differences[(i)]		
	L	M	H	S.D.	L v. M	M v. H	L v. H
n	20	12	20				
GLUC	554	603	690	73	NS	* *	* * *
FFA	265	266	240	46	NS	NS	NS
KB	43.2	37.8	38.7	10.0	NS	NS	NS
TP	62.0	60.4	60.7	4.8	NS	NS	NS
ALB	25.5	24.3	26.6	2.2	NS	* *	NS
GLOB	36.5	36.1	34.1	5.1	NS	NS	NS
UN	120	104	75.7	11.9	* * *	* * *	* * *
Ca	85.5	86.6	93.5	4.7	NS	* * *	* * *
Mg	23.9	25.0	26.2	1.8	NS	NS	* * *
P	71.8	70.5	59.2	10.0	NS	* * *	* * *
PCV	26.9	27.1	26.5	3.8	NS	NS	NS

(i) Using appropriate 't' test.

CHAPTER 4

SHEEP EXPERIMENTS

Undernutrition of hill ewes is commonly found in winter under UK conditions, and supplementary feeding must be given if large-scale ewe mortality is to be avoided. Such feeding is costly and to gain the maximum economic benefit, the composition and timing of the supplementation must be carefully controlled. When and what to feed depends upon the degree and nature of the undernutrition and the state of the body reserves, all of which are unknown under hill conditions.

Recent work has shown a relationship between the nutritional status of a number of essential nutrients and ovine blood composition, and there is some hope that a system of nutritional monitoring of hill sheep based upon changes in blood composition can be developed. Such a system would provide information not only on the type of supplementary feeding to give, but also whether or not the supplementation had been successful.

The present experiment represents a crude attempt to model the nutrition of the hill ewe in winter. The quality and quantity of the diet offered to the animals were both varied in a manner considered to be similar to that occurring on the hill. The availability of hill herbage to sheep varies throughout the winter and from hirsel to hirsel, so that the D.M. intake of individual animals may often be less than appetite. The digestibility of hill herbage also varies considerably, ranging from low in mid- and late winter, the period usually coincident with early pregnancy, to high in spring, usually coincident with lactation.

The experiment is presented in two parts. The first (Experiment S1) deals with the effects of level of feeding and of pregnancy and lactation

on blood composition; the second (Experiment S2) with the within-day changes in blood composition when the food intakes of groups of animals were offered in one, two or three feeds per day. The latter experiment served two purposes, firstly to determine the time of day at which the effects of the treatment on blood composition were maximal, and secondly to determine at what frequency of feeding prandial variation in ovine blood composition disappeared.

Experiment S1. Variations in ovine plasma composition attributable to physiological status and to level of nutrition

INTRODUCTION

In the sheep, relationships between level of intake of energy (Annison, 1960; Reid & Hinks, 1962b; Russel et al, 1967; Russel & Doney, 1969; Sykes & Field, 1972b), protein (Preston et al, 1965; Sykes & Field, 1973) and minerals (Chicco et al, 1973; Field et al, 1975) and the plasma concentrations of those constituents used as indices of the intakes of the individual nutrients have been well documented. There are also reports of pregnancy and lactation affecting plasma composition, particularly concentrations of those constituents used as indices of energy status (Reid & Hinks, 1962a; Ross & Kitts, 1969; Karihaloo et al, 1970) and protein status (Fell et al, 1968; Mackie & Fell, 1971; Robinson et al, 1973; Sykes & Field, 1973). There are few comparisons in the literature of level of nutrition and of physiological state as sources of variation in plasma composition (Davies, Johnston & Ross, 1971).

The present investigation was carried out to investigate the changes in ovine plasma composition produced by undernutrition at levels considered to be similar to those which occur under field conditions, to monitor those changes throughout pregnancy and lactation, and to compare the mean plasma composition of the undernourished animals with the corresponding composition in a control group of well-nourished animals.

ANIMALS AND MANAGEMENT

Thirty-two $5\frac{1}{2}$-year old Scottish Blackface ewes, weighing 39-56 kg with a mean of 44.4 $\pm$ 3.7 kg, were mated at pasture to Scottish Blackface rams. Ovulation was synchronized using progesterone-impregnated pessaries (Gordon, 1971), and mating took place 16-18 days after pessary removal; twelve ewes were served a second time some 16 days later.

Three to four weeks after mating, the ewes were brought indoors and allocated at random to one of two groups. Group R consisted of 24 animals and was offered a diet calculated to meet 70% of the ewe's requirements for ME, N, Ca, P and Mg in early pregnancy, late pregnancy and lactation. Some of the requirements for pregnancy and lactation were based on modifications of those given by the Agricultural Research Council (A.R.C., 1965) for a ewe bearing a single lamb of 6 kg at birth and giving a maximum milk yield of 2.3 kg/day (Peart, 1967). The second group, containing 8 animals, was offered the same diet as Group R animals but in amounts sufficient to meet appetite.

Three diets were used: diet EP was offered to the ewes from the beginning of pregnancy up to 40 days before parturition, diet LP until parturition and

diet LA during lactation. The diets were obtained by varying the proportions of individual ingredients of a basic diet consisting of oat hulls, starch, sugar, dried skim milk and blood meal (Table S1.1). Diets of very similar compositions have been used previously at rates of feeding similar to those used here (Sykes, unpublished), and so estimates of D.M. and N digestibilities were already available. The estimates were confirmed in subsequent balance trials, the details of which will not be given here. Sykes & Dingwall (1976) noted that there was only a small difference in the digestibilities of D.M. and N between animals on restricted intakes and animals fed to appetite, so that in the present work the same digestibility figures were used for Groups R and A when calculating nutritional status.

The ME contents of the diets were calculated using the following relationships:

1 g digestible dry matter = 4 Kcal digestible energy (Blaxter, 1962)

and digestible energy x 0.82 = metabolizable energy (Blaxter, Clapperton & Martin, 1966)

The digestible N contents of the diets were estimated by determining their total N content in Kjeldahl digestions and multiplying the resulting figures by the N digestibilities of the diets. The analytical methods for determining the Ca and Mg contents of the the diets were those of Suttle & Field (1969), P concentrations in the acid digests of the diets being determined by the method of Robinson <u>et al</u> (1971).

It was calculated that offering 0.34 of EP, 0.65 of LP and 1.6 kg/day of LA would acheive the required levels of undernutrition at the different stages of pregnancy and during lactation. To prevent the occurence of digestive disorders, all animals were offered 0.2 kg/day of chopped hay.

The hay was drawn from a single batch and had a mean D.M. of 90%, D.M. digestibility of 66% and N digestibility of 67% (Sykes, unpublished). The mean composition of the hay was 7.4 g N, 2.9 g Ca, 2.7 g P and 0.8 g Mg per kg D.M.

Group R animals were kept in individual pens and were offered their total daily ration of semi-purified diet and hay in a single feed at 08.00h. Only rarely were there any diet refusals. Group A animals were kept collectively in a semi-covered yard: individual dietary intakes were unknown, and all calculations of nutritional status were based on the mean amount consumed per head. All animals had free access to tap water.

After parturition, the lambs from both groups of ewes remained with their dams either in the pens or in the semi-covered yard. Lambs could thus consume the rations offered to their dams: the amounts so consumed were small until lambs were around 4 weeks old. Each lamb was weighed shortly after birth and at weekly intervals thereafter.

Ewes were weighed and blood sampled on consecutive days at fortnightly intervals throughout pregnancy and lactation: both operations took place from 08.00h, before Group R animals were fed. Ewes were also weighed at shearing, shortly after parturition and at the end of the experiment.

Plasma samples were assayed for GLUC, FFA, KB, TP, ALB, GLOB, UN, Ca, P and Mg; for technical reasons the results of the TP (and GLOB) assays were unsatisfactory and are not presented here.

RESULTS

Owing to an error in formulation, all of the EP and LP diets and some of the LA diets were Mg deficient, resulting in the death of 4 lactating ewes

from hypomagnesaemic tetany. The results from these ewes, and those from the three barren ewes and the three which produced twins, have been discarded. There remained 22 ewes which produced and suckled single lambs: 16 in Group R and 6 in Group A.

Intake of nutrients

Tables S1.3, S1.4 and S1.5 show respectively the estimated mean amounts of each of the macro-nutrients available to or ingested by the Group R animals in early pregnancy, late pregnancy and in lactation. The tables also give estimates at these times of the requirements of the ewe for the nutrients, and approximate figures are given for the intakes of and requirements for nutrients by the Group A animals.

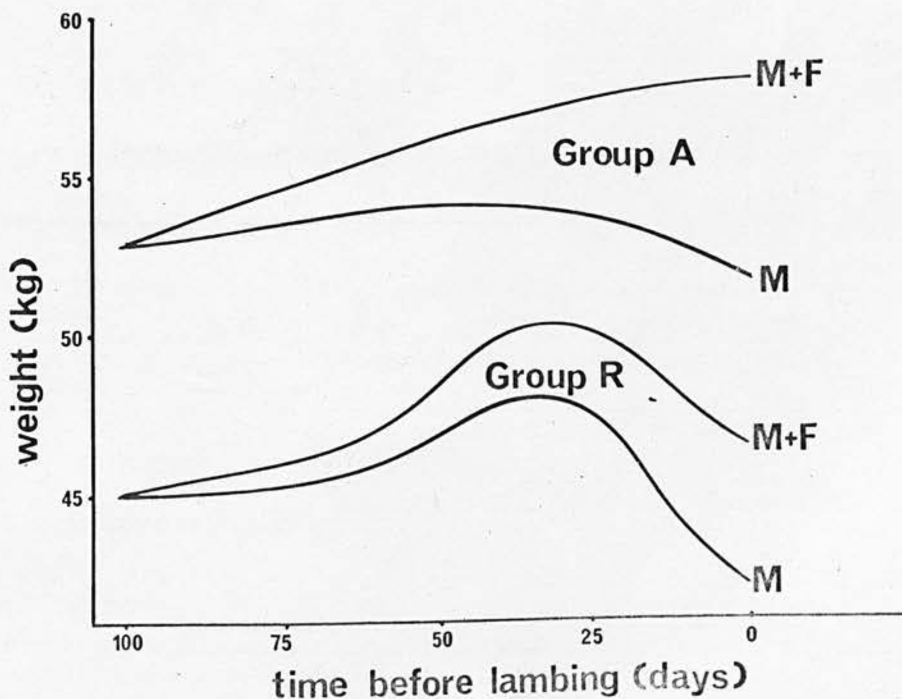
For ME and N, the intake figures were calculated from the composition, rate of feeding and digestibility of the diets: they are the amounts actually available to the ewe. For the minerals the intake figures were derived solely from the composition and rate of feeding of the diet, the availability of the minerals to the ewe being considered in the estimation of the ewe's requirements for those minerals.

The Mg content of both pregnancy diets was around 0.02%, so that the total Mg intakes of the Group R animals in early and late pregnancy respectively were around 0.21 and 0.28 g/day, the corresponding figures for Group A being 0.49 and 0.63 g/day. The estimated Mg requirements of animals in early and late pregnancy are around 0.6 and 0.9 g/day respectively (assuming an endogenous loss of 2.5 mg/kg liveweight/day and an availability of 20%: A.R.C., 1965), so that throughout pregnancy both groups of animals were Mg-deficient.

Liveweight changes of ewes and lambs

Both groups of ewes increased in liveweight from the beginning of the experiment until around 30-40 days before lambing. From then until the end of the experiment Group A animals maintained liveweight at around 58 kg, whereas the mean weight of Group R animals steadily declined to around 40 kg thirty days after lambing (Fig. S1.01).

The changes in maternal liveweight throughout pregnancy are masked by the concomitant increases in the weight of the gravid uterus. Changes in maternal liveweight (M) may be estimated by subtracting from the weight of the dam, fetus and uterus (M + F) the estimated weights of the gravid uterus at different stages of pregnancy (A.R.C., unpublished):



From the above curves, it can be seen that the ewes in both groups increased in weight until around 35 days before lambing, followed in Group R animals by a rapid decline in weight until parturition. This decline coincided approximately with the change from early to late pregnancy rations. Table S1.6 shows for both groups the percentage change in maternal liveweight from mating to shortly after lambing, along with the birth weights of the lambs.

The mean growth rates of both groups of lambs were similar at around 0.26 kg/day until approximately 30 days after birth, when there was a marked increase in the growth rate of Group A lambs (Fig. S1.02).

Milk yield

Milk yields of the ewes were calculated from the growth rates of the lambs using the assumption of Peart (cited by Sykes & Dingwall, 1975) that 6 grams of milk are required for each gram of liveweight gain. Peak growth rates of the lambs were seen around 10 days after birth, and corresponded to maximum milk yields in Groups R and A of 1.58 and 1.66 kg/day respectively.

Changes in plasma composition: effect of level of feeding

The degree of variation in plasma composition produced by level of nutrition in early pregnancy, late pregnancy and lactation was estimated by comparing the overall mean plasma compositions of Groups R and A in these periods (Table S1.7). No comparisons of plasma composition were made between the different feeding periods. In general the mean plasma composition of Group A animals reflected their higher plane of nutrition, but statistically significant ($P < 0.05$) group differences in plasma composition

were restricted to Ca and P in early pregnancy and to KB, ALB and Ca in lactation.

The effect of group on plasma composition was in some cases modified by the effect of duration of feeding, resulting in significant ($P < 0.05$) interaction effects. The latter were noted in early pregnancy for plasma concentrations of KB and Ca in late pregnancy for GLUC, ALB and Ca and in lactation for GLUC and ALB.

Effect of duration of feeding

For statistical analyses and for ease of presentation data from chemical assays of plasma samples were apportioned into six, four and five 10-day time periods within early pregnancy, late pregnancy and lactation respectively. The changes in the mean plasma composition of both groups of animals throughout the experiment are shown by 10-day period in Figs. S1.03 - S1.09.

Within each of the three feeding periods the D.M. intakes of the individual animals in Group R were kept constant, so that any variations in their plasma composition within the periods were considered to have been caused by changing physiological state. The significances of 10-day time periods within each feeding period as sources of variation in Group R plasma composition are shown in Table S1.8. Inferences cannot be drawn from the changing plasma compositions of Group A animals because direct measurements of the changing D.M. intake of individual animals within this group were not made. The following results refer only to Group R animals.

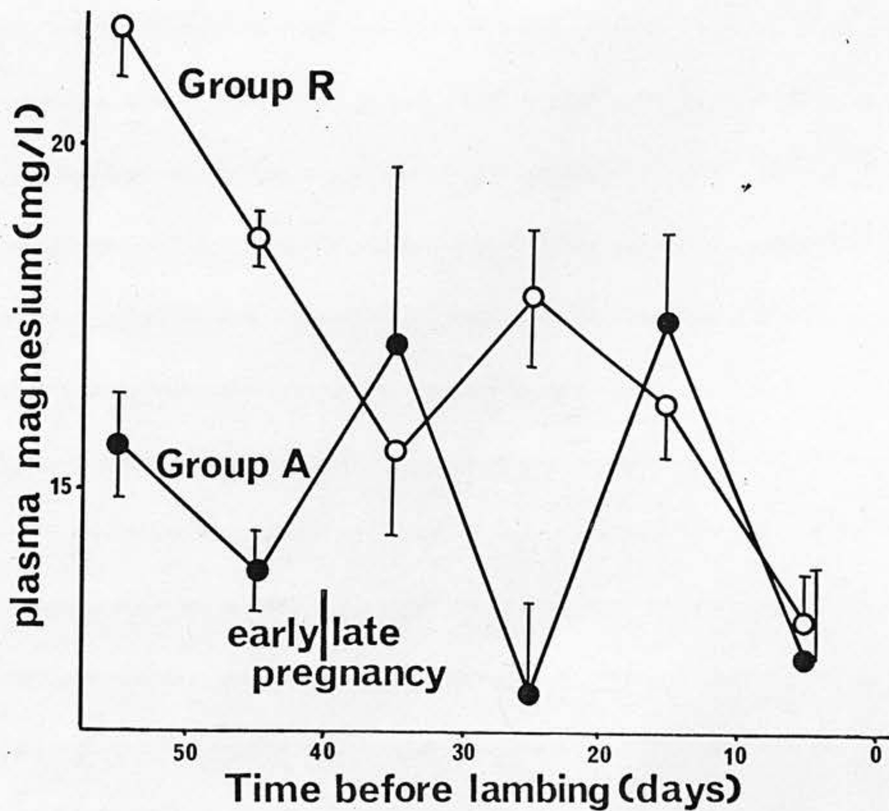
Plasma GLUC levels declined in early pregnancy from 610 mg/l in the first 10-day time period to 490 mg/l in the sixth, the change in late pregnancy being a transient rise to 575 mg/l in the third time period (Figure S1.03).

Plasma FFA concentrations increased from 371 to 866 μ equiv/l in early pregnancy, reaching 961 μ equiv/l in the 10-day time period immediately prior to parturition (Figure S1.04). Figure S1.05 shows the statistically non-significant ($P > 0.05$) variations in plasma KB concentrations.

Plasma ALB concentrations declined in early pregnancy to 23.9 g/l from levels of around 27 g/l, falling steadily in late pregnancy from 25.0 to 20.7 g/l. In lactation, plasma ALB levels increased from 22.3 to 28.5 g/l (Figure S1.06). Plasma UN levels in lactation were constant at 147 mg/l apart from the values of 92.3 mg/l observed in the fourth period after parturition (Fig. S1.07).

Plasma Ca concentrations increased in lactation from 93.5 to 109 mg/l (Figure S1.08), while plasma P concentrations peaked at 61.8 mg/l in the second 10-day time period after parturition (Figure S1.09).

The ewes received Mg-supplementation at different stages of lactation, so that the results of Mg assays of plasma samples taken from lactating animals will not be presented here. The mean plasma Mg concentration of Group R animals was 20.2 mg/l in early pregnancy and 15.6 mg/l in late pregnancy, these levels being significantly ($P < 0.01$) greater than the corresponding levels in Group A (14.7 and 14.8 mg/l). The following figure shows the declining plasma Mg concentrations of both groups of pregnant animals, the time relationships of the Group R animals in both early and late pregnancy being statistically significant ($P < 0.05$). The interaction between group and time was significant ($P < 0.05$) in late pregnancy:



DISCUSSION

The main aim of the present work was to investigate the changes in ovine plasma composition produced by undernutrition throughout pregnancy and lactation. It is thus essential to be able to define in each of the feeding periods the exact level of the undernutrition imposed on the Group R animals.

Energy parameters

The rations used in early pregnancy provided the Group R animals with an estimated 0.08 MJ ME / kg W / day, a figure approximately 62% of that given by Sykes & Field (1972b) for the maintenance requirement of the pen-fed, non-pregnant ewe. The corresponding figure for the Group A animals was 0.26 MJ / kg W / day. No allowances have been made in these calculations for the ME requirements of the developing fetus, as these are known to be small

in the first 100 days of gestation (Cloete, 1939).

Such a dietary ME deficit should have led to a loss of fat and protein from the maternal body. In the absence of data on changes in body composition it is difficult to be precise about how much of each type of tissue would have been lost. Firstly, there was a concomitant protein deficiency which may have favoured protein catabolism, and secondly, it is not known with what efficiency body tissues are used as energy sources for maintenance.

It can be calculated that body tissue should have theoretically provided 140 MJ for maintenance. Assuming calorific values of 38.5 and 17.1 MJ/kg respectively for fat and protein, and that the two are catabolized in the approximate ratio (w:w) of 4 : 1 (Russel, unpublished), this energy deficit is equivalent to a loss of around 4 kg of body tissues. It must be stressed that this value is probably a minimal one, in that 100% efficiency of utilization has been assumed.

Contrary to theoretical considerations the net weight of the ewes increased during early pregnancy. A number of possibilities can be advocated to explain this observation. One is that the body tissues were replaced by water: it is known that there is an increase in the volume of extracellular water in late pregnancy (Sykes & Field, 1972a), but there are no specific reports on this aspect in early pregnancy. This explanation does not seem probable as the accumulation of water necessary to explain the observed weight changes (estimated 4 kg body tissue loss, plus 3 kg body weight gain) would require an increase of around 70% in the volume of extracellular water.

Another possibility is that the estimated intake of ME is in error. This again would appear unlikely, in that the digestibility of the organic matter (DOM) in the EP diet was determined directly in a balance trial (Coggins &

Field, unpublished), and the other factors used in the conversion of DOM to ME were common to all calculations of ME requirements. The most probable explanation therefore is that the maintenance requirement of energy proposed by Sykes & Field (1972b) is an overestimate and that the energy deficits of the present ewes were less than theoretical considerations would indicate.

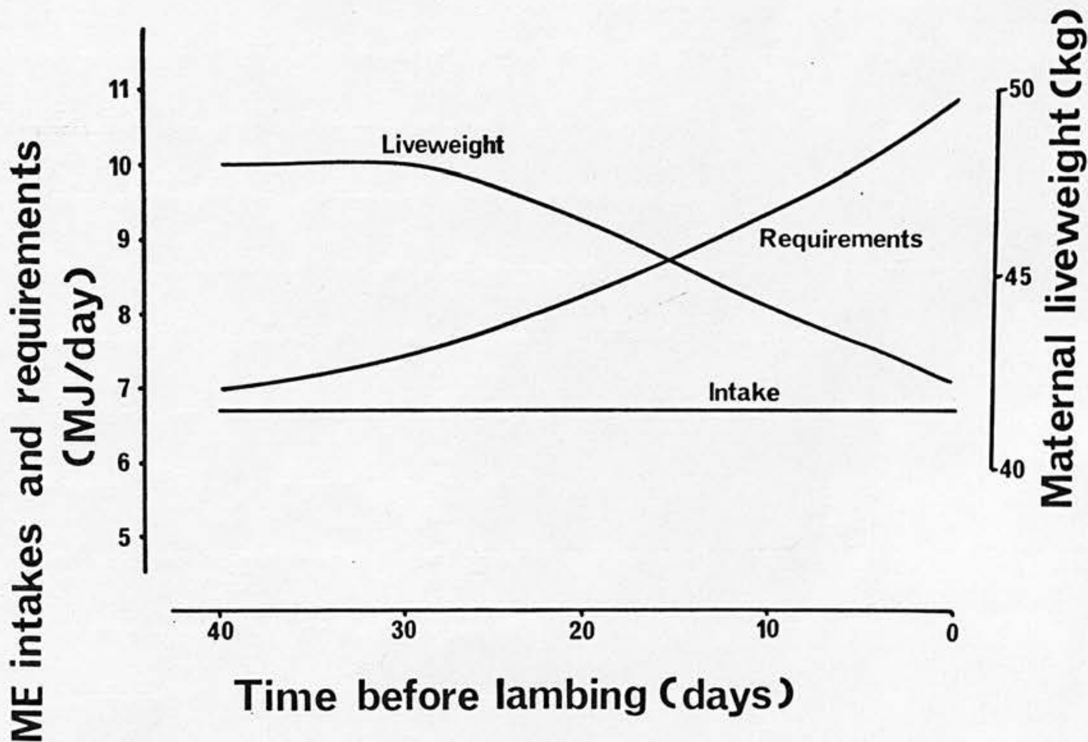
That the Group R animals were energy deficient in early pregnancy is suggested by the finding that the plasma FFA levels in this group were much higher than those normally observed in animals gaining in liveweight (Annison, 1960). The significance of this finding is lessened however by the fact that the FFA levels in the adequate energy intake group were not significantly different from those of the Group R animals, the marked tendency for the Group R values to be higher than the corresponding ones for Group A (Fig. S1.04) being masked by the large individual differences within the groups.

It is clear from the above discussion that a precise estimate of undernutrition is only possible if data is available on body composition. Even then an accurate estimate of body change is only possible if the changes in the energy content of the body are large relative to individual differences in the energy content of the pre-slaughter group, even though individual differences can often be measured by covariance analysis on body weight. It is thus not possible in the present experiment to reach any firm conclusions about the value of FFA in describing undernutrition in the ewes in early pregnancy.

The declining plasma GLUC levels of both groups of animals in early pregnancy are unlikely to have been the direct result of the increasing GLUC requirements of the developing fetus (Reid, 1968), because as mentioned above the fetus is relatively small until around 50 days <u>pre-partum</u> (Cloete,

1939). In addition, the decline was of similar magnitudes in both groups whereas more pronounced effects of an increased drain of nutrients to the fetus would have been expected in animals on restricted intakes of ME. The fall may have resulted from hormonal changes associated with pregnancy.

The situation regarding the relationship between the theoretical energy deficiency of the ewes and their body weight was much clearer in late pregnancy, in that the energy deficient ewes lost weight. That the ewes were energy deficient throughout late pregnancy can be clearly seen from the following figure, where the ME intake and requirements of the pregnant ewes are shown together with the corresponding change in maternal body weight.



One interesting result which has a bearing on the interpretation of data on FFA levels is the fact that the cumulative energy deficit of the ewes during

this period was 120 MJ, which would correspond to a mobilization of fat and protein in the approximate ratio (w:w) of 1 : 9. Thus there appeared to be a selective mobilization of protein rather than fat in late pregnancy, but the exact ratio will depend in part on the efficiency with which those body tissues are used for energy, and on the increase in body water. Whether this switch is due to a reduction in the fat reserves, or to the concurrent protein deficiency, cannot be resolved with the present experimental design.

The finding that the plasma concentrations of the energy parameters remained effectively constant throughout late pregnancy was surprising, and may indicate the relative insensitivity of these parameters to energy or GLUC shortages. One would expect the concentrations of FFA and KB to increase, and those of GLUC to decline, as the requirements of the fetus for energy increased throughout pregnancy against a constant dietary intake (Reid, 1968).

It is generally accepted that the concentration of FFA in the plasma of severely undernourished ewes in late pregnancy will lie between 1100 and 2500 μ equiv/l (Annison, 1960; Patterson, 1963; Russel et al, 1967). The plasma concentration of FFA in the Group R animals never rose above 900 μ equiv/l and averaged about 800 μ equiv/l, indicating that either the animals were not in severe energy deficit or, as suggested above, little fat was mobilized to meet the energy deficit.

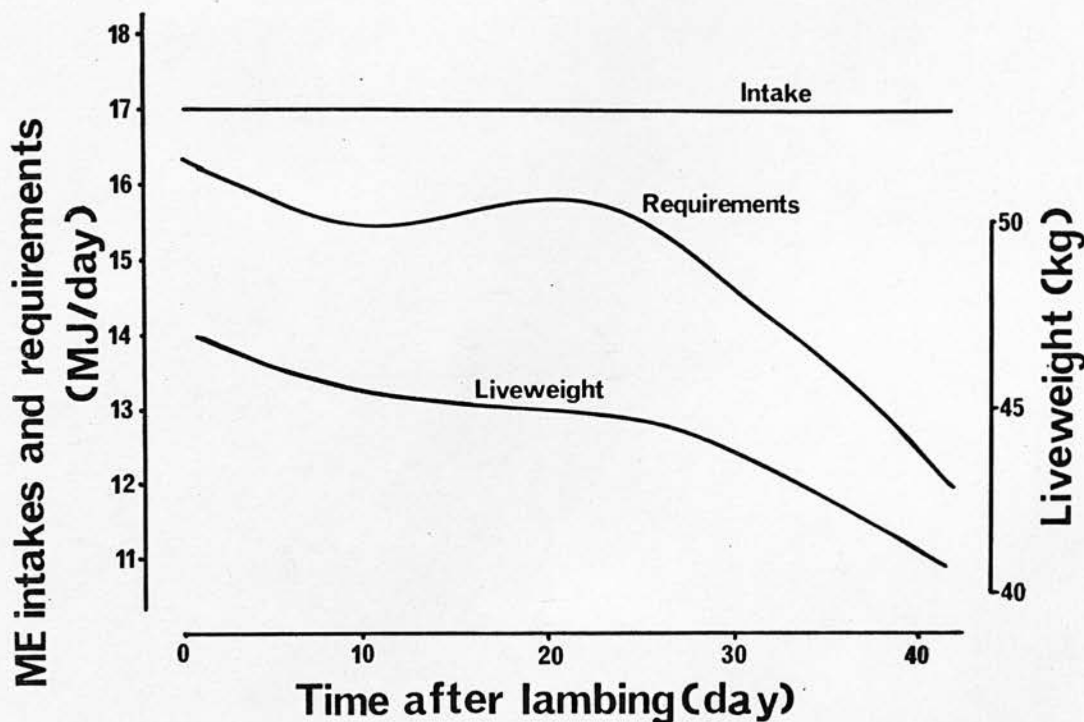
In late as in early pregnancy it was not possible to maintain plasma FFA levels in Group A below 300-400 μ equiv/l, although the ewes were calculated to be consuming excess energy over their requirements and maintained net liveweight. Sykes & Field (1972b) could not maintain the plasma FFA levels of animals fed virtually ad libitum below 650-700 μ equiv/l, postulating that hormonal changes occurring during pregnancy may influence concentrations

of this plasma constituent. Heaney & Lodge (1975) suggested that in the monotocous ewe fed to appetite and in positive energy balance, elevation of plasma FFA levels is a normal occurence, indicative of fat mobilization, but not necessarily indicative of inadequate energy intakes. The involvement of the mobilized fat in the caloric homeostasis of the ad libitum fed ewe in late pregnancy is unknown.

No significant difference in the birthweights of the lambs from Group R and A animals was found, despite the fact that the ewes in the former group were subjected to a combined energy and protein deficiency. Sykes & Field (1972b) found a significant effect on lamb birthweight of a protein and energy deficiency of the same order as that used in the present study, but not with an energy deficiency alone.

It is interesting to compare the overall changes in maternal weight of the Group R animals relative to those of Robinson & Forbes (1966) and of Sykes & Field (1972b), who used comparable deficiencies of energy and protein. The Group R animals lost 0.7% body weight, much less than the losses of -12 and -21% reported respectively by the above workers. No explanation can be suggested for these conflicting results.

Little can be said about the relationship between the plasma concentrations of the energy parameters and level of nutrition in lactation, because as shown in the following figure and in Table S1.5 animals in both groups received adequate amounts of energy and protein. The daily milk yields of both groups were lower than the yields predicted from single-suckling ewes on planes of nutrition similar to those used here (Peart, 1967), and this is the main reason why the Group R ewes were not undernourished in lactation as originally intended.



Despite the adequate energy intake of both groups, plasma FFA concentrations remained above the resting level (200-300 μ equiv/l: Annison, 1960). The same observation of relatively high plasma FFA concentrations in ovine lactation has been reported by Reid & Hinks (1962a), and has been tentatively attributed to fat mobilization to support lactation.

Protein parameters

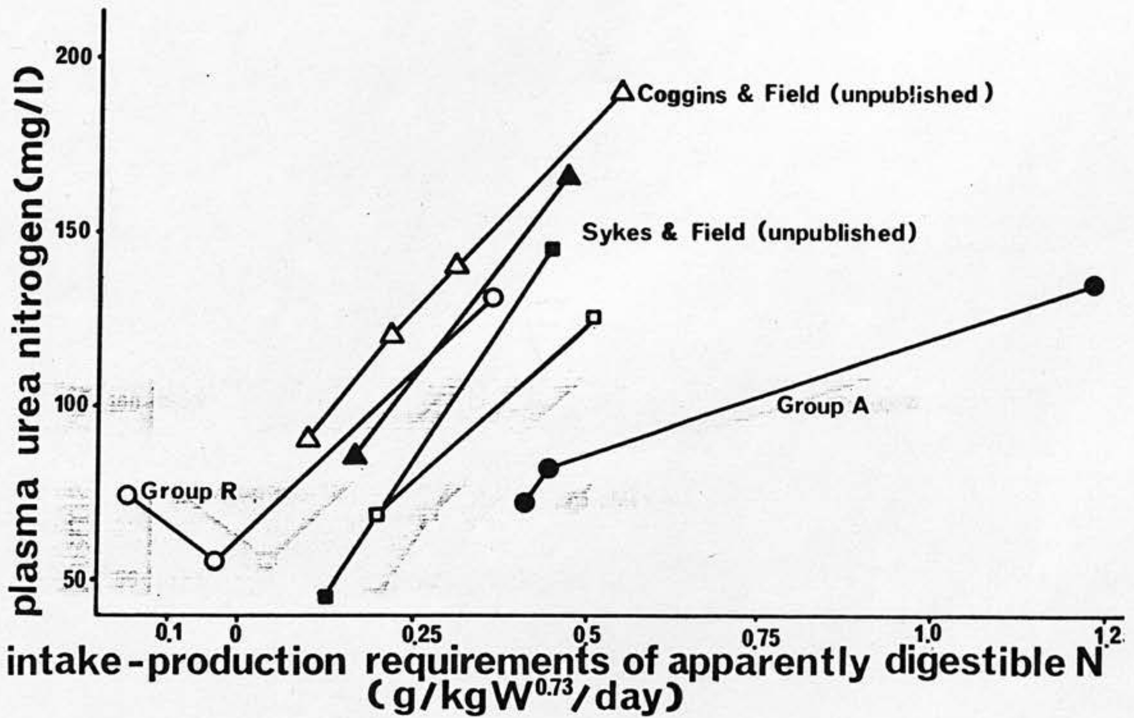
Apart from some short-term fluctuations in plasma ALB levels in both groups of ewes, particularly in Group R in early pregnancy and in Group A associated with the dietary change in mid-pregnancy, the changes were confined to Group R animals and were characterized by a fall in late pregnancy and an increase in lactation.

Changes in plasma ALB concentrations occur slowly because of the long half-life (around 15 days: Mackie & Fell, 1971), and may be caused a priori by a reduced ALB synthesis, increased ALB catabolism or changes in the volume of extracellular water (Barcroft et al, 1939). It is generally thought that protein deficiency affects protein synthesis rather than degradation (Rothschild et al, 1970), and the fall in Group R can be attributed to reduced protein synthesis arising from the protein deficiency, combined with an increase in the volume of extracellular water. Sykes & Goodhew (unpublished) have shown that ovine plasma volume remains constant until the 15th week of pregnancy, after which there is a linear increase until parturition. Unless further protein is added to the plasma pool an inevitable reduction of up to 30% may occur in the plasma ALB concentrations of protein-deficient animals as the result of plasma dilution (Sykes & Field, 1973).

In early lactation the reverse situation is thought to have occurred, namely increased ALB synthesis in a response to the improved N intakes, combined with the decreases in the volume of extracellular water which are known to occur in early lactation (Barcroft et al, 1939).

A surprising feature of the present work was the failure to find any difference in the concentrations of UN in plasma between Groups R and A, despite the fact that the N intake of the latter group was around three times greater than that of the former. It is generally recognized that plasma UN concentrations are related to N intake (Preston et al, 1966) or, more accurately, to the N absorbed in excess of requirements (Sykes, 1976). The following figure gives the latter relationship for the Group R and A animals, together with those for balance trials using the same diets (Coggins & Field, unpublished), and for feeding

trials with ewes consuming other semi-purified diets (Sykes & Field, unpublished).



The slopes of the different relationships were very similar, apart from that of Group A which was relatively flat. Another distinguishing feature was the relatively high plasma UN values associated with the N deficits, a possible explanation of which is the amount of UN originating from the catabolism of body protein in response to the energy and protein deficiencies in late pregnancy.

The reason for the different relationship for the Group A ewes is probably associated with their high D.M. intakes. The above figure relates only to

apparently digestible N, so that the well-known (A.R.C., 1965) increase in endogenous faecal loss of N with increasing D.M. intake will not be involved. Such a high D.M. intake, particularly in lactation, will lead to high voluntary intakes of water. It is known that increased urine flow causes increased urinary losses of urea (Cocimano & Leng, 1967; Thornton, 1970b), and hence the equilibrium between urea production and excretion will be established at a lower plasma urea concentration.

One further possibility lies in the different patterns of plasma UN concentration during the day between the restricted and *ad libitum* fed animals. The plasma UN levels of the Group R animals were always at maxima at 08.00h, the time of blood sampling in the present experiment, whereas those of the Group A animals were relatively constant throughout the day. Thus the pre-feeding values may represent erroneously high estimates of the plasma UN concentrations relative to N intake for the Group R animals. The same situation holds for the other studies quoted here, in that pre-feeding plasma samples were taken.

Mineral parameters

It is now generally accepted that ruminants absorb Ca from the gastro-intestinal tract according to requirements. However, the consequences of this biological control are less well understood. It means that for a given Ca requirement, efficiency of absorption of dietary Ca is inversely related to Ca intake and that for a given Ca intake, availability is related to requirements. The question as to whether a diet is deficient in Ca available for absorption will be best answered by considering dietary intake (I) scaled by requirements (R). If the value of I/R is low, less than 1.5, the probability is high that the diet is deficient in Ca (Field, unpublished). Using this measurement of

dietary Ca adequacy, Group R animals were Ca-deficient only in pregnancy, the I/R ratios being 0.94, 1.21 and 1.74 for early pregnancy, late pregnancy and lactation respectively.

This assessment of the difference in Ca status between Groups R and A is reflected in the higher value of plasma Ca in the latter group of animals throughout pregnancy. However, it must be stressed that both sets of plasma Ca concentrations would be considered normal and, therefore, plasma Ca concentration has not been a good index of dietary Ca deficiency. The last finding confirms the conclusions of Sykes & Field (1972a).

There was no doubt that the Group R animals were P-deficient during pregnancy, the dietary P intakes being of the same order as the animals' P requirements. This group difference in P status was not reflected in plasma P concentration; in fact during early pregnancy the reverse situation was seen. This poor relationship between P status and plasma P levels confirms the findings of Sykes & Dingwall (1976).

In lactation the dietary P intake of the Group R animals was marginally adequate, requiring efficiencies of absorption of around 0.60, a value well within the known range of efficiencies of absorption of dietary P. The difference in P absorption between the groups was reflected in the plasma P levels, but why this should have been only in lactation and not in pregnancy is difficult to understand.

Due to an error in diet formulation both groups of animals were Mg-deficient during pregnancy and at the onset of lactation. This deficiency led to severe hypomagnesaemia in both groups, but the severity was more marked in the Group A animals, despite their higher dietary intake of Mg. Assuming that this group difference in plasma Mg levels reflected differences in Mg status,

the higher D.M. intakes associated with the Group A animals may have increased endogenous faecal losses of Mg through increased digestive secretions, or by lowering the efficiency of absorption of dietary Mg. There is no detailed information on either of these points because the effect of level of feeding on Mg nutrition has not been investigated.

Experiment S2: Variations in the blood composition of sheep of different physiological states attributable to prandial effects and to nutritional status

INTRODUCTION

The chemical composition of blood is being used more and more in the investigation of nutritional status, both under experimental and field situations. It is general practice to blood sample at some fixed time of day, thus removing any within-day variation in blood composition and ensuring that valid comparisons of the effects of the experimental treatment can be made on a within-experiment basis. However, difficulties arise when attempting to generalize the results of individual experiments, as no two experiments have the same experimental conditions. To assess the restriction imposed by this practice information is required on the extent of within-day variation in blood composition, and an understanding of the major variables responsible for the observed variation.

That diurnal variations in concentrations of the energy parameters in sheep blood occur is well known (Annison, 1960; Reid & Hinks, 1962b; Ambo et al, 1973; Bassett, 1974b), but there is no comparable information on those plasma constituents used as indices of ovine protein and mineral status. It is reasonable to postulate that the diurnal variation will be influenced by the extent of undernutrition and by the frequency of feeding at a given level of undernutrition. Opportunity has been taken in the present experiment to

investigate the significance of these two variables.

The effect of physiological state has also been included for the sole reason that the GLUC requirements, and indirectly the concentration of KB in blood, are determined mainly by the requirements of GLUC for fetal development and for the synthesis of lactose in milk.

ANIMALS AND MANAGEMENT

This experiment took place within Experiment S1, using essentially the same animals, management and diets.

The Group R animals were divided into three experimental groups, designated R1, R2 and R3 in pregnancy and RL1, RL2 and RL3 in lactation, the number indicating the daily feeding frequency during the observational period. The daily feed for the restricted group was partioned into 1, 2 or 3 equal feeds and given at 08, 08 and 20.00, and 08, 16 and 24.00h respectively. Group A animals had continuous access to the same diet as the restricted group throughout the experiment.

Three sampling periods, one in each feeding period, were undertaken: the two in pregnancy were at 70 and 20 days pre-partum, and the one in lactation at 36 days post-partum. Sampling in lactation was performed in 2 parts because of the 16-day gap in parturition dates.

To enable the animals to become accustomed to the feeding and blood sampling regime a dummy run was performed for the 6 days immediately preceeding, and differing from the actual sampling period only in that no blood was actually taken. Animal accomodation was illuminated continuously throughout the dummy runs and sampling periods.

The ewes were blood sampled according to a schedule which ensured that

a complete set of blood samples was available for each ewe. The set consisted of samples taken at 08. 10, 12, 16, 20 and 24.00h. Individual sample times during a day were allocated at random, but no more than 6 animals were bled at any one time and blood sampling preceeded feeding if the time of the two operations coincided.

RESULTS

The results refer to those ewes which produced or suckled single lambs, and all data on twin-bearing ewes was discarded. There were thus 7 animals in Group A, 5 in R1, 6 in R2 and 8 in R3 in pregnancy, and 6 in each group in lactation.

The results are presented as graphs (Figs. S2.01 - S2.17), as mean values for each group over the 6-day sampling periods (Tables S2.2, S2.4, S2.6), together with the level of significance between groups, R groups alone or with Group A, between different times of sampling, and the interaction between these variables (Tables S2.1, S2.3 and S2.5).

Diurnal variation in blood composition was restricted to concentrations of FFA, KB, UN and PCV in all sampling periods, Mg in early pregnancy, P in late pregnancy and TP, ALB and GLOB in lactation.

The pattern of diurnal variation in plasma FFA concentrations in the restricted groups was characterized by an increase before and a decrease after a feed. Hence the frequency of peaks was the same as that of feeding, and in most cases the pre-feeding concentrations reflected the size and temporal separation of the individual feeds. The extents of the prandial changes were more pronounced in late than in early pregnancy, and were greatest in lactation. Plasma FFA levels in Group A showed little change during the day.

It is difficult to generalize about the pattern of plasma KB concentrations in the restricted group, because the pattern changes with physiological state of the animal. What can be said is that plasma concentrations appeared to increase after a meal, so that in early pregnancy the most variation was in Group R1 and the least in R3. Different results were obtained in late pregnancy, where the variations in Group R3 were at least as marked as those in Group R1 and occurred at much higher levels. In lactation the changing plasma KB concentrations in Groups RL1 and RL2 were similar to those noted in pregnancy, while there were two pronounced peaks in Group RL3. The plasma KB values for Group A showed much less diurnal variation than did Group R levels, but there was a tendency for values to be minimal at 08.00h.

Plasma UN concentrations in Group R1 animals showed similar postprandial responses in each sampling period, declining markedly from maxima shortly after feeding to minima some 8-10h later. With the exception of the falling UN values in Group R2 animals from 08 to 12.00h in early pregnancy, plasma UN concentrations in the remaining Group R animals in early and in late pregnancy were effectively constant throughout the day. In the lactating animals much more pronounced prandial fluctuations were noted, the number of peaks corresponding to the number of daily feeds, with only limited evidence of an effect of size of the individual feeds. Plasma UN levels in Group A were erratic in early pregnancy, and showed only small declines throughout the day in late pregnancy and in lactation.

Blood PCV levels usually declined after feeding and, in the two samplings in pregnant animals where the diurnal changes were not particularly pronounced, there was no evidence to suggest effects of size of the individual feeds upon the extent of the postprandial responses. The most marked changes were seen

in the Group R3 animals. Diurnal variations were much more pronounced in lactation, where all three R groups showed declining PCV levels after the 08.00h feeding with smaller declines shown after some of the other feeds. The greatest overall change was in Group RL2 animals. In late pregnancy and in lactation PCV levels in Group A were constant throughout the day, but in early pregnancy levels declined marginally from maxima at 08.00h to minima at 16.00h.

Plasma Mg concentrations in early pregnancy declined in Groups R2 and R3 between 08 and 10.00h. In Group R1 levels were effectively constant within this period, but increased slowly from minima at 12.00h to maxima some 8h later. There was also a decline in A group plasma Mg levels, from maxima at 12.00h to minima 4h later.

In late pregnancy Groups R1 and R2 both showed pronounced postprandial declines in plasma P levels, the extent of the changes being somewhat greater in the former group than in the latter. In Group R3 plasma P levels declined slowly from 08 to 20.00h, rising to maxima at 24.00h. With the exception of the high values at the 20.00h samplings plasma P levels in Group A were relatively constant throughout the day.

The diurnal changes in the plasma concentrations of the circulating proteins in the lactating Group R animals were similar in each sub-group, and consisted of small declines from maxima at 08.00h, followed by gradual increases throughout the day. Apart from ALB in Group RL2 there was little evidence of postprandial declines at any other time of day, the most pronounced time effects being in Group RL3. Diurnal changes in Group A were the reverse of those seen in Group R, and consisted of small decreases during the day from maxima at 10.00h.

Plasma FFA levels in Group R1 in late pregnancy were lower than the corresponding levels in Groups R2 and R3, this being the only significant difference in blood composition between groups R1, R2 and R3.

Differences in blood composition between Groups R and A were similar to those shown in Experiment S1. In early and in late pregnancy the mean concentrations of TP, ALB, GLOB and Ca in plasma, and of blood PCV, were higher in Group A than in Group R with the reverse effect being noted for Mg in early pregnancy and FFA in late pregnancy. In late pregnancy and lactation plasma UN levels were higher in Group A than in Group R, a similar effect being noted in plasma P levels in the lactating animals.

DISCUSSION

For a blood constituent to display prandial variation it can be hypothesized that the act of feeding, or the increased production rate of a nutrient in the rumen, must alter blood composition either directly or indirectly through the nutritional status of the animal to which the blood constituent is sensitive. It will be shown that, although the observed prandial variations in FFA, KB, UN and PCV levels were induced in different ways, the mechanisms all fulfilled the above conditions.

Energy parameters

The changes in plasma FFA levels during the day can be used as evidence of the changing energy status within the day. It is known that in times of dietary energy shortage, fat from adipose tissue is mobilized and transported in the form of FFA to the liver for oxidation. The resultant elevated plasma FFA levels are usually proportional to the extent of the energy deficiency up to a maximum determined by the rate at which fat can be mobilized. The response of plasma FFA concentrations to a dietary energy deficit is extremely

rapid because of the very short half-life of the FFA molecule in the blood.

The present results show clearly that for animals on a restricted diet given in discrete amounts, their energy status showed cyclical changes during the day, associated with feeding and occurring irrespective of the overall energy status of the animal. Furthermore, the duration of the energy adequacy depended upon the energy content of the diet and the energy requirements of the animal. Thus the post-feeding period of energy adequacy was longest with the single feed and shortest with the three times daily feeding regime, and was shortest in lactation and longest in early pregnancy.

The diurnal change in plasma KB concentrations can be attributed in part to the increased rate of production of butyric acid in the rumen following a meal and the subsequent conversion of the butyrate to β -hydroxybutyrate, the predominant KB in blood, in the ruminal wall. The diurnal changes in KB in early pregnancy were consistent with this explanation, the post-feeding increase being maximal for the single feed and minimal for the three feeds regime. However, for the diurnal changes in late pregnancy and early lactation other explanations must be invoked. One possibility is that the production of KB from fatty acids and their subsequent oxidation was materially altered by the pattern of feeding. It is not possible from the present data to assess the truth of this hypothesis.

A surprising facet of this section of the work was the complete absence of significant effects of feeding upon plasma GLUC concentrations. Ovine plasma GLUC levels have in the past been shown to increase steadily after feeding (Ambo et al, 1973; Bassett, 1974b), but whether this is a true finding or whether it is due to the effect of the stress of repeated blood sampling on

blood GLUC levels (May et al, 1974) is not known. Recent studies by Hodgson & Mellor (unpublished) favour the former in that pregnant ewes accustomed to serial blood samplings showed marked increases in plasma GLUC levels after a feed. The present finding, together with the fall observed after feeding in the cattle experiments, highlights the variation in the response of ruminant plasma GLUC levels to feeding.

It is possible that the markedly different postprandial responses in the plasma GLUC concentrations of the animals used in this study may have been mediated through differential actions of insulin (Bassett, 1974a; b). Fortunately information was available on the insulin concentrations of the animals used in the experiment investigating the prandial variations in bovine blood composition (Experiment C3), and also of the animals used in the present experiment.

In the former experiment plasma insulin levels increased non-significantly from around 0.30 - 0.35 ng/ml 1h pre-feeding to around 0.38 - 0.42 ng/ml 3.5h post-feeding (Coggins & Field, 1976b). The only significant changes in plasma insulin concentrations in the present experiment were from 08 to 10.00h in Group R1 animals in late pregnancy, the rise being rom 0.4 to 0.9 ng/ml (Coggins & Field, unpublished). These results indicate that insulin is either not involved in the differing postprandial responses, or that the elevations in plasma insulin levels after feeding are rapid and of short duration.

Offering the total daily ration in progressively smaller daily instalments had the predictable effect of reducing the extent of the individual prandial fluctuations in plasma FFA and KB concentrations. However, the variations were not eliminated completely in the animals fed most frequently, nor were they eliminated in the

animals fed *ad libitum*. Techniques have been devised which reduce the diurnal variation to very low levels by feeding animals 24 times a day at hourly intervals (Hodgson & Mellor, unpublished).

Protein parameters

In general the plasma UN concentrations of the Group R animals decreased markedly after a feed, and this change may be explained either in terms of a decrease in UN production or an increase in the drain of UN from the body. The first possibility is considered much less likely, because of the rapid rate of ruminal de-amination of ingested protein known to occur after feeding (Lewis, 1957). The fall was probably due to the increase in the volume of saliva associated with feeding (Thornton, 1970b; Harrop & Philippson, 1974).

The postprandial decline in plasma UN concentrations shown here is in direct contrast to the results of the cattle experiments, where levels either remained constant (Experiment C3) or increased (Experiment C2) following a feed. There are a number of differences between the experiments which would possibly explain this anomaly.

Relative to sheep, the cattle consumed diets with lower D.M. concentrations and higher N contents relative to requirements. Silage, the predominant fraction of the cattle rations, has a relatively high proportion of non-protein nitrogen (Edwards, unpublished) and all of these factors would result in lower UN outputs in saliva and higher productions of ammonia in the rumen.

The postprandial declines in the plasma TP, ALB and GLOB levels of the lactating Group R animals were unexpectedly large when one considers that the half-lives of these circulating proteins are measured in days rather than in hours (Mackie & Fell, 1971). The changes were probably brought about

by postprandial increases in the volume of distribution of plasma proteins, as evidenced by the concurrent changes in blood PCV levels.

This latter hypothesis is supported by recent findings (Christopherson & Webster, 1972; Dooley & Williams, 1975) showing initial postprandial increases, followed by declines, in ovine blood PCV levels. It is not clear in the present work why the significant changes in plasma protein concentrations were restricted to the lactating animals, as postprandial declines in blood PCV levels were noted in all three sampling periods. Similarly it is difficult to understand why the significant prandial changes in plasma concentrations of the protein parameters were restricted to the 08.00h feed, there being virtually no changes in Groups RL2 and RL3 following their other daily feeds.

Mineral parameters

The diurnal changes in plasma Mg concentrations in early pregnancy were of marginal significance statistically and are considered to be of low significance biologically.

No other workers have reported such a marked postprandial fall in plasma P concentrations as was found here in the R1 and R2 groups in late pregnancy: although Unshelm & Rappen (1968) noted postprandial falls in bovine plasma P concentrations, the changes were of small magnitudes compared with those presented here. The only relevant work in sheep (Ternouth, 1968) has shown small increases in plasma P concentrations after a feed.

The physiological explanation for the changing plasma P levels remains unclear, particularly as the changes were unique to late pregnancy. The explanation is probably related to factors involved in the control of salivary P production, since the site of dietary P absorption is the small intestine and

consequently the lag-phase between feeding and absorption would be too great to cause the short-term fluctuations observed here in plasma P concentrations.

SUMMARY AND CONCLUSIONS

Summary

Experiment S1 investigated the blood composition of groups of ewes consuming different amounts of the same diet throughout pregnancy and lactation. One group of animals was given rations intended to produce severe energy, protein and mineral undernutrition, while a second group was allowed continuous access to the same diet.

Milk yields of all animals were lower than expected, and as a result all lactating animals consumed excess nutrients over their requirements for those nutrients. In early pregnancy animals gained in weight so that it was difficult to assess their actual nutritional status as the weight changes may have been due to accumulations of water.

Despite these large differences between the groups in their mean nutritional status, the only plasma constituents to show significant group effects were Ca and P in early pregnancy, and KB, ALB and Ca in lactation, suggesting that the chosen plasma constituents do not accurately reflect nutritional status. In contrast, concentrations of several plasma constituents varied significantly within each feeding period and, as the changes occurred in both groups, they were considered to have been caused by physiological and biochemical changes associated with pregnancy and lactation.

The most significant changes were in plasma concentrations of GLUC, FFA and ALB in early pregnancy, ALB in late pregnancy, and GLUC, KB, ALB UN, Ca and P in lactation.

The second sheep experiment investigated the within-day variation in the blood composition of the animals used in the first sheep experiment. To differentiate between diurnal and prandial variations in blood composition some of the undernourished group of animals were fed in two or three equal feeds per day at 12- or 8-hourly intervals.

The most pronounced prandial variations were shown in plasma concentrations of FFA, UN and P, which declined after feeding, and KB, which increased. Blood PCV levels also declined after feeding. The effect of frequency of feeding on the postprandial response was consistent in the instance of FFA, where each feed was preceeded by an increase and followed by a fall in plasma concentration. For the other plasma constituents which exhibited prandial variation the pattern changed with each feeding period.

Plasma concentrations of the circulating proteins fell after feeding in lactating animals, probably as a result of changes in extracellular volume. The prandial changes in plasma concentrations of FFA and KB agree well with observations made in the cattle experiments, but those of UN and P did not. The most unexpected result was the absence of significant prandial variations in plasma GLUC concentrations.

Conclusion

Concentrations of those blood constituents examined here were undoubtedly affected by nutritional status, but the effects were small compared with prandial variations and with the effects of stage of pregnancy and lactation.

Table S1.1. Composition and analysis of semi-purified diets

	Diet		
	EP	LP	LA
Ingredient (%)			
Ground oat hulls	25.4	19.5	20.0
Whole oat hulls	25.3	19.5	20.0
Starch	14.5	18.2	15.7
Sugar	15.0	18.2	15.7
Blood meal	2.5	3.8	4.9
Dried skim milk	6.0	9.3	12.1
Arachis oil	3.0	3.0	3.0
Water	3.0	3.0	3.0
Dicalcium phosphate	-	0.2	0.5
Calcium carbonate	0.3	0.7	0.7
Sodium bicarbonate	2.5	2.5	2.5
Sodium chloride	1.5	1.5	1.5
Calcined magnesite (see text)	-	-	-
Sodium sulphate	0.3	0.3	0.3
Vitamin A & D premix[i] (g/100 kg)	6.0	4.5	4.5
Vitamin E premix[ii] (g/100 kg)	30.0	20.0	30.0
Supplements, trace elements	iii	iii	iii
D.M.[iv] digestibility[v] (%)	48.0	57.0	62.0
N digestibility[v]	50.0	57.0	65.0
Analysis (% in D.M.)			
N	0.96	1.28	1.71
Ca	0.27	0.37	0.45
P	0.11	0.19	0.27
Mg (see text)	-	-	-

i 50,000 I.U. vitamin A, 12,500 I.U. vitamin D/g.

ii 200 I.U. vitamin E/g.

iii Composition and inclusion rate as given by Sykes & Dingwall (1975).

iv Mean D.M. content of all diets was 91%

v Sykes (unpublished).

Table S1.2. Rate of feeding and weights of animals (± S.D.) during early pregnancy, late pregnancy and lactation

	Group	
	R	A
Early pregnancy (diet EP)		
Weight (kg)	46.6 ± 3.5	48.3 ± 4.8
SPD D.M. intake (kg/day)	0.34 ± 0.026	1.71
Late pregnancy (diet LP)		
Weight (kg)	46.4 ± 3.4	55.5 ± 7.3
SPD D.M. intake (kg/day)	0.65 ± 0.05	2.4
Lactation (diet LA)		
Weight (kg)	46.2 ± 5.0	54.3 ± 6.8
SPD D.M. intake (kg/day)	1.81 ± 0.19	3.03
Estimated maximum milk yield (kg/day)[i]	1.58 ± 0.52	1.66 ± 0.32

i See Figure S1.02

Table S1.3. Estimated nutrient balances (± S.D.) during early pregnancy

	Group	
	R	A
ME balance (MJ/day)		
Intake	3.84 ± 0.17	12.8
Requirements[i]	6.24 ± 0.47	6.47 ± 0.64
Intake/requirements (%)	61.7 ± 1.9	198
N balance (g/day)		
Intake	2.98 ± 0.12	9.51
Requirements[ii]	5.08 ± 0.28	5.23 ± 0.38
Intake/requirements (%)	58.6 ± 0.9	181
Ca balance (g/day)		
Intake	1.43 ± 0.07	5.12
Requirements[iii]	2.97 ± 0.11	3.04 ± 0.17
Intake/requirements (%)	48.1 ± 0.5	168
P balance (g/day)		
Intake	0.86 ± 0.03	2.36
Requirements[iv]	1.51 ± 0.06	1.55 ± 0.08
Intake/requirements (%)	57.0 ± 1.0	152

i 0.134 MJ/kg W/day. (Sykes & Field, 1972b)
ii Maintenance requirement 0.16 g/kg $(W)^{0.73}$/day (Robinson & Forbes, 1966; pregnancy requirement 0.14 g/kg $(W)^{0.73}$/day (Sykes, unpublished).
iii Endogenous loss 16 mg/kg/day (Field & Suttle, 1969), requirement 0.6 g/day (Sykes, unpublished); 45% availability (A.R.C., 1965).
iv Endogenous loss 12 mg/kg/day, requirement 0.35 g/day (Sykes & Dingwall, 1976); 60% availability (A.R.C., 1965).

Table S1.4. Estimated nutrient balances (± S.D.) during late pregnancy

	Group	
	R	A
ME balance (MJ/day)		
Intake	6.70 ± 0.38	20.3
Requirements[i]	10.4 ± 0.45	11.6 ± 0.9
Intake/requirements (%)	64.3 ± 2.2	175
N balance (g/day)		
Intake	6.09 ± 0.35	18.8
Requirements[ii]	11.3 ± 0.57	12.9 ± 1.28
Intake/requirements (%)	53.9 ± 0.2	145
Ca balance (g/day)		
Intake	2.92 ± 0.18	9.35
Requirements[iii]	4.84 ± 0.10	5.17 ± 0.24
Intake/requirements (%)	60.2 ± 2.1	181
P balance (g/day)		
Intake	1.72 ± 0.09	5.02
Requirements[iv]	2.35 ± 0.06	2.53 ± 0.06
Intake/requirements (%)	73.2 ± 1.7	198

i Maintenance requirement 0.134 MJ/kg W/day (Sykes & Field, 1972b), pregnancy requirement 4.18 MJ/day. (Sykes, unpublished)

ii Maintenance requirement 0.16 g/kg $(W)^{0.73}$/day (Robinson & Forbes, 1966; pregnancy requirement 0.52 g/kg$(W)^{0.73}$/day. (Sykes, unpublished)

iii Endogenous loss 16 mg/kg/day (Field & Suttle, 1969), pregnancy requirement 1.44 g/day (Sykes, unpublished); 45% availability (A.R.C., 1965)

iv Endogenous loss 12 mg/kg/day, pregnancy requirement 0.86 g/day (Sykes & Dingwall, 1976); 60% availability (A.R.C., 1965).

Table S1.5. Estimated nutrient balances (± S.D.) during lactation

	Group	
	R	A
ME balance (MJ/day)		
Intake	17.0 ± 1.6	27.4
Requirements[i]	16.4 ± 3.6	18.0 ± 2.6
Intake/requirements (%)	107 ± 25	152
N balance (g/day)		
Intake	18.9 ± 2.0	35.0
Requirements[ii]	15.2 ± 4.3	16.4 ± 2.5
Intake/requirements (%)	124 ± 53	213
Ca balance (g/day)		
Intake	8.65 ± 0.89	14.1
Requirements[iii]	8.66 ± 2.42	9.28 ± 1.06
Intake/requirements (%)	105 ± 4.1	152
P balance (g/day)		
Intake	5.36 ± 0.53	8.67
Requirements[iv]	4.60 ± 1.45	5.30 ± 0.91
Intake/requirements (%)	116 ± 4.4	163

i 0.134 MJ/kg W/day (Sykes & Field, 1972b); 6.46 MJ/kg milk (Peart, 1967).

ii 0.16 g/kg $(W)^{0.73}$/day (Robinson & Forbes, 1966); 8g/kg milk (Peart, 1967).

iii Endogenous loss 16 mg/kg/day (Field & Suttle, 1969), 2g/kg milk (Ashton & Youssef, 1966), 45% availability (A.R.C., 1965).

iv Endogenous loss 12 mg/kg/day (Sykes & Dingwall, 1976); 1.6 g/kg milk (Ashton & Youssef, 1966); 60% availability (A.R.C., 1965).

Table S1.6. Effect of ME and N intake on ewe and lamb performance

	Intake		Ewe weight change	Lamb birth weight
	ME	N		
	(MJ/kg $(W)^{0.73}$)	(g/kg $(W)^{0.73}$)	(%)	(g/kg $(MW)^{i\ 0.73}$)
Sykes & Field, 1972b				
Group LP	0.343	0.136	-21.3	15.9
" HP	0.343	0.304	-18.0	20.8
Present work				
Group R	0.406	0.370	- 0.78	24.2
" A	1.080	1.000	19.4	27.4

i MW = Weight of ewe at mating. (See Donald & Russell, 1970)

Table S1.7. Mean group concentrations (± S.E.) of plasma constituents in early pregnancy, late pregnancy and in lactation

	Early pregnancy Group		Late pregnancy Group		Lactation Group	
	R	A	R	A	R	A
n	64	24	49	18	76	27
GLUC	529 ± 13.7	571 ± 19.3	488 ± 15.4	627 ± 29.1	578 ± 14.0	688 ± 24.3
FFA	652 ± 47.0	411 ± 65.9	800 ± 35.5	408 ± 66.9	833 ± 35.2	453 ± 60.7
KB	27.7 ± 1.38	26.0 ± 1.94	37.0 ± 6.73	25.2 ± 12.7	32.9 ± 2.99	34.6 ± 5.17
ALB	27.3 ± 0.49	29.3 ± 0.69	22.8 ± 0.37	26.8 ± 0.70	25.0 ± 0.30	26.4 ± 0.51
UN	56.3 ± 3.9	73.1 ± 5.5	73.8 ± 3.68	80.8 ± 6.94	131 ± 5.7	134 ± 9.9
Ca	99.8 ± 0.76	103 ± 0.1	95.9 ± 0.74	106 ± 0.1	100 ± 0.9	105 ± 1.6
P	57.7 ± 1.07	51.9 ± 1.50	49.6 ± 1.65	51.3 ± 3.11	54.7 ± 1.29	67.4 ± 2.2

Table S1.8. Significances of within-feeding period variations in the mean plasma composition of Group R animals

	Early pregnancy	Late pregnancy	Lactation
	$F_{5,58}$	$F_{3,45}$	$F_{4,71}$
GLUC	* * *	*	NS
FFA	* *	*	NS
KB	NS	NS	NS
ALB	* *	* *	* * *
UN	NS	NS	*
Ca	NS	NS	* * *
P	NS	NS	*

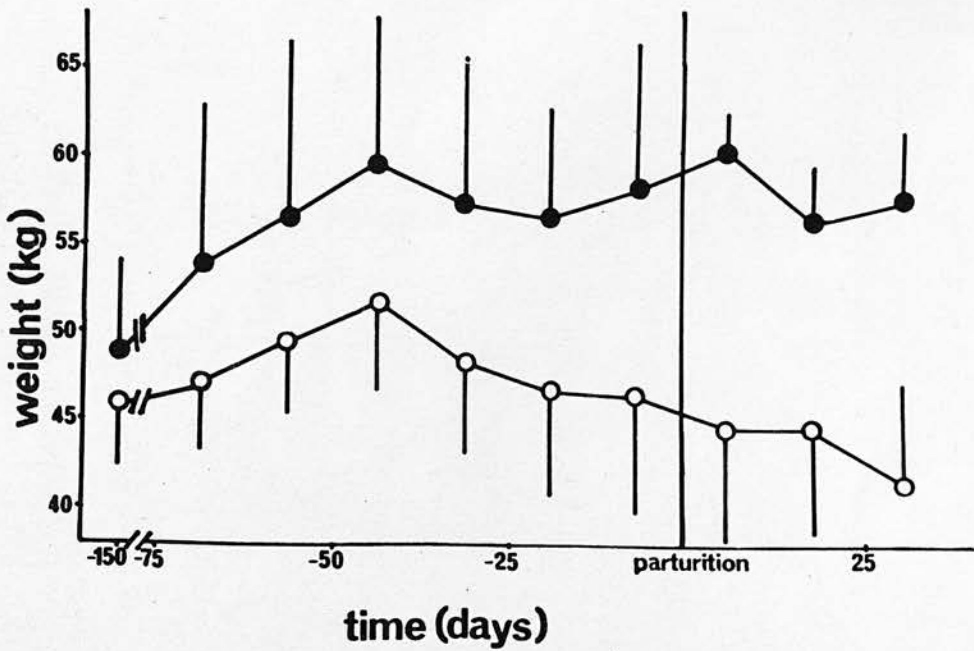


Figure S1.01

Weight changes (± S.D.) in 2 nutritional groups of sheep throughout pregnancy and early lactation.

●—● Group A, ○—○ Group R

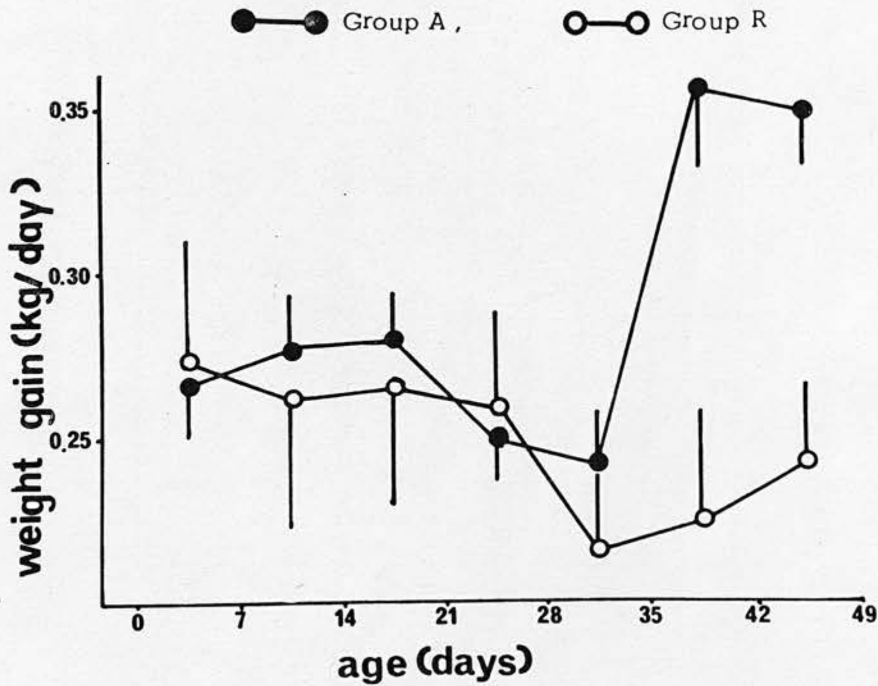
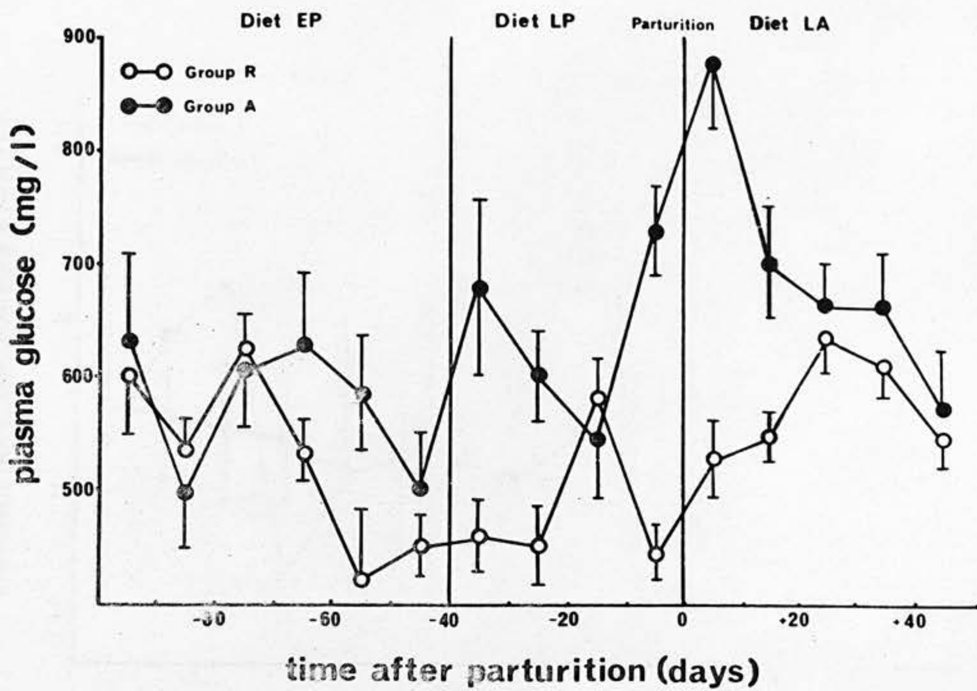
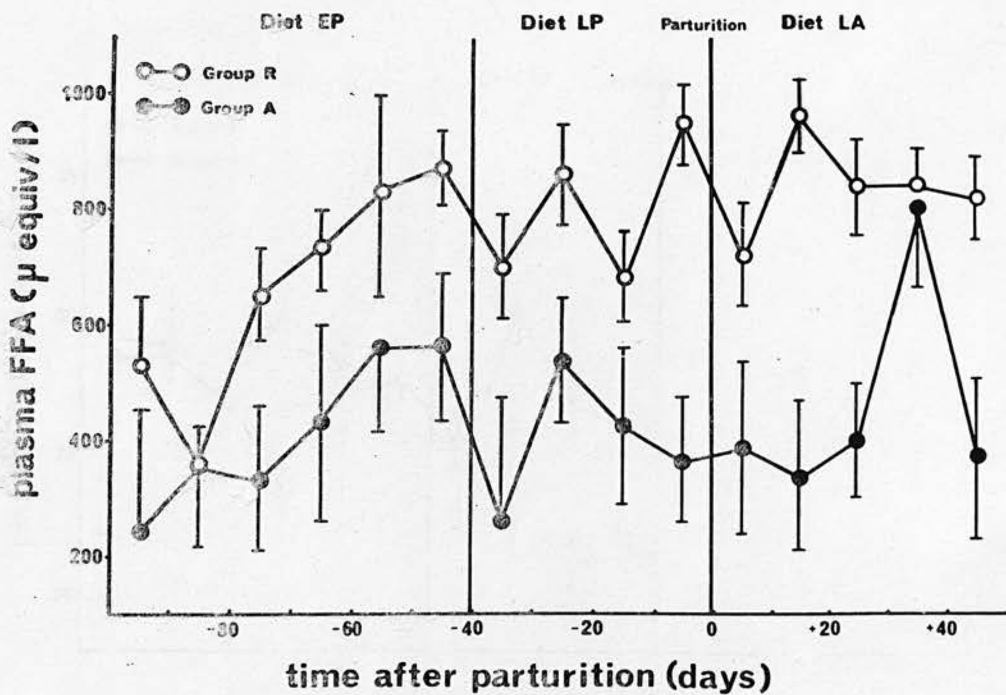


Figure S1.02

Growth rates (± S.D.) of lambs from 2 nutritional groups of lactating sheep.

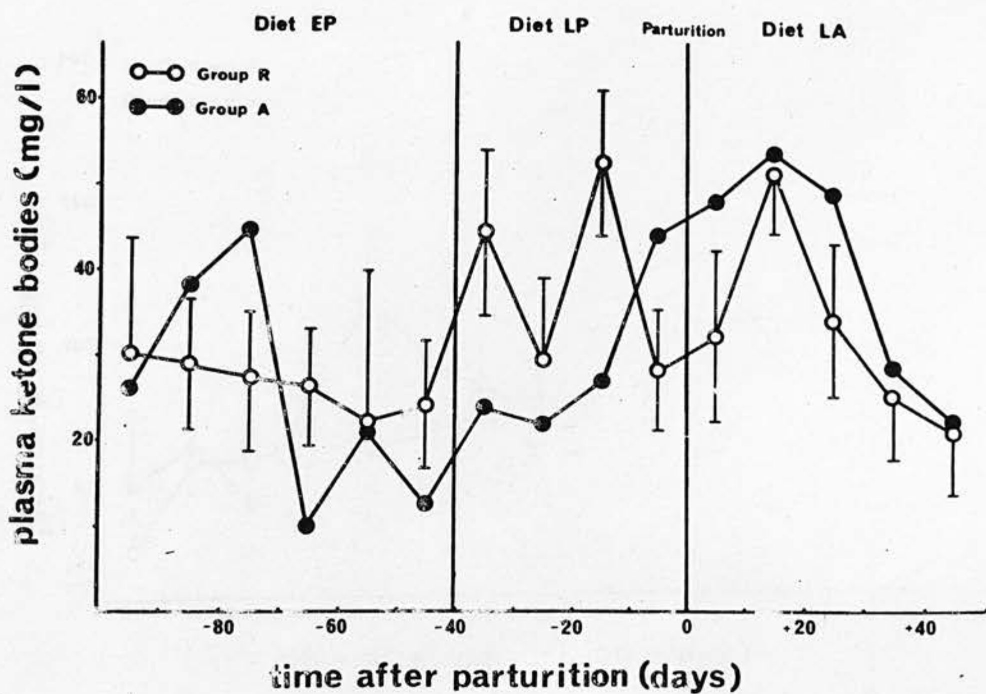
●—● Group A, ○—○ Group R

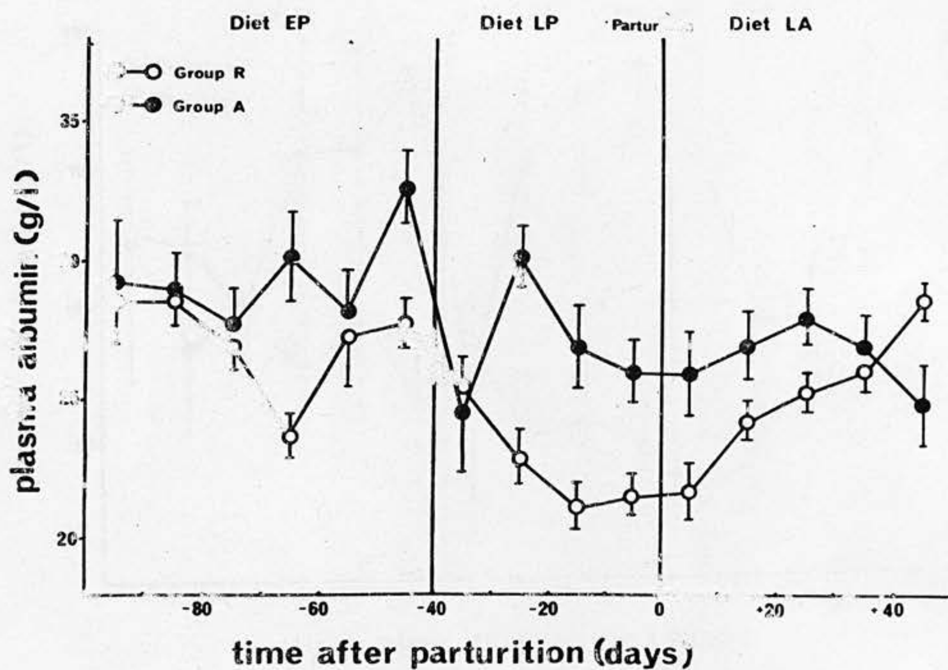




Figures S1.03, S1.04

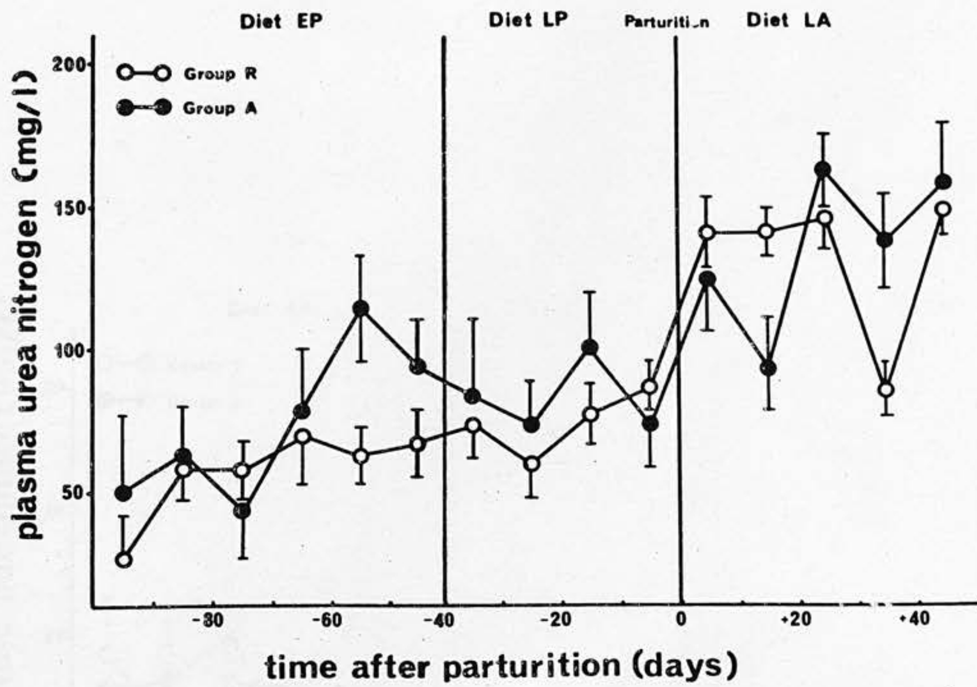
Changes in plasma glucose and FFA concentrations (± S.E.) in 2 nutritional groups of sheep throughout pregnancy and early lactation.

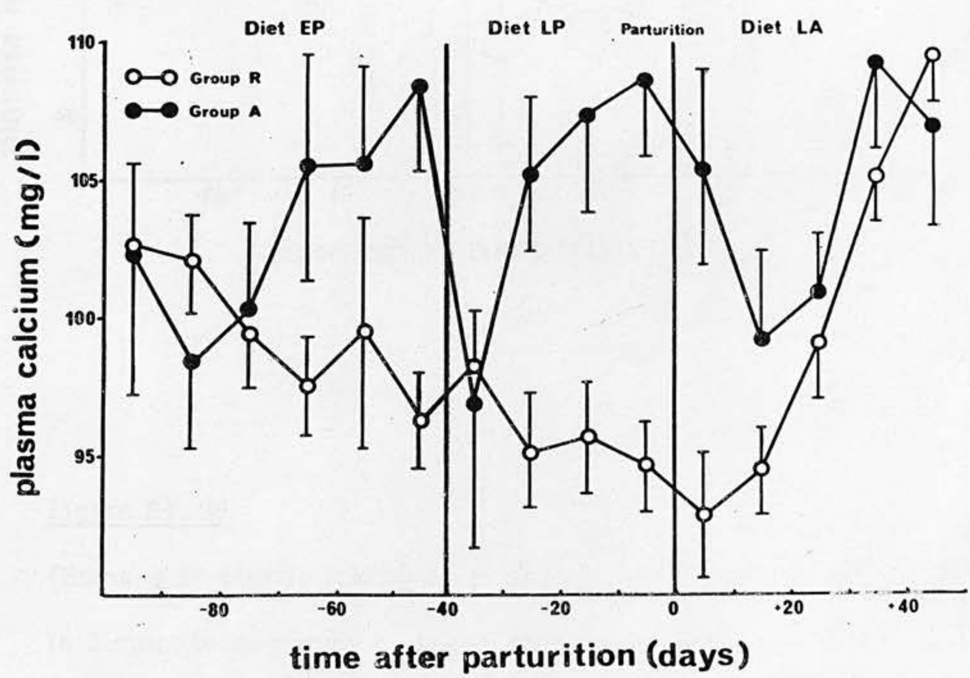




Figures S1.05, S1.06

Changes in plasma ketone body and albumin concentrations (± S.E.) in 2 nutritional groups of sheep throughout pregnancy and early lactation.





Figures S1.07, S1.08

Changes in plasma urea nitrogen and calcium concentrations (± S.E.) in 2 nutritional groups of sheep throughout pregnancy and early lactation.

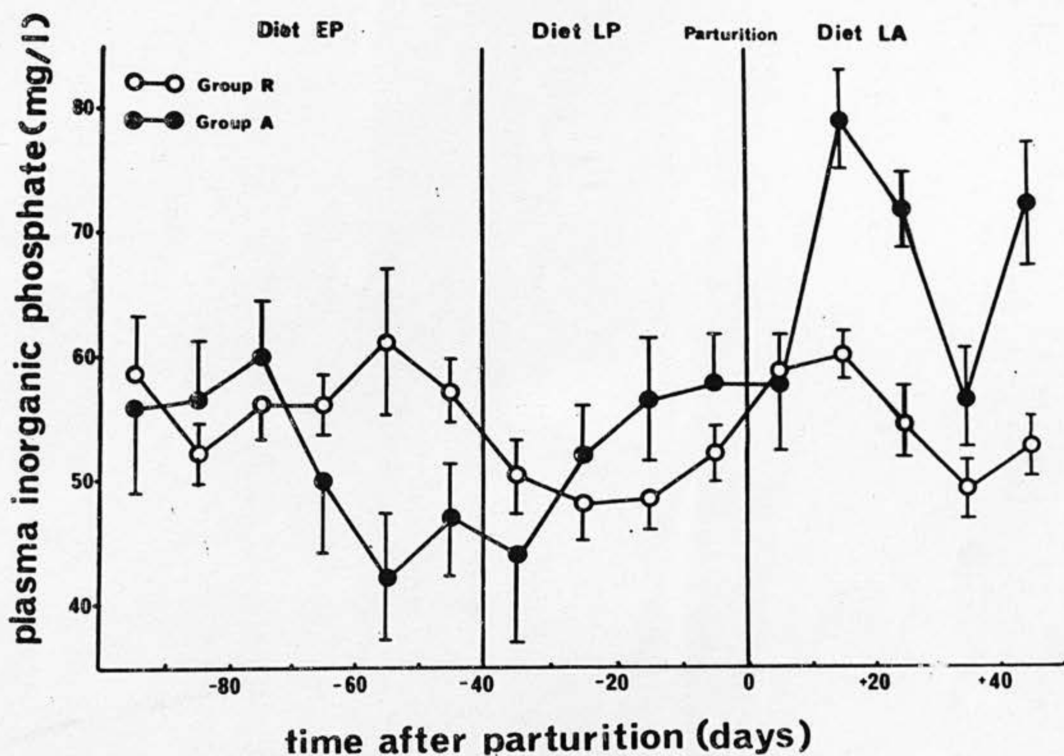


Figure S1.09

Changes in plasma inorganic phosphate concentrations (± S.E.) in 2 nutritional groups of sheep throughout pregnancy and early lactation.

Table S2.1. Significances of sources of variation in blood composition during early pregnancy

	Group		Time		Interaction	
	(R + A)	R	(R + A)	R	(R + A)	R
	$F_{3,22}$	$F_{2,22}$	$F_{5,110}$	$F_{5,110}$	$F_{15,110}$	$F_{10,110}$
GLUC	NS	NS	NS	NS	NS	NS
FFA	NS	NS	* * *	* * *	NS	* *
KB	NS	NS	* * *	* * *	* * *	* * *
TP	* *	NS	NS	NS	NS	NS
ALB	* *	NS	NS	NS	NS	NS
GLOB	*	NS	NS	NS	NS	NS
UN	NS	NS	*	* *	NS	* *
Ca	*	NS	NS	NS	NS	NS
P	NS	NS	NS	NS	NS	NS
Mg	* *	NS	NS	*	NS	NS
PCV	* *	NS	*	*	* *	NS

Table S2.2. Least-squares mean group concentrations (± S.E.) of blood constituents during early pregnancy

	Group			
	A	R1	R2	R3
n	42	30	36	48
GLUC	607 ± 10	571 ± 11	554 ± 10	584 ± 9
FFA	319 ± 18.4	377.0 ± 21.8	368.0 ± 19.8	354.0 ± 17.2
KB	30.3 ± 1.23	31.0 ± 1.45	30.7 ± 1.33	27.4 ± 1.15
TP	78.3 ± 0.34	75.1 ± 0.41	69.3 ± 0.37	69.3 ± 0.32
ALB	25.8 ± 0.27	21.7 ± 0.32	22.2 ± 0.29	22.0 ± 0.25
GLOB	52.5 ± 0.28	53.4 ± 0.34	47.1 ± 0.31	47.3 ± 0.27
UN	78.1 ± 2.97	68.9 ± 3.51	71.6 ± 3.21	67.1 ± 2.78
Ca	105 ± 0.6	95.8 ± 0.78	95.4 ± 0.71	98.5 ± 0.61
P	54.6 ± 1.06	48.1 ± 1.26	50.0 ± 1.15	51.4 ± 0.99
Mg	15.9 ± 0.22	19.7 ± 0.26	21.4 ± 0.23	20.4 ± 0.20
PCV	47.7 ± 0.44	42.5 ± 0.53	41.6 ± 0.48	43.2 ± 0.42

Table S2.3. Significances of sources of variation in blood composition during late pregnancy

	Group		Time		Interaction	
	(R + A)	R	(R + A)	R	(R + A)	R
	$F_{3,22}$	$F_{2,22}$	$F_{5,110}$	$F_{5,110}$	$F_{15,110}$	$F_{10,110}$
GLUC	NS	NS	NS	NS	NS	NS
FFA	* * *	*	* * *	* * *	* * *	* * *
KB	NS	NS	* *	* *	* *	*
TP	* * *	NS	NS	NS	NS	NS
ALB	* * *	NS	NS	NS	NS	NS
GLOB	* *	NS	NS	NS	NS	NS
UN	*	NS	* * *	* * *	* *	* * *
Ca	* * *	NS	NS	NS	NS	NS
P	NS	NS	* * *	* * *	* * *	* * *
Mg	NS	NS	NS	NS	NS	NS
PCV	* * *	NS	* * *	* * *	NS	NS

Table S2.4. Least-squares mean group concentrations (± S.E.) of blood constituents during late pregnancy

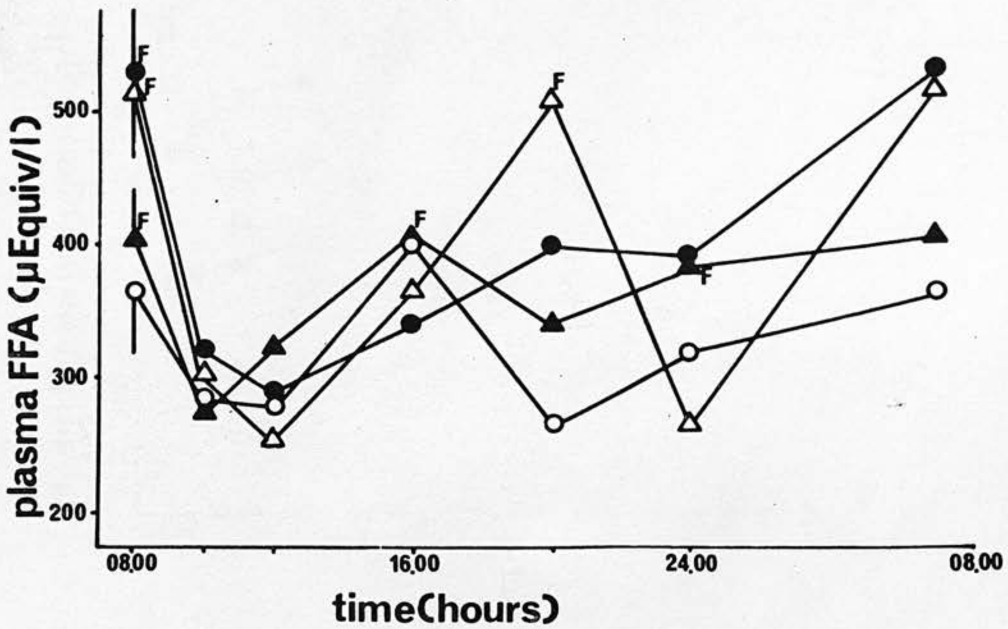
	Group			
	A	R1	R2	R3
n	42	30	36	48
GLUC	643 ± 20.1	630 ± 23.8	594 ± 21.7	593 ± 18.8
FFA	242 ± 12.1	285 ± 14.3	441 ± 13.1	433 ± 11.3
KB	43.3 ± 4.45	35.0 ± 5.27	70.1 ± 4.81	95.2 ± 4.16
TP	74.2 ± 0.34	66.0 ± 0.41	60.1 ± 0.37	62.9 ± 0.32
ALB	25.1 ± 0.24	19.1 ± 0.28	19.7 ± 0.26	20.1 ± 0.22
GLOB	49.1 ± 0.33	46.9 ± 0.39	40.4 ± 0.35	42.8 ± 0.31
UN	68.9 ± 2.09	47.5 ± 2.47	42.5 ± 2.26	46.1 ± 1.95
Ca	105 ± 0.8	95.4 ± 0.95	93.1 ± 0.87	97.3 ± 0.76
P	50.2 ± 0.77	47.0 ± 0.91	44.2 ± 0.84	43.7 ± 0.72
Mg	16.2 ± 0.21	16.1 ± 0.24	16.0 ± 0.22	18.2 ± 0.19
PCV	48.2 ± 0.34	35.2 ± 0.39	33.6 ± 0.39	37.8 ± 0.31

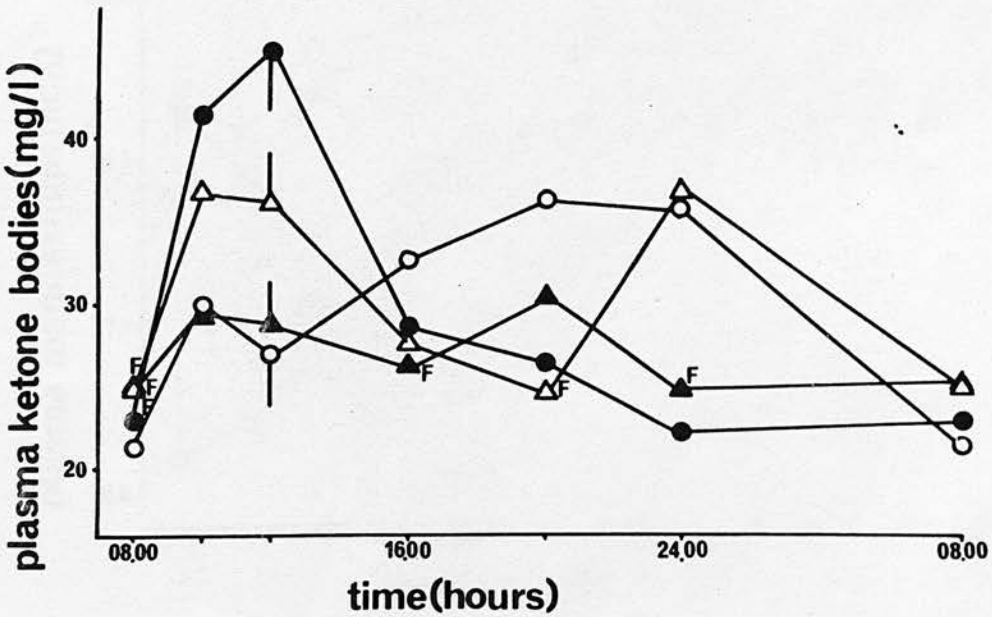
Table S2.5. <u>Significances of sources of variation in blood composition during lactation</u>

	Group		Time		Interaction	
	(R + A)	R	(R + A)	R	(R + A)	R
	$F_{3,20}$	$F_{2,20}$	$F_{5,100}$	$F_{5,100}$	$F_{15,100}$	$F_{10,100}$
GLUC	NS	NS	NS	NS	NS	NS
FFA	NS	NS	* * *	* * *	* * *	* * *
KB	NS	NS	* *	*	*	*
TP	NS	NS	*	* *	NS	NS
ALB	NS	NS	NS	* *	NS	NS
GLOB	NS	NS	*	*	NS	NS
UN	* *	NS	* * *	* * *	* * *	* * *
Ca	NS	NS	NS	NS	NS	NS
P	*	NS	NS	NS	NS	NS
Mg	NS	NS	NS	NS	NS	NS
PCV	* * *	* * *	* * *	* * *	*	NS

Table S2.6. Least-squares mean group concentrations of blood constituents during lactation

	Group				S.E.
	A	R1	R2	R3	
n	36	36	36	36	
GLUC	627	600	668.	659	17.2
FFA	302	455	389	362	29.1
KB	38.4	34.6	31.5	44.5	3.70
TP	82.6	76.0	78.5	82.0	0.66
ALB	27.1	22.7	26.1	24.0	0.59
GLOB	55.5	53.3	52.4	58.0	0.66
UN	91.8	58.6	69.3	47.4	3.28
Ca	109	105	105	105	1.0
P	61.7	50.3	52.6	51.3	1.36
Mg	17.7	15.9	19.3	18.0	0.37
PCV	48.1	37.1	41.9	34.3	0.36



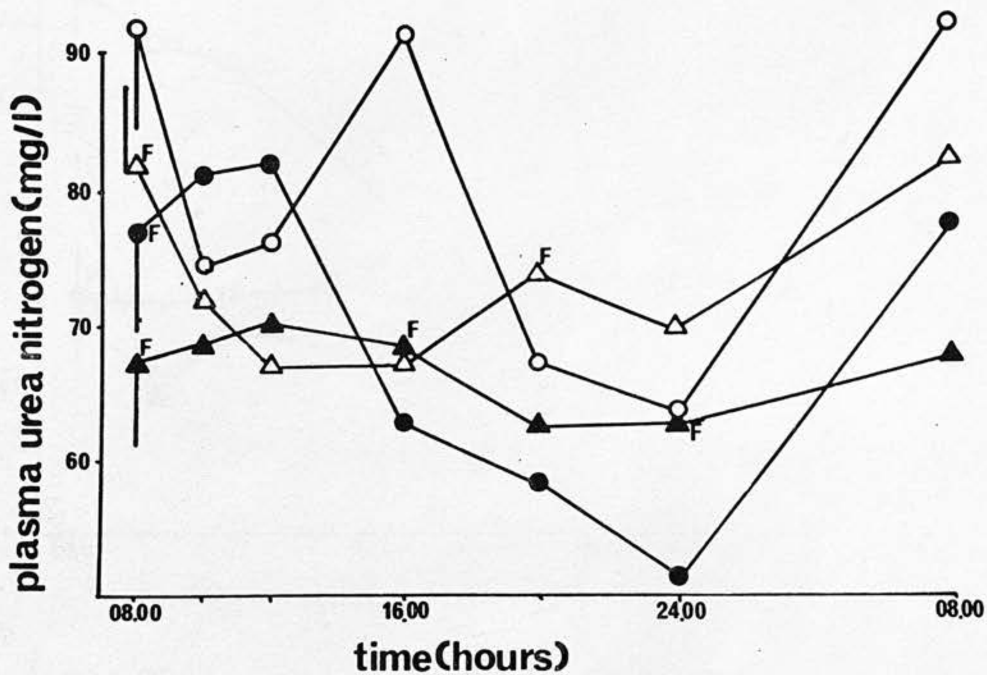


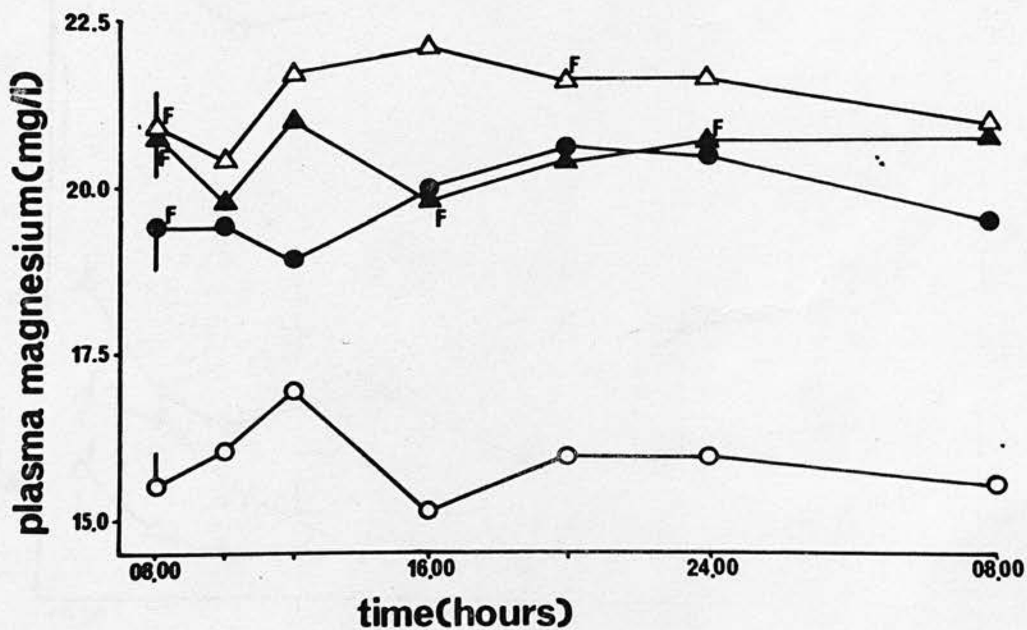
Figures S2.01, S2.02

Least-squares mean values (± S.E.) of plasma FFA and ketone body concentrations in 4 groups of sheep during early pregnancy.

Group A, Group R1, Group R2, Group R3

F = Feeding



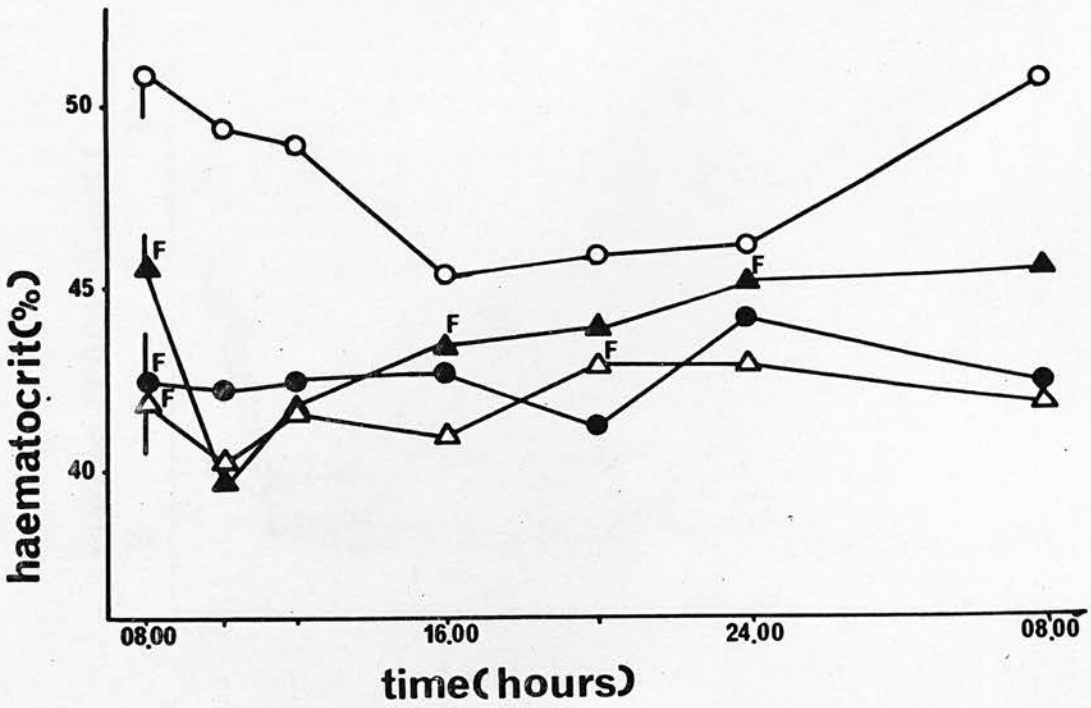


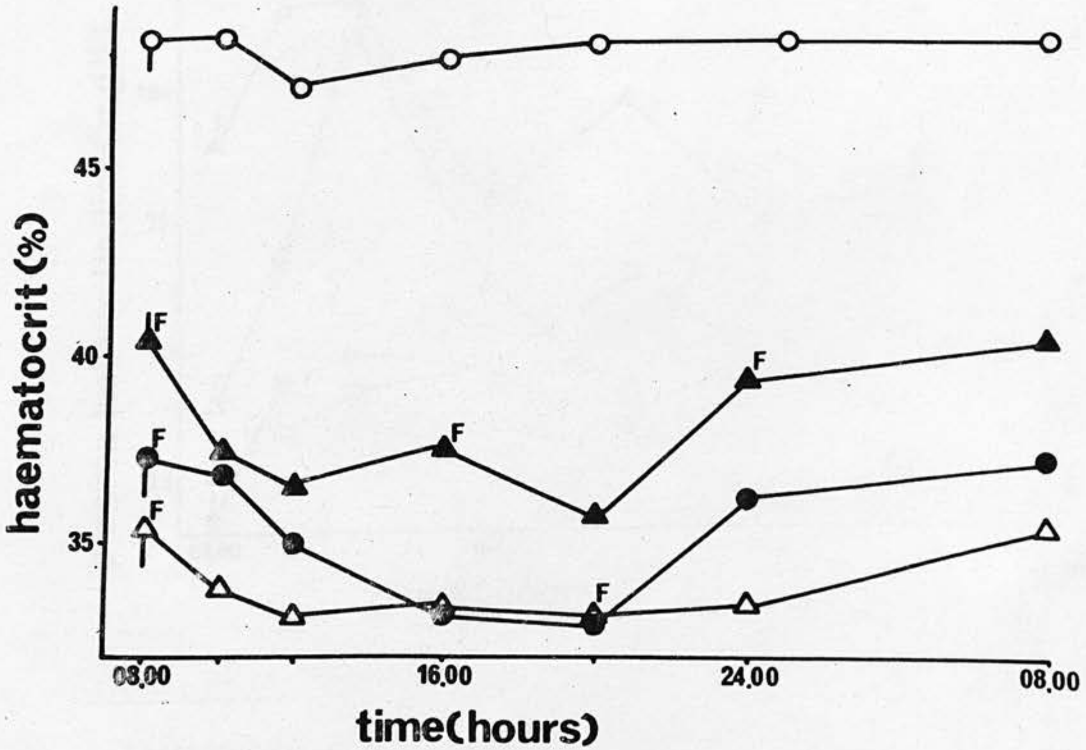
Figures S2.03, S2.04

Least-squares mean values (± S.E.) of plasma urea nitrogen and magnesium concentrations in 4 groups of sheep during early pregnancy.

○—○ Group A, ●—● Group R1, △—△ Group R2, ▲—▲ Group R3

F = Feeding



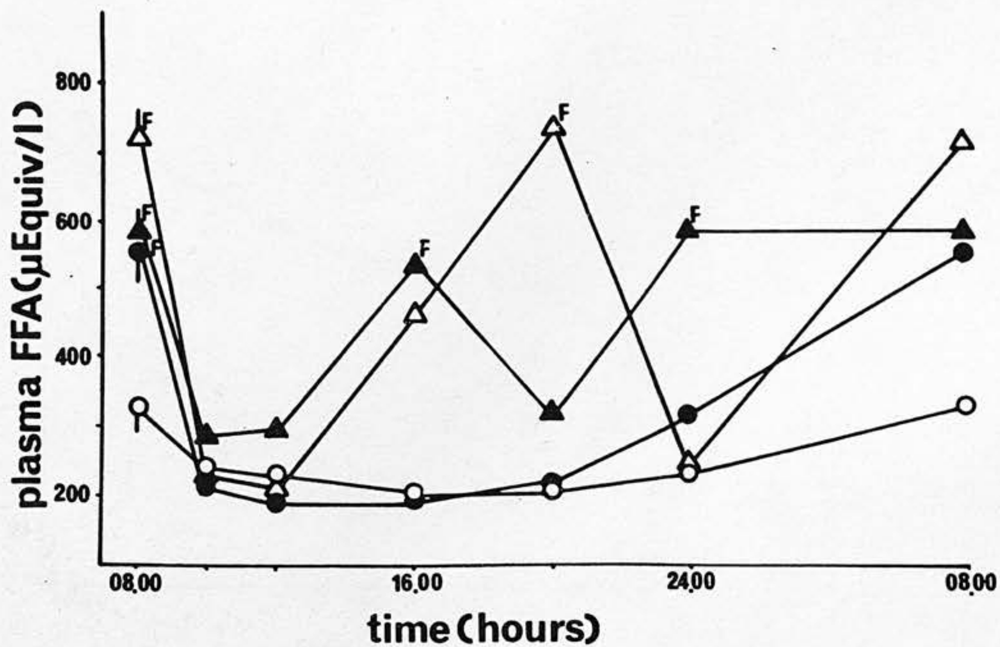


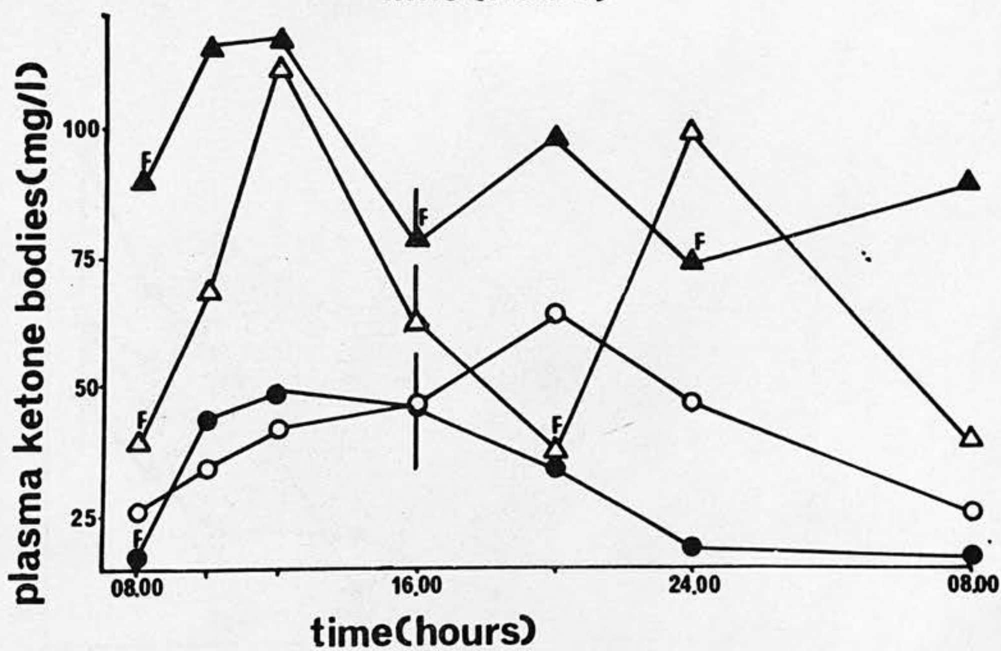
Figures S2.05, S2.10 (lower)

Least-squares mean values (± S.E.) of blood haematocrit levels in 4 groups of sheep during early and late (lower) pregnancy.

O—O Group A, ●—● Group R1, △—△ Group R2, ▲—▲ Group R3

F = Feeding



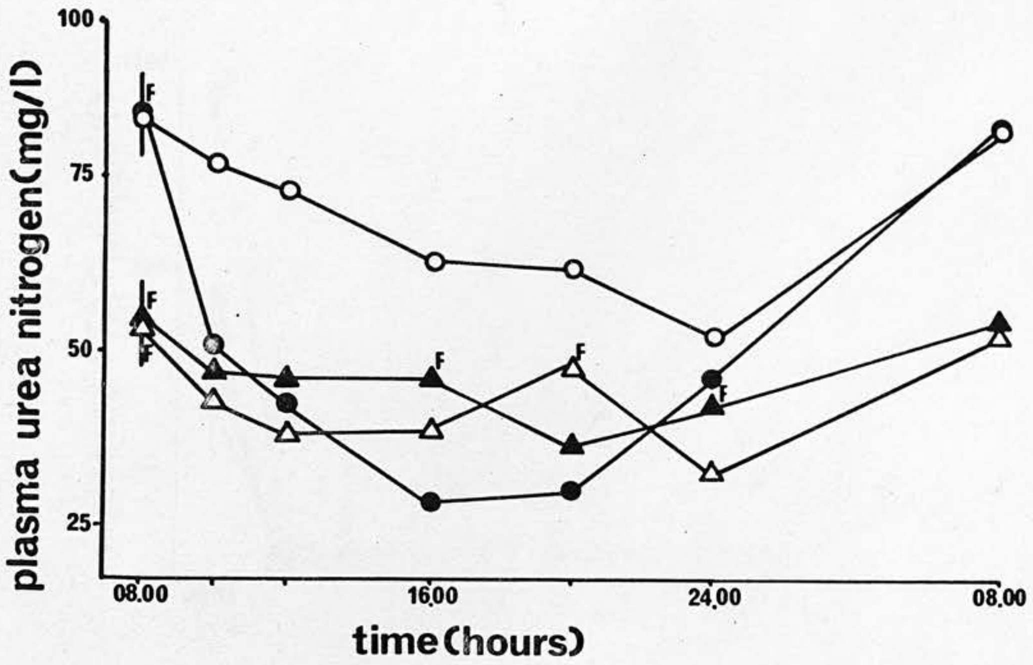


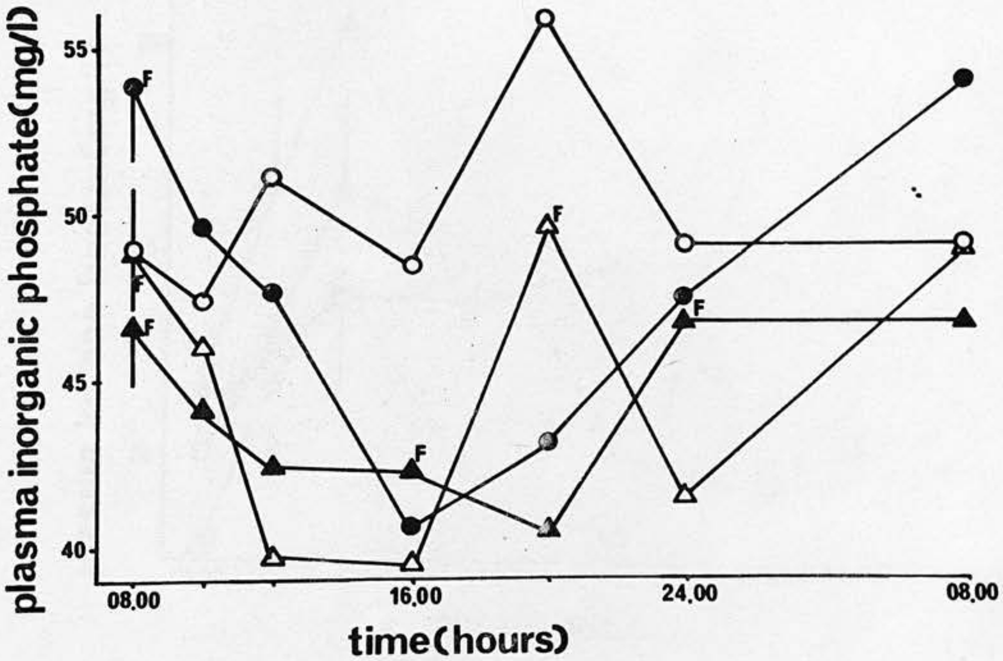
Figures S2.06, S2.07

Least-squares mean values (± S.E.) of plasma FFA and ketone body concentrations in 4 groups of sheep during late pregnancy.

Group A, Group R1, Group R2, Group R3

F = Feeding



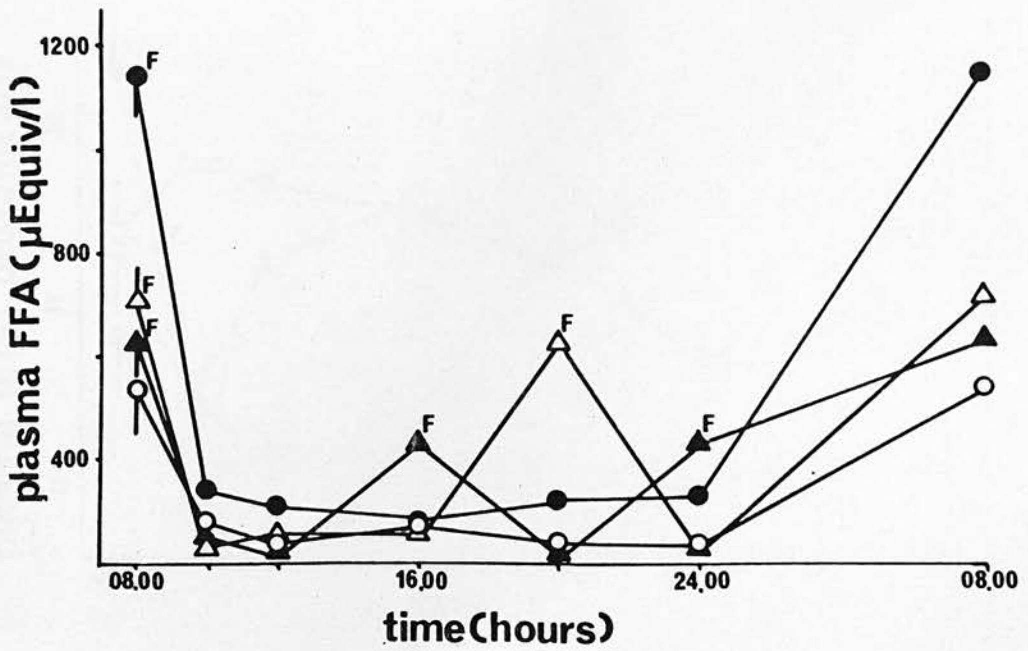


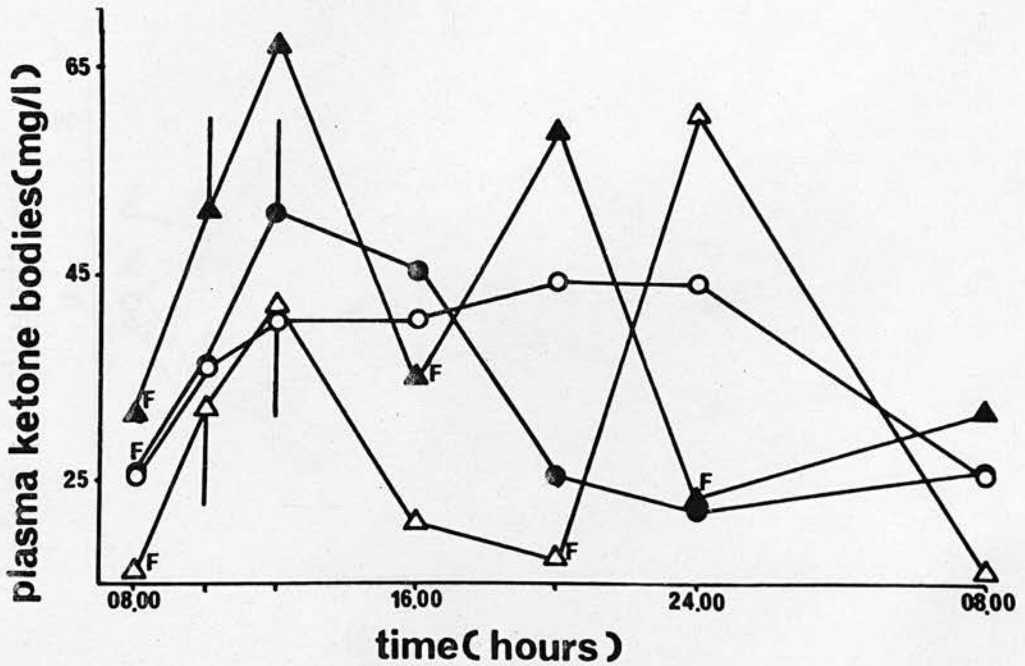
Figures S2.08, S2.09

Least-squares mean values (± S.E.) of plasma urea nitrogen and inorganic phosphate concentrations in 4 groups of sheep during late pregnancy.

○—○ Group A, ●—● Group R1, △—△ Group R2, ▲—▲ Group R3

F = Feeding



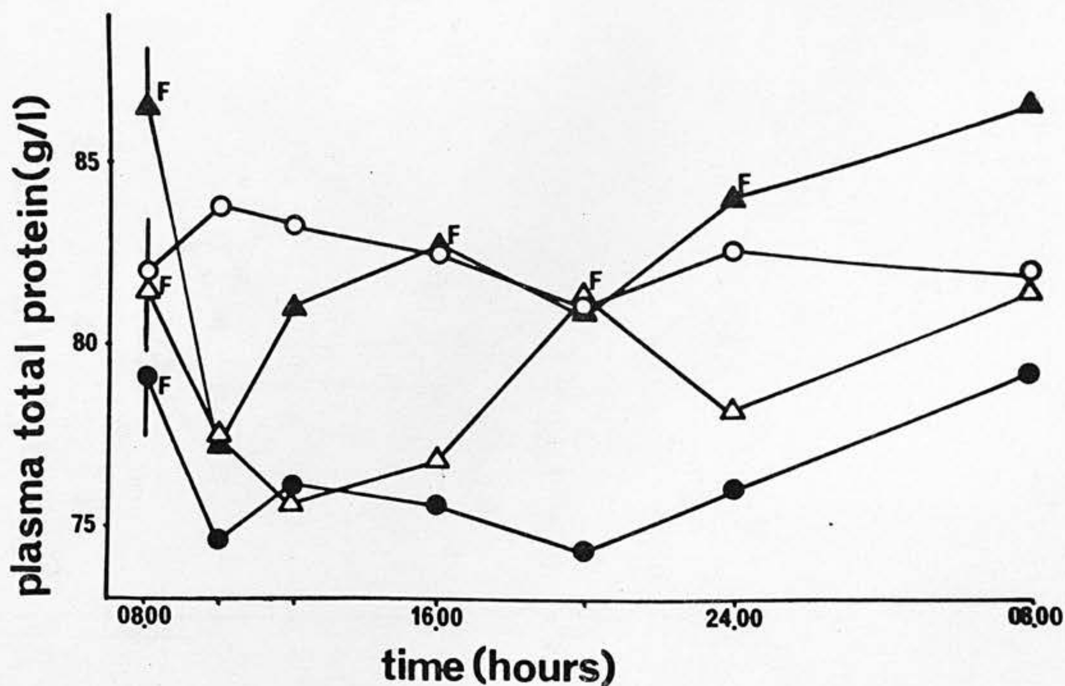


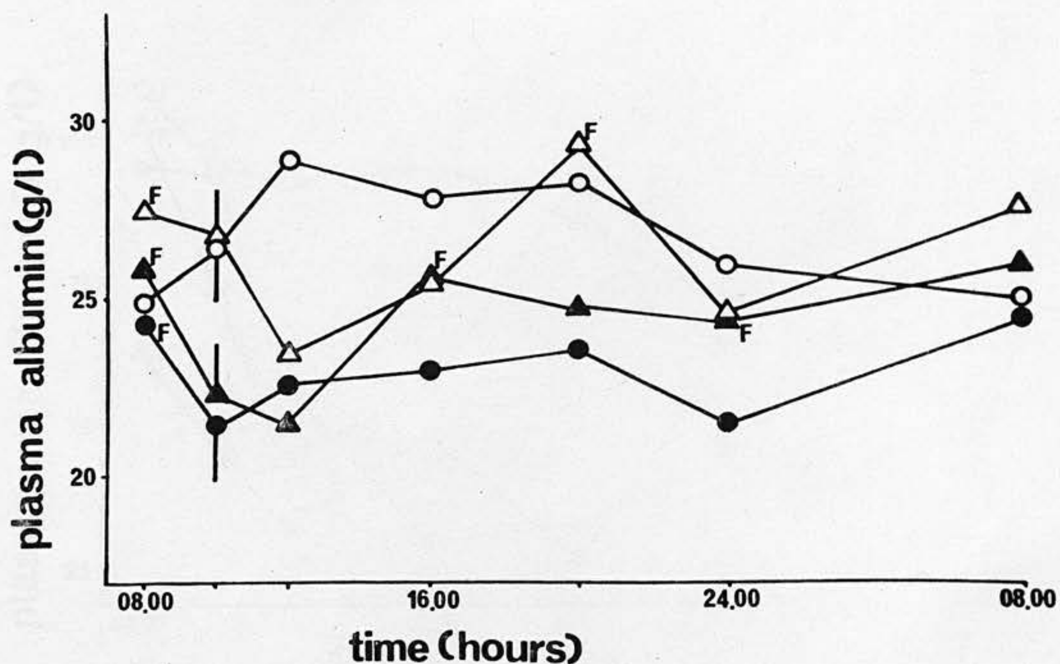
Figures S2.11, S2.12

Least-squares mean values (± S.E.) of plasma FFA and ketone body concentrations in 4 groups of sheep during early lactation.

O–O Group A, ●–● Group RL1, △–△ Group RL2, ▲–▲ Group RL3

F = Feeding



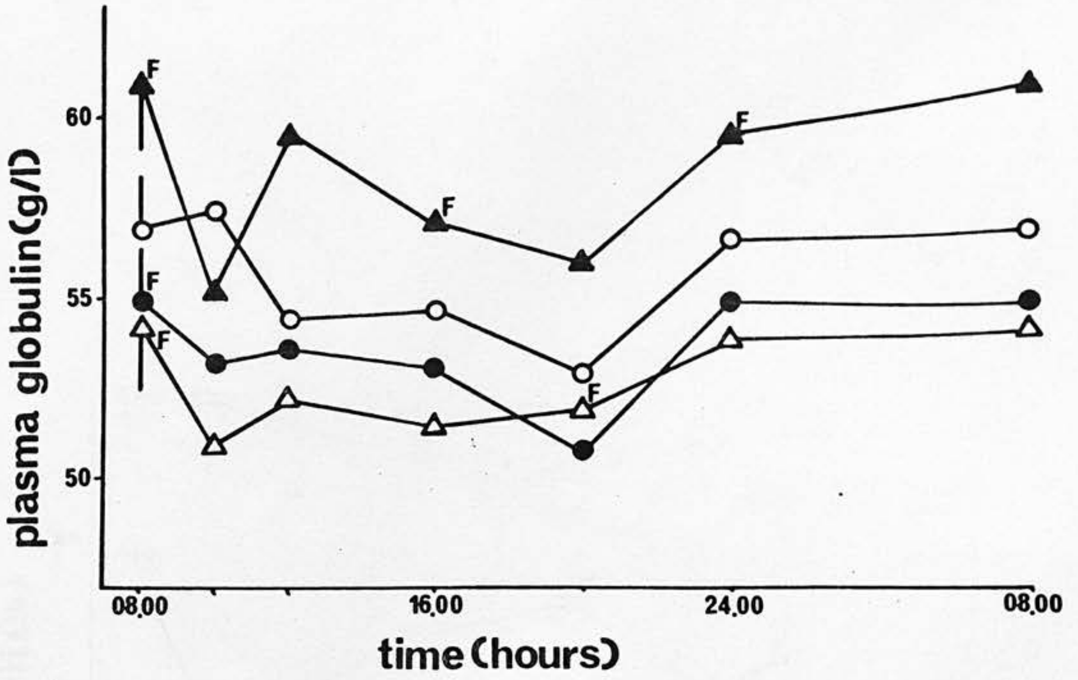


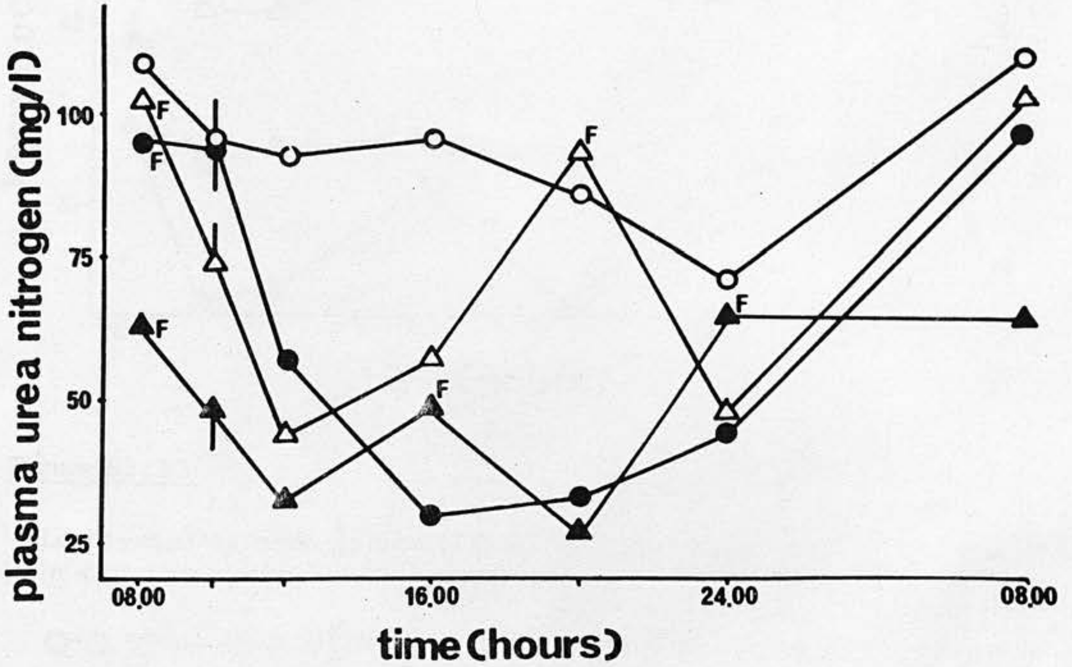
Figures S2.13, S2.14

Least-squares mean values (± S.E.) of plasma total protein and albumin concentrations in 4 groups of sheep during early lactation.

O–O Group A, ●–● Group RL1, △–△ Group RL2, ▲–▲ Group RL3

F = Feeding





Figures S2.15, S2.16

Least-squares mean values (± S.E.) of plasma globulin and urea nitrogen concentrations in 4 groups of sheep during early lactation.

○—○ Group A, ●—● Group RL1, △—△ Group RL2, ▲—▲ Group RL3

F = Feeding

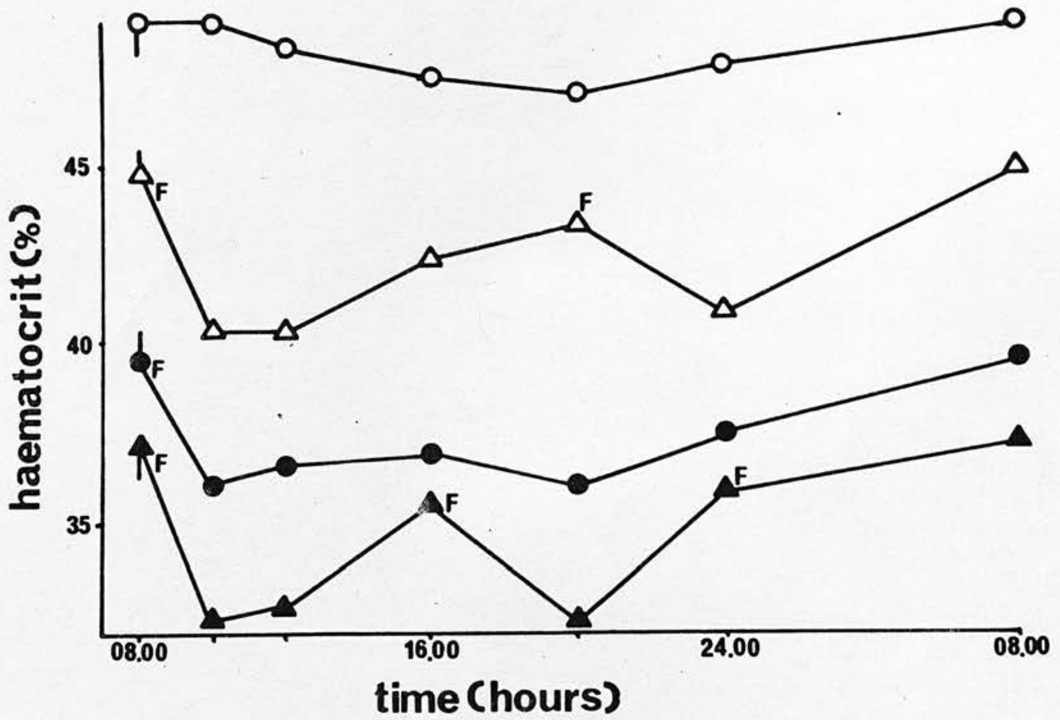


Figure S2.17

Least-squares mean values (± S.E.) of blood haematocrit levels in 4 groups of sheep during early lactation.

Group A, Group RL1, Group RL2, Group RL3

F = Feeding

ACKNOWLEDGEMENTS

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DECLARATION

I declare that this thesis has been composed by myself and that subject to the above acknowledgements, the work described here is my own.

C.R.E. Coggins 11/xi/76

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Appendix I. Typical computer print-out from an automated chemical assay

The computer programme consisted of two parts: a quality control section and a results section.

Quality Control

Experiment number.

First lab. number.

Number of peaks.

Versatol readings before drift (the X co-ordinates from the pencil follower).

Regression coefficient and residual variance (of the best line in a graph of the X co-ordinates of the standards v. the concentrations of the standards).

Uncorrected Versatol concentrations (initial and final).

Actual Versatol concentrations (as given by manufacturers).

Versatol multiplication factor.

Actual and estimated concentrations of standards.

Results

Pool plasma concentrations.

Mean and S.D. of pool plasma concentrations.

Drift-corrected Versatol concentrations (initial and final).

Sample concentrations, 1-64.

Results section, as above, for duplicate run.

Missing values.

Duplicate difference $>$ 3 x median duplicate difference in samples:

Mean of duplicate sample concentrations.

Other calculations (e.g. subtraction of albumin blanks, calculation of globulins).

Appendix II. Typical computer print-outs from non-automated assays

Free Fatty Acids

Experiment number.

First lab. number.

Number of samples.

Regression coefficient and residual variance of best line through standards.

Actual pool plasma concentration.

Estimated pool plasma concentration.

Multiplication factor.

Actual and estimated concentrations of standards.

Results.

Ketone Bodies

In KB assays the computer calculated concentrations in standards and unknowns from the manual input of the optical densities (O.D.) of the reaction products.

Experiment number?

First lab number?

O.D. butyrate?

O.D. distilled acetone?

O.D. undistilled acetone?

O.D. blank?

O.D. samples?

Results

Number of samples.

A/4 + B/3/4.

Results.

Appendix III. Typical LSMLGP output (from Experiment C2, omitting most of the actual data): listing of constants, least-squares means and standard errors

RHM name	Row Code	Independent Variable	Number of Observations	Constant Estimate	LSM	SE
Copper	1	MU	217	95.39	95.39	0.79
"	2	Group L	80	- 8.00	87.32	1.29
"	3	Group M	68	12.50	107.8	1.41
"	0	Group H	69	- 4.49	90.83	1.40
"	4	Time 09.00h	44	- 0.47	94.85	1.75
"	5	Time 15.00h	44	1.55	96.88	1.75
"	6	Time 17.00h	44	2.89	98.22	1.75
"	7	Time 19.00h	41	- 0.79	94.53	1.83
"	0	Time 22.00h	44	- 3.17	92.15	1.75
"	8	Cow L1	10			
"	9	Cow L2				
		Cow H10				
"	27	Group X Time, L X 09.00h				
"	28	Group X Time, L X 15.00h				
		Group X Time, H X 22.00h				
Glucose	1	MU	217	755.7	755.7	4.3

RHM = Right hand member

LSM = Least-squares mean

SE = Standard error

Appendix IV. Typical LSMLGP output (from Experiment C2, omitting most of the actual data): least-squares analysis of variance

COPPER

Source		df	Sum of Squares	Mean Squares	F
Total		217	108116	2452.6	18.15
			⋮	⋮	⋮
Total reduction		34			
MU		1			
Group		2			
Time		4			
Within Group :	L	7			
	M	6			
	H	6			
Group X Time		8			
Remainder		183			

GLUCOSE

Appendix V. Residual correlation coefficients between blood constituents in Experiment C1
(df = 213)

KB	0.00						
TP	-0.00	0.15*					
ALB	-0.01	-0.00	0.41***				
GLOB	0.00	0.16*	0.83***	-0.14*			
UN	-0.09	0.15*	0.07	0.02	0.08		
Ca	0.39***	0.00	-0.10	0.12	-0.18**	-0.23***	
Mg	0.05	-0.03	-0.05	0.15*	-0.13	0.18**	0.14
P	0.18**	0.08	0.00	-0.04	0.02	0.00	0.44
	GLUC	KB	TP	ALB	GLOB	UN	Ca

Appendix VI. Residual correlation coefficients between blood constituents in Experiment C2

(df = 181)

	GLUC	FFA	KB	TP	ALB	GLOB	UN	Ca	Mg	P
FFA	0.33***									
KB	-0.25***	-0.00								
TP	0.23**	0.00	-0.44***							
ALB	0.02	-0.25***	-0.29***	0.54***						
GLOB	0.25***	0.12	-0.37***	0.91***	0.15*					
UN	0.14*	0.12	-0.21**	0.09	0.22**	-0.00				
Ca	-0.00	-0.06	0.07	0.35***	0.29***	0.28***	-0.17*			
Mg	-0.15*	-0.00	-0.02	-0.27***	0.00	-0.33***	0.04	-0.23**		
P	-0.15*	-0.19**	-0.09	-0.24***	0.03	-0.31***	-0.02	0.21**	-0.00	
Cu	0.15*	-0.04	0.00	0.25***	-0.01	0.30***	0.05	0.20**	-0.17*	-0.1

Appendix VII. Residual correlation coefficients between plasma constituents of Group R animals in Experiment S1

Early pregnancy (df=56) (above diagonal, left); Late pregnancy (df=43) (below diagonal, left); Lactation (df=69) (right)

	GLUC	FFA	KB	ALB	UN	Ca	P	GLUC	FFA	KB	ALB	UN	Ca	P
GLUC		-0.24	-0.48***	-0.40**	0.11	-0.16	-0.23							
FFA	-0.39**		0.28*	0.36**	-0.03	0.09	0.22	-0.09						
KB	-0.26	0.09		0.32*	0.05	-0.02	0.32*	-0.19	0.08					
ALB	-0.17	0.25	-0.12		-0.00	0.50***	0.12	0.14	-0.01	-0.33**				
UN	-0.17	0.08	0.00	0.04		0.02	-0.25*	-0.29	0.15	-0.09	0.37**			
Ca	-0.06	-0.09	0.00	0.20	-0.26		0.17	-0.05	-0.07	-0.26*	0.40***	0.22		
P	0.08	0.17	-0.18	-0.10	0.09	0.38**		0.06	0.01	0.11	0.00	0.09	-0.15	

Late pregnancy (df=43) Lactation (df=69)

Appendix VIII. Residual correlation coefficients between blood constituents in Experiment S2 (early pregnancy)

Group K (df = 78)

	GLUC	FFA	KB	TP	ALB	GLOB	UN	Ca	P	Mg	PCV
GLUC		*-0.28	*-0.28	0.20	0.20	0.05	-0.12	0.10	*-0.22	**0.34	*-0.25
FFA	0.00		0.08	0.14	*0.26	0.10	***0.36	0.15	**0.30	0.11	0.05
KB	***-0.38	-0.02		-0.10	-0.12	0.01	0.09	0.01	0.00	-0.17	0.09
TP	**0.30	*0.20	**-0.28		***0.49	***0.62	*0.23	*0.26	0.01	*0.26	-0.01
ALB	*0.21	**0.29	*-0.19	***0.58		***-0.36	*0.27	-0.09	0.10	*0.29	-0.04
GLOB	0.16	0.05	-0.14	***0.63	-0.25		0.02	***0.36	-0.10	0.03	0.01
UN	0.16	0.41	*-0.24	***0.38	*0.21	**0.27		0.02	**0.32	0.13	0.06
Ca	0.13	0.01	-0.03	***0.31	0.16	*0.21	-0.10		-0.03	**0.34	0.02
P	*-0.23	0.04	*0.23	0.02	0.10	-0.08	-0.04	0.12		*0.23	0.14
Mg	***0.40	0.01	***-0.37	*0.23	0.17	0.13	*0.20	0.16	***0.47		-0.01
PCV	*-0.21	0.08	0.13	-0.01	-0.02	-0.02	0.08	-0.01	0.10	-0.02	
	GLUC	FFA	KB	TP	ALB	GLOB	UN	Ca	P	Mg	PCV

Groups K, A (df = 108)

Appendix IX. Residual correlation coefficients between blood constituents in Experiment S2 (late pregnancy)

Group R (df = 78)

	GLUC	FFA	KB	TP	ALB	GLOB	UN	Ca	P	Mg	PCV
GLUC		-0.11	-0.02	-0.11	***-0.56	**0.30	*-0.25	-0.17	0.05	0.00	-0.01
FFA	-0.11		-0.15	-0.08	0.01	-0.13	0.07	-0.09	0.04	-0.19	-0.15
KB	0.01	-0.15		*-0.25	-0.02	*0.24	*-0.24	-0.19	-0.01	*0.26	-0.05
TP	-0.18	-0.04	*-0.21		***0.41	***0.72	-0.10	***0.58	0.03	-0.12	**0.33
ALB	***-0.54	0.05	-0.00	***0.38		**-0.32	0.16	***0.58	*-0.23	-0.04	*0.25
GLOB	*0.20	-0.09	*0.21	***0.74	***0.31		-0.00	0.17	0.21	-0.10	0.15
UN	*0.21	0.18	*-0.19	**0.23	*0.24	0.09		0.05	0.07	-0.09	0.00
Ca	-0.08	-0.09	-0.08	***0.40	***0.47	0.08	-0.06		0.01	*0.24	0.15
P	0.05	-0.03	0.00	-0.05	-0.16	0.07	-0.00	*0.24		-0.00	-0.13
Mg	-0.16	-0.11	0.12	-0.10	-0.03	-0.09	0.03	***-0.40	-0.07		0.12
PCV	0.04	-0.05	-0.06	***0.33	0.13	**0.26	0.05	0.13	-0.14	0.00	
	GLUC	FFA	KB	TP	ALB	GLOB	UN	Ca	P	Mg	PCV

Groups R, A (df = 108)

Appendix X. Residual correlation coefficients between blood constituents in Experiment S2 (lactation)

Group K (df = 73)

	GLUC	FFA	KB	TP	ALB	GLOB	UN	Ca	P	Mg	PCV
GLUC		-0.55	-0.11	0.06	-0.07	0.15	0.04	0.16	0.12	0.03	0.09
FFA	-0.05		-0.06	*0.23	0.12	0.16	**0.30	-0.00	**-0.34	0.14	**0.34
KB	-0.01	-0.08		-0.16	0.03	0.19	**0.32	0.05	-0.15	-0.19	0.14
TP	0.01	*0.25	-0.13		***0.47	***0.68	*0.24	***0.59	-0.02	0.17	0.17
ALB	-0.11	0.14	0.05	***0.43		**-0.30	-0.01	*0.27	-0.16	**0.35	0.03
GLOB	0.12	0.12	-0.16	***0.58	***0.47		**0.26	***0.43	0.11	-0.08	0.17
UN	-0.07	***0.32	**0.26	*0.24	0.13	0.10		0.08	-0.05	-0.03	0.09
Ca	0.13	-0.01	0.05	***0.56	**0.27	**0.31	-0.03		***0.43	-0.11	0.22
P	-0.03	**-0.29	0.13	-0.03	-0.12	0.07	0.02	**0.32		**0.30	-0.03
Mg	0.01	0.09	-0.16	0.17	**0.33	-0.11	-0.02	-0.08	*-0.20		-0.08
PCV	-0.00	***0.35	0.11	0.18	0.06	0.13	0.12	*0.22	-0.00	-0.13	
	GLUC	FFA	KB	TP	ALB	GLOB	UN	Ca	P	Mg	PCV

Groups (K+A)(df = 98)

Appendix XI. Equations used to calculate ME content of silage and concentrates in Experiment C3 (courtesy, R.A. Edwards)

Silage

ME = 17.4 - 0.2 (MAD-F %) (if silage D.M. = $<$ 25%)

ME = 16.4 - 0.188 (MAD-F %) (if silage D.M. = $>$ 25%)

Concentrates

Group L

ME = 0.137 CP% + 0.318 EE% + 0.091 CF% + 0.122 NFE%

Group M

ME + 0.12 CP% + 0.291 EE% + 0.078 CF% + 0.137 NFE%

Group H

ME = 0.166 CP% + 0.274 EE% + 0.072 CF% + 0.145 NFE%

PUBLICATIONS

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Diurnal variation in the chemical composition of plasma from lactating beef cows on three dietary energy intakes

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SUMMARY

Twenty-two beef cows, producing on average 5·5 kg 4% fat corrected milk per day, were offered diets of rolled barley and silage such that eight animals were consuming 70%, seven 95% and seven 120% of their estimated energy requirements. Blood samples were taken at intervals during the day and the plasma concentrations of glucose, free fatty acids (FFA), ketone bodies, total protein, albumin, globulin, urea nitrogen, calcium, inorganic phosphate, magnesium and copper determined.

Diurnal variation was found in the plasma concentration of glucose, FFA, ketone bodies, urea nitrogen, albumin, calcium and magnesium; it was particularly marked for the first four constituents and appeared to be related to feeding.

There were no overall significant differences between groups in the mean daily concentrations of the measures of energy status (glucose, FFA, ketone bodies), but significant ($P < 0·001$) prefeeding group differences were found in glucose and ketone body concentrations. No significant differences between groups were found for the remaining constituents.

There was a postprandial depression in plasma glucose concentration, which was positively correlated with FFA concentration.

INTRODUCTION

Concentrations of a range of blood constituents, obtained from single blood samplings, have been used as indices of the nutritional status both of experimental sheep (Reid & Hinks, 1962; Russel, Doney & Reid, 1967; Sykes & Field, 1973) and of commercial dairy herds (Payne *et al.* 1970). In cattle fed low-roughage diets concentrations of several of these constituents (glucose, FFA, ketone bodies, urea nitrogen) have shown significant diurnal variation (Allcroft, 1933; Radloff, Schultz & Hoekstra, 1966; Thornton, 1970; Hove & Blom, 1973), whereas others have shown less pronounced variation (Unshelm & Rappen, 1968; Unshelm, 1969; Hagemeister & Unshelm, 1970). There is currently no information on the extent of diurnal variation in the blood composition of energy-deficient beef cows. Undernutrition in commercial beef herds may be of importance as farmers attempt to minimize production costs by reducing the maternal plane of nutrition, with a resulting depletion of body reserves (Economides *et al.* 1973).

In the present work the diurnal variation in the blood composition of beef cows fed a typical high-roughage diet of silage and barley has been determined and compared with the variation attributable to level of nutrition. As measures of energy status we used the plasma concentration of glucose, and also of FFA and ketone bodies (Bowden, 1971); of protein status total protein, albumin, globulin and urea nitrogen, and of mineral status calcium, magnesium, inorganic phosphate and copper.

MATERIALS AND METHODS

Animals and management

Twenty-two lactating White Shorthorn × Galloway (Blue-grey) cows from the Boghall herd of the Edinburgh School of Agriculture, neck-tied in a conventional byre, were allocated at calving into three groups: L (eight animals), M (seven) and H (seven). The groups received feed allowances designed to provide 90, 125 and 175% respectively of their estimated (ARC, 1965) energy requirements for maintenance, based on *post-partum* live weight. The diet, offered from parturition, consisted of a fixed ratio of a silage–barley mixture with overall energy and nitrogen concentrations of 10·5 MJ metabolizable energy (ME) and 11·6 g

nitrogen (N) per kg dry matter (D.M.). Values of 9·6 and 12·9 MJ/kg D.M. and 12·0 and 10·6 g N/kg D.M. were assumed for the ME and N concentrations of the silage and of the barley respectively (R. A. Edwards, personal communication).

All animals were machine-milked twice daily (at 07.10 and 15.00 h); they were offered approximately 5 kg silage at 07.00h and the remainder of the total daily ration at 13.30 h. Silage refusals were removed and weighed each morning before fresh silage was offered; all animals had access to tap water at all times. The byre was illuminated continuously during the experiment, and also during a 10-day 'dummy run' immediately prior to the experiment.

Experimental design

The blood sampling programme commenced some 2–3 months after parturition. The mean age, weight, stage of lactation and milk yield of the groups are shown in Table 1 and corresponding rations consumed, with estimated energy and nitrogen balances, are given in Table 2.

Each animal was bled once daily over a 10-day period at one of five times during the day: 09.00, 15.00, 17.00, 19.00 and 22.00 h. The sampling programme was arranged so that two independent blood samples were obtained for each animal at each time of day, thus preventing unrepresentative plasma constituent concentrations arising from the stress of previous sampling(s) on the same day (Gartner, Ryley & Beattie, 1966). A dummy run was performed in an attempt to accustom the animals to the environment in which they were subsequently bled: it was identical to the actual experiment except that no blood samples were taken.

Jugular blood samples were taken using heparinized copper-free Vacutainers (Beckton-Dickinson & Co. Ltd, Rutherford, New Jersey), and plasma obtained within 15 min by centrifugation at 1300 *g* for 10 min. Plasma was kept at −20 °C in polystyrene tubes prior to chemical analysis.

Chemical analysis

Plasma samples were analysed for total protein albumin and copper; and deproteinized (using 4% trichloroacetic acid) plasma samples for glucose urea nitrogen, calcium and inorganic phosphate using modifications of the following automated methods: total protein, Weichselbaum (1946) albumin, Ness, Dickerson & Pastewka (1965) copper, Summers (1960); glucose, Trinder (1969) urea nitrogen, Marsh, Fingerhut & Miller (1965) calcium, Gitelman (1967); inorganic phosphate Fiske & Subbarow (1925). Magnesium concentra

Table 1. *Age, weight and milk production of the cows during the blood-sampling period*

	Group			Pooled
	L	M	H	S.D.
Age (years)	7·0	10·0	9·5	3·6
Weight (kg)	464	479	489	52
Weight as % of weight at parturition	84	85	91	5
Mean daily 4% FCMY* (kg)	5·46	5·51	5·50	2·02
Cumulative milk yield from parturition (kg)	390	326	530	212
Days *post-partum*	61	52	80	28

S.D. = standard deviation.
* fat corrected milk yield

Table 2. *Rations consumed by the cows, with estimated energy and nitrogen balances, during the blood sampling period*

	Group			Pooled
	L	M	H	S.D.
Rations consumed (kg/day)				
Fresh silage offered	16·8	23·6	31·9	1·6
Fresh silage refusals	—	0·95	2·88	1·25
Silage D.M. intake	3·64	4·90	6·26	0·36
Fresh barley offered	1·68	2·43	3·16	0·13
Barley D.M. intake	1·25	1·81	2·36	0·10
Energy balance (MJ/day)				
Intake Silage	34·6	47·1	60·6	3·5
Barley	16·7	23·2	29·6	1·3
Total	51·3	70·3	90·2	4·4
Requirement				
Maintenance*	48·9	49·7	50·6	9·5
Lactation†	24·6	24·6	24·6	9·1
Total	73·5	74·3	75·2	9·0
Intake/Requirement (%)	70·0	95·0	120	12·8
Nitrogen balance (g/day)				
Intake Silage	43·6	58·8	75·0	4·3
Barley	13·3	19·2	24·9	1·1
Total	56·9	78·0	100	5·1
Requirement				
Maintenance*	18·0	18·7	19·0	2·0
Lactation†	42·0	42·2	42·2	15·5
Total	60·0	60·9	61·2	15·2
Intake Requirement	98·5	140	175	48

S.D. = standard deviation.
* 0·489 MJ/kg $(W)^{0.75}$; 0·039 g N/kg W (A.R.C., 1965).
† 4·51 MJ, 7·68 g N/kg 4% fat corrected milk (A.R.C., 1965).

tion in the deproteinized plasma samples was estimated using atomic absorption spectrophotometry. Glucose, urea nitrogen, calcium, inorganic phosphate and magnesium estimations were standardized against control sera (Versatol: William Warner Diagnostics Ltd., Eastleigh, Hants). Globulin values were taken as the arithmetical difference between total protein and albumin values; these two estimations were made using bovine albumin standards (N × 6·25).

All estimations were performed in batches and each batch analysed twice; if the assessment of the mean difference between duplicates, within batches was greater than 2% then the results for the batch were rejected and samples re-analysed.

FFA concentrations were estimated using the Dole (1956) extraction followed by the colorimetric determination of Duncombe (1963); ketone body concentrations were measured as described by Reid (1960).

Statistical analysis

A least-squares, maximum likelihood general purpose program (Harvey, 1968) was used to determine the variation in blood composition due to time of blood sampling, group, and the (group × sampling time) interaction; the program also determined the significance of this variation for each variable. Correlations between blood constituent concentrations were determined after the effects of group, time and the interaction between group and time had been removed.

The least-squares technique involves the principle of attributing equal weight to each class of a given effect, thus making it possible to study the effect of a certain factor without it being biased by the uneven distribution of other effects. Since each class of each source of variation exerts an equal effect irrespective of the number of observations in the class, the least-squares means often differ from the simple overall means.

RESULTS

The significance of group, time of day and the (group × time of day) interaction as sources of variation in plasma composition is shown in Table 3.

The groups maintained similar concentrations of most of the plasma constituents (Table 4), the only statistically significant group difference being the tendency of group M animals to maintain higher levels of globulin and total protein than groups L or H. This was found to be due to two animals in group M which were maintaining relatively high globulin concentrations of 51·4 and 51·8 g/l (cf. overall mean of 42·9 g/l).

Table 3. *Significance levels of sources of variation in plasma composition*

	Group $F_{2,19}$	Time $F_{4,183}$	Interaction $F_{8,183}$
Glucose	NS	***	NS
FFA	NS	***	NS
Ketone bodies	NS	***	NS
Total protein	*	NS	NS
Albumin	NS	***	NS
Globulin	*	NS	NS
Urea nitrogen	NS	***	NS
Calcium	NS	***	NS
Inorganic phosphate	NS	NS	NS
Magnesium	NS	***	NS
Copper	NS	NS	NS

F = Variance ratio. NS = $P > 0·05$.
* $0·01 < P < 0·05$; *** $P < 0·001$.

There was highly significant ($P < 0·001$) diurnal variation in the plasma concentration of glucose, FFA, ketone bodies, albumin, urea nitrogen, calcium and magnesium (Table 4); for each constituent the diurnal variation was similar in each group.

The marked diurnal variation in plasma concentrations of glucose, FFA and ketone bodies is shown in Figs. 1–3: for these measures of energy status the variations appeared to be related to feeding in that glucose and FFA concentrations decreased, and ketone bodies increased, shortly after feeding. Although no overall group effects were observed for the energy measures, pre-feeding (09.00 h) concentrations of glucose and ketone bodies in group L were significantly ($P < 0·001$) different from those in groups M or H. Concentrations of FFA were similar in all groups at all times.

Total protein, albumin and globulin concentrations were relatively constant throughout the day, although the small post-prandial increase in plasma albumin concentration was statistically highly significant. Plasma urea nitrogen concentration also increased after feeding (Fig. 4) although no significant difference in pre-feeding urea nitrogen concentration could be detected between the groups.

The magnitude of the statistically highly significant diurnal variation in plasma calcium and magnesium concentrations was low; inorganic phosphate and copper concentrations were constant throughout the day.

Of the 55 residual correlation coefficients (Table 5) 32 were statistically significant ($P < 0·05$), the largest being that between globulin and total protein ($r = +0·91$, $P < 0·001$). There was a positive correlation ($r = 0·33$, $P < 0·001$) between glucose and FFA.

Table 4. *Variation in constituent concentrations attributable to the effects of group and time*

	Overall mean (±S.D.)	Group least-squares means (±S.E.) Group			Time least-squares means (±S.E.) Time (h)				
		L	M	H	09.00	15.00	17.00	19.00	22.00
n	217	80	68	69	44	44	44	41	44
Glucose (mg/l)	756 ± 91	737 ± 7·0	774 ± 7·6	760 ± 7·6	835 ± 9·5	790 ± 9·5	705 ± 9·6	711 ± 10·0	735 ± 9·5
FFA (μ-equiv./l)	358 ± 144	381 ± 17·6	378 ± 19·2	312 ± 19·0	509 ± 23·8	451 ± 23·8	267 ± 23·8	259 ± 24·9	287 ± 23·8
Ketone bodies (mg/l)	24·6 ± 9·1	25·3 ± 0·74	22·7 ± 0·81	25·5 ± 0·8	19·2 ± 1·01	17·4 ± 1·01	27·9 ± 1·01	29·4 ± 1·05	29·2 ± 1·01
Total protein (g/l)	74·9 ± 5·5	72·6 ± 0·39	78·2 ± 0·43	74·4 ± 0·42	74·9 ± 0·53	74·7 ± 0·53	76·4 ± 0·53	74·9 ± 0·55	74·5 ± 0·53
Albumin (g/l)	31·9 ± 3·0	31·8 ± 0·16	30·8 ± 0·17	33·2 ± 0·17	31·2 ± 0·21	31·4 ± 0·21	32·7 ± 0·21	32·3 ± 0·22	32·0 ± 0·21
Globulin (g/l)	42·9 ± 5·9	40·7 ± 0·33	47·3 ± 0·35	41·2 ± 0·35	43·6 ± 0·44	43·2 ± 0·44	43·7 ± 0·44	42·6 ± 0·46	42·5 ± 0·44
Urea nitrogen (mg/l)	68·8 ± 27·0	65·3 ± 1·85	63·9 ± 2·01	77·9 ± 2·00	59·7 ± 2·50	64·2 ± 2·50	74·4 ± 2·51	79·4 ± 2·61	67·2 ± 2·50
Calcium (mg/l)	100 ± 4	100 ± 0·3	100 ± 0·4	100 ± 0·3	102 ± 0·5	99·2 ± 0·48	99·8 ± 0·48	100 ± 0·5	103 ± 0·5
Inorganic phosphate (mg/l)	48·8 ± 8·3	50·7 ± 0·68	48·1 ± 0·74	47·3 ± 0·74	49·9 ± 0·92	48·1 ± 0·92	48·5 ± 0·93	48·3 ± 0·96	48·8 ± 0·92
Magnesium (mg/l)	22·7 ± 2·4	23·3 ± 0·20	21·7 ± 0·20	22·9 ± 0·20	22·3 ± 0·25	21·8 ± 0·25	22·5 ± 0·26	22·8 ± 0·27	23·8 ± 0·26
Copper (mg/l)	0·95 ± 0·22	0·87 ± 0·013	1·07 ± 0·014	0·91 ± 0·014	0·95 ± 0·017	0·97 ± 0·017	0·98 ± 0·017	0·94 ± 0·018	0·92 ± 0·017

n = Number of observations. S.D. = Standard deviation. S.E. = Standard error.

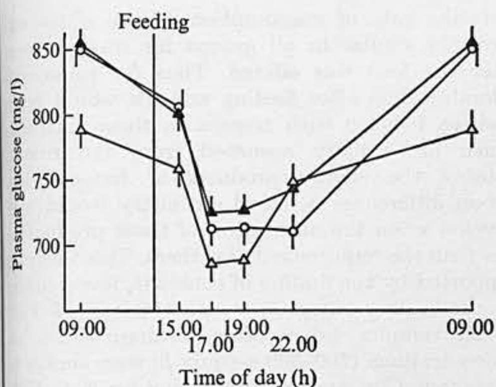

Fig. 1. Least-squares mean values (±S.E.) of plasma glucose concentrations in three groups of lactating beef cows at five times during the day. △—△, Group L; ▲—▲, group M; ○—○, group H.

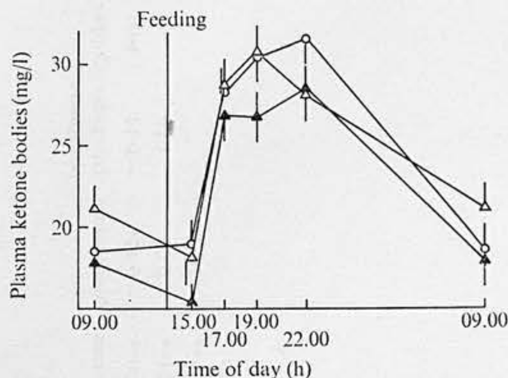

Fig. 3. Least-squares mean values (±S.E.) of plasma ketone body concentrations in three groups of lactating beef cows at five times during the day. △—△, Group L; ▲—▲, group M; ○—○, group H.

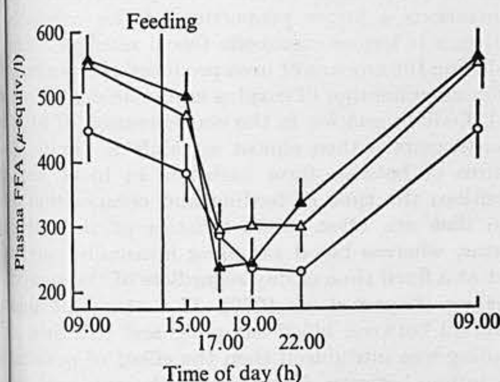

Fig. 2. Least-squares mean values (±S.E.) of plasma FFA concentrations in three groups of lactating beef cows at five times during the day. △—△, Group L; ▲—▲, group M; ○—○, group H.

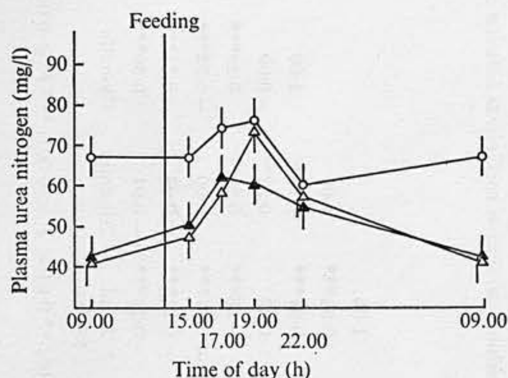

Fig. 4. Least-squares mean values (±S.E.) of plasma urea nitrogen concentrations in three groups of lactating beef cows at five times during the day. △—△, Group L; ▲—▲, group M; ○—○, group H.

DISCUSSION

The results show clearly that for glucose, FFA, ketone bodies and urea-nitrogen diurnal variation in plasma concentration is of greater importance than the variation resulting from a 76% difference in D.M. intake and a 71% difference in energy status (Table 2). Most of the diurnal variation was associated with feeding.

The postprandial changes in plasma concentrations of the energy measures are qualitatively similar to results obtained from high-yielding (up to 40 kg milk/day) dairy cows fed adequate amounts of low-roughage diets (Radloff *et al.* 1966; Hove & Blom, 1973). However, the changes described in the present work were much more rapid than those reported by either group of earlier workers; the rapid rise in ketone body concentrations immediately after feeding is thought to be due to the conversion of silage butyrate to β-hydroxybutyrate during ruminal absorption (Kronfeld, 1972). Maximum urea-nitrogen concentrations were observed 8 h after feeding; a finding in agreement with previous work (Thornton, 1970) in beef cattle given a basal low-nitrogen diet supplemented by varying amounts of urea.

The absence of group differences in constituent concentrations (in particular concentrations of glucose, FFA and ketone bodies) until approximately 20 h after the main feed of silage and barley was offered may be related to the nature of the

Table 5. *Residual correlation coefficients between constituents*

	Glucose	FFA	Ketone bodies	Total protein	Albumin	Globulin	Urea nitrogen	Calcium	Magnesium	Inorganic phosphate	Copper
Glucose	1·00										
FFA	0·33***	1·00									
Ketone bodies	−0·25***	−0·00	1·00								
Total protein	0·23**	0·00	−0·44***	1·00							
Albumin	0·02	−0·25***	−0·29***	0·54***	1·00						
Globulin	0·25***	0·12	−0·37***	0·91***	0·15*	1·00					
Urea nitrogen	0·14*	0·12	−0·21**	0·09	0·22**	−0·00	1·00				
Calcium	−0·00	−0·06	−0·07	0·35***	0·29***	0·28***	−0·17*	1·00			
Magnesium	−0·15*	−0·00	0·02	−0·27***	0·00	−0·33***	−0·04	−0·23**	1·00		
Inorganic phosphate	−0·15*	−0·19**	−0·09	−0·24***	0·03	−0·31***	−0·02	0·21**	−0·00	1·00	
Copper	0·15*	−0·04	0·00	0·25***	−0·01	0·30***	0·05	0·20**	−0·17*	−0·14	1·00

* $0{\cdot}01 < P < 0{\cdot}05$; ** $0{\cdot}01 < P < 0{\cdot}001$; *** $P < 0{\cdot}001$ (181 D.F.).

diet; the rate of consumption of the silage was probably similar in all groups for several hours after the feed was offered. Thus for some considerable time after feeding animals would be in positive balance with respect to those nutrients which are rapidly absorbed from the rumen, notably the energy products of fermentation. Group differences in blood chemistry would only develop when the absorption of these products is less than the requirements for them. This theory is supported by the finding of constant, low (relative to pre-feeding samples) concentrations of FFA in all samples taken after feeding; these low concentrations (200–300 μ-equiv./l) were similar to those found by Annison (1960) and by A. C. Field (unpublished) in well-nourished sheep and cattle respectively.

The low overall concentrations of urea nitrogen (relative to those found by Payne *et al.* 1970) are probably due to the low concentration (7·3 %) of protein in the diet: at low dietary nitrogen concentrations a larger proportion of the available nitrogen is lost as metabolic faecal nitrogen, thus reducing the amount of urea produced systemically from de-amination of surplus amino-acids.

Prandial variation in the concentration of blood constituents is then almost certainly a significant source of between-farm variation in blood composition: the time of feeding and composition of the diet are often characteristics of individual farms, whereas blood sampling is usually carried out at a fixed time of day regardless of the time of feeding (Payne *et al.* 1970). If a standard time interval between blood sampling and the time of feeding was introduced then the effect of prandial variation between farms would be minimal for animals consuming similar rations, thus allowing more meaningful comparisons of blood chemistry to be made. Prandial variation may also contribute to within-farm variation in blood composition as the diet and time of feeding of dry cows often differs from that of high-yielding cows.

Correlations between blood constituent concentrations have been examined by several workers (Radloff *et al.* 1966; Erfle, Fisher & Sauer, 1974), as well as using various species, breeds, ages and physiological states of animals different workers have tended to use different methods for the estimation of these coefficients. We have used simple residual correlation coefficients. Several of the correlations which we found are at variance with those presented by other workers; their value in the biochemical assessment of nutritional status is probably low. One previously unreported correlation is the positive relationship between glucose and FFA: this is difficult to explain using current theories on the mechanisms involved in caloric homeostasis in ruminants.

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CHANGES IN PLASMA CONCENTRATIONS OF GLUCOSE, FREE FATTY ACIDS, KETONE BODIES, THYROXINE AND INSULIN OF LACTATING BEEF COWS IN RELATION TO TIME OF FEEDING AND ENERGY STATUS.

by

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SUMMARY

In single-suckling beef enterprises the main variable cost is the nutrition of the mother : it is in the farmer's interest to minimize feed costs, without sacrificing animal performance. To assess the adequacy of dietary regimens, some system for monitoring energy status must be devised.

To investigate the relevance of the true (free fatty acids - FFA) and possible ancillary (glucose, ketone bodies (KB), thyroxine and insulin) parameters of energy status, pre- and post-feeding blood samples were taken on two consecutive days from three groups of lactating beef cows. Animals were consuming a silage/concentrate diet providing 81 (Group L : 20 animals), 107 (Group M : 12 animals) and 124% (Group H : 20 animals) of their estimated daily metabolizable energy (ME) requirements.

Differences in blood composition attributable to energy status were not detectable in post-feeding samples (apart from higher glucose levels in Group H compared with Groups L and M). At the pre-feeding sampling only thyroxine was capable of differentiating between all the groups, although there were other significant group differences. Feeding was associated with marked changes in the concentrations of all blood constitutents except insulin.

INTRODUCTION

The economic implications of sub-standard performance in animal production (through an increased incidence of metabolic disorders such as bovine ketosis and ovine pregnancy toxaemia, along with reduced reproductive performance), and the contributions which can be made to such performance by undernutrition, are both well known. Together they make it desirable to be able to assess the adequacy of rations in supplying nutrients, particularly energy, to animals so that costly undernutrition can be avoided. The assessment of nutritional status is becoming increasingly important as farmers attempt to maintain enterprise profitability by either reducing feed costs and maintaining production, by maintaining feed costs and increasing overall production, or by combinations of these two procedures.

For animals consuming fixed amounts of rations of known compositions, nutritional adequacy can be calculated by comparing the intake of individual nutrients with the estimated requirements for those nutrients. In beef herds the level and composition of the food intake of individual animals is usually unknown, so that dietary adequacy cannot be directly determined and indirect measures of nutritional status must be used.

In the past, concentrations of 3 plasma constituents (glucose, FFA, KB) have been used as parameters of energy status (Bowden, 1971), although there is little published work on the comparative effectiveness of these predictors (Erfle, Fisher and Sauer, 1974; Fisher, Donnelly, Hutton and Duganzich, 1975). It is considered possible that plasma hormone concentrations may act as additional parameters of energy status, although there is at present little evidence to support this concept.

Previous work in lactating beef cows consuming high-roughage rations (Coggins and Field, 1976) provided evidence for marked diurnal variations, which were probably prandial in origin, in the plasma concentrations of glucose, FFA and KB. Further work was required to verify these results, and also to determine the importance of energy status and time of feeding as sources of variation in plasma hormone concentrations.

For these reasons time of feeding and nutritional status were compared as sources of variation in the plasma composition of lactating beef cows.

MATERIALS AND METHODS

Pre- and post-feeding blood samples were taken from three nutritional groups of lactating beef cows, designated L, M and H, which received food allowances of silage and concentrates (Table I) providing 90, 125 and 175% respectively of their estimated (Agricultural Research Council, 1965) daily ME requirements for maintenance (based on liveweight 12 hr after parturition). No ME allowances were made for milk production.

The animals used were Hereford x British Friesian cows, aged 4-6 yr (mean 4.4 $\pm$ 0.6), and were part of a long-term nutrition experiment. A preliminary report of the latter was given by Somerville, Lowman and Edwards (1976).

Possible effects of stress on blood composition were minimized by taking the blood samples on consecutive days : at 08.00 and 12.30 hr in Group L (fed at 09.00 hr), at 10.00 and 14.30 hr in Group M (fed at 11.00 hr) and at 09.00 and 13.30 hr in Group H (fed at 10.00 hr). The order of sampling was random within each group on each occasion.

Plasma glucose, FFA and KB concentrations were estimated using modifications

of the methods described by Coggins and Field (1976). Plasma thyroxine concentrations were estimated using the method of Seth, Rutherford and McKenzie (1975), and plasma insulin concentrations using a double antibody radioimmunoassay (Hales and Randle, 1963).

Statistical analyses were made using Student's t-test.

RESULTS

Table 2 shows the mean weight, milk yield and stage of lactation of animals in each of the groups. At the time of sampling, animals in Groups L, M and H respectively were considered to be undernourished, adequately nourished and more than adequately nourished (in terms of their daily ME intake/requirements ratios). These considerations were confirmed by the trends observed in rates of weight change before and after the experiment.

Tables 3 and 4 show the mean pre- and post-feeding plasma constituent concentrations for each of the groups, along with the significances of the group differences (pre- and post-feeding) and of the prandial changes within each group.

Pre-feeding glucose concentrations in Group L were lower than equivalent levels in Groups M and H; post-feeding levels in Group H were higher than levels in Groups L and M. The marked postprandial fall in levels was highly significant in all groups. Similar concentrations (690 mg/l) were maintained by Group L animals pre-feeding as by Group H animals post-feeding.

The higher pre-feeding FFA levels in Group L than in Groups M or H were the only significant effect of group on concentrations of this metabolite. The lowering of these high pre-feeding levels in Group L to post-feeding levels

similar to those in Groups M and H was the only significant time effect.

Pre-feeding KB levels were higher in Group H than in Groups L and M and there were no significant group differences post-feeding; in all groups KB levels were increased markedly by feeding.

Pre-feeding thyroxine concentrations were differentiated according to group, with low levels in Group L and high levels in Group H. The effect of feeding on thyroxine concentrations was different in each group, resulting in similar post-feeding levels in all groups.

No significant effect of group on plasma insulin concentrations was detectable, with mean group levels only marginally increased by feeding.

The changes in plasma composition over a $4\frac{1}{2}$ hr time period were generally of greater significance than the changes due to a 50% difference in ME status and a 45% difference in DM intake.

DISCUSSION

It is important to differentiate between ME status and glucose status : the ruminant has a general requirement for energy and a specific requirement for glucose. Of the three more commonly used parameters of energy status, only FFA are a true measure of caloric sufficiency as hypoglycaemic ketosis may develop without necessarily involving a caloric deficiency (Adler, 1970).

The pre-feeding FFA levels in the undernourished animals (Group L), although higher than the equivalent levels in weel-nourished animals, were below the levels (600 - 1500 μ equiv/l) observed by A. C. Field (unpublished) in well nourished dairy cattle, and were well below the levels (up to 2500 μ equiv/l) observed in undernourished sheep (Reid and Hinks, 1962; Russel, Doney and Reid, 1967). There is little information on the ability of

animals which have lost large proportions of their bodyweight (up to 30% in Group L animals) to mobilize fat reserves, and it is conceivable that after prolonged undernutrition this ability may be markedly reduced. As pointed out by Somerville et al (1976), the results demonstrate the ability of the undernourished beef cow to maintain milk production by mobilizing body reserves.

The absence of significant group differences in glucose and KB levels may be related to the low glucose requirements of all the groups, resulting from their uniformly low milk yields. Nevertheless, similar findings were reported by Fisher et al (1975) in animals producing larger quantities of milk than the animals described here.

The greater number of significant group differences pre-feeding than post-feeding is probably due to the bulky nature of the food. The rate of consumption of the silage was probably similar in all groups for some time after feeding, so that for several hours animals would be in positive balance with respect to those nutrients which are rapidly absorbed from the rumen. Group differences in blood composition would only develop when the absorption of these products was less than the requirements for them. This theory is supported by the results of the previous work (Coggins and Field, 1976), and in the present work by the finding of no group differences 3½ hr after feeding but significant group differences 23 hr after feeding.

Pre-feeding plasma thyroxine concentrations may be an effective parameter of energy status, as the nutritionally distinct groups could be easily distinguished according to their thyroxine levels. Plasma insulin levels would appear to be of little value in the biochemical assessment of nutritional adequacy.

There is some disagreement as to the nature of the postprandial change in bovine plasma glucose levels as both rises (Allcroft, 1933; Holmes and Lambourne, 1970; Bowden, 1973) and falls (Radloff, Schultz and Hoekstra, 1966; Hove and Blom, 1973; Coggins and Field, 1976) have been reported. It is possible that the nature of the response may be modified by the composition of the diet : markedly different rations were used by the above groups of workers. Prandial changes in plasma glucose (and KB) levels in the present work were very similar to those reported by Coggins and Field (1976).

The variable prandial increase in plasma thyroxine concentrations between the groups is though to reflect the change in nutritional status of the groups at feeding : changes in animals with restricted intakes (Group L) were much more pronounced than changes in animals fed virtually <u>ad libitum</u> (Group H). The lack of effect of feeding on plasma insulin levels was surprising in view of the concomitantly large changes in plasma glucose concentrations, and also because of previous reports of prandial variation in bovine insulin levels (Hove and Blom, 1973; Hove, 1974).

It is obvious that the biochemical assessment of the energy status of animals consuming rations at pre-determined intervals requires an appreciation of the inherent prandial fluctuations in blood composition. Prandial variations may be minimized by taking all samples at a fixed time relative to feeding, reversing the current recommendation (Payne, Dew, Manston and Faulks, 1970) to sample at a fixed time of day, irrespective of the time of feeding. However, even at the time of day at which group differences are maximized (i.e. immediately pre-feeding), plasma composition is only of limited value in the prediction of energy status.

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Table 1 Composition and rate of feeding of rations offered to three groups of lactating beef cows.

(a) Ration composition.[i]

		Silage	Concentrates		
			Group L	Group M	Group H
Dry matter (DM) (%)		28.4	85.3	84.5	85.0
Modified acid digest fibre	% in DM	30.8	-	-	-
Crude protein (CP)	% in DM	12.6	41.2	20.7	11.1
Digestible CP	% in DM	7.81	37.7	15.7	8.4
Crude fibre	% in DM	-	7.2	4.6	3.7
Ether extract	% in DM	-	0.5	0.4	0.7
Nitrogen free extract	% in DM	-	33.2	62.6	78.1
ME (MJ/kg DM)		10.6	10.5	11.5	13.1

(b) Rations consumed (kg/day)[ii]

	Group			Pooled
	L	M	H	SD
Fresh silage offered	18.8	25.0	36.3	2.7
Silage DM intake	5.28	7.02	9.65	1.03
Fresh concentrates offered	0.85	1.02	1.38	0.08
Concentrate DM intake	0.72	0.87	1.17	0.07

i R.A. Edwards, personal communication.

ii S.H. Somerville, personal communication.

Table 2 Estimated ME (MJ/day) and N (g/day) intake/requirements ratios of three groups of lactating beef cows.

	Group L	Group M	Group H	Pooled SD
No. of animals	20	12	20	
Wt (kg)	393	409	500	49
Wt at parturition (kg)	530	498	525	49
Days post partum	123	138	132	29
Date of cessation of wt loss (days post partum)	115	103	64	12
Estimated milk yield	7.25	7.04	8.71	1.63
ME intake	63.6	84.8	118	11
ME requirements[i]	79.3	79.6	95.1	10
Intake/requirements (%)	80.8	107	124	13.6
N intake	109	110	136	14.7
N requirements[ii]	71	70.1	86.4	13
Intake/requirements (%)	159	161	160	32

i 0.489 MJ/kg $(Wt)^{0.75}$ (A.R.C., 1965)
5 MJ/kg milk (R.A. Edwards, personal communication).

ii 0.039 g/kg Wt; 7.68 g/kg milk (A.R.C., 1965).

Table 3 Mean values of plasma metabolite concentrations in 3 nutritional groups of lactating beef cows before and after feeding.

	Group			Pooled	Group difference		
	L	M	H	SD	L v. M	M v. H	L v. H
Glucose (mg/l)							
Pre-feeding	690	755	753	79	***	NS	***
Post-feeding	554	603	690	73	NS	**	***
Time effect	***	***	**				
FFA (μ equiv/l)							
Pre-feeding	496	282	251	145	***	NS	***
Post-feeding	265	266	239	46	NS	NS	NS
Time effect	***	NS	NS				
KB (mg/l)							
Pre-feeding	13.9	11.8	17.3	3.7	NS	***	***
Post-feeding	14.2	37.8	38.7	10.0	NS	NS	NS
Time effect	***	***	***				

Table 4 Mean values of plasma hormone concentrations (ng/ml) in 3 nutritional nutritional groups of lactating beef cows before and after feeding

	L	M	H	Pooled SD	Group difference L v. M	M v. H	L v. H
Thyroxine							
Pre-feeding	57.3	68.5	83.7	10.0	*	**	***
Post-feeding	72.3	75.2	83.8	14.3	NS	NS	NS
Time effect	***	**	NS				
Insulin							
Pre-feeding	0.304	0.311	0.352	0.103	NS	NS	NS
Post-feeding	0.386	0.385	0.426	0.200	NS	NS	NS
Time effect	NS	NS	*				

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